

Meta-omics of the Engineered Water Cycle

(Nitrogen Cycling in Wastewater Treatment)

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<http://www.columbia.edu/~kc2288/>

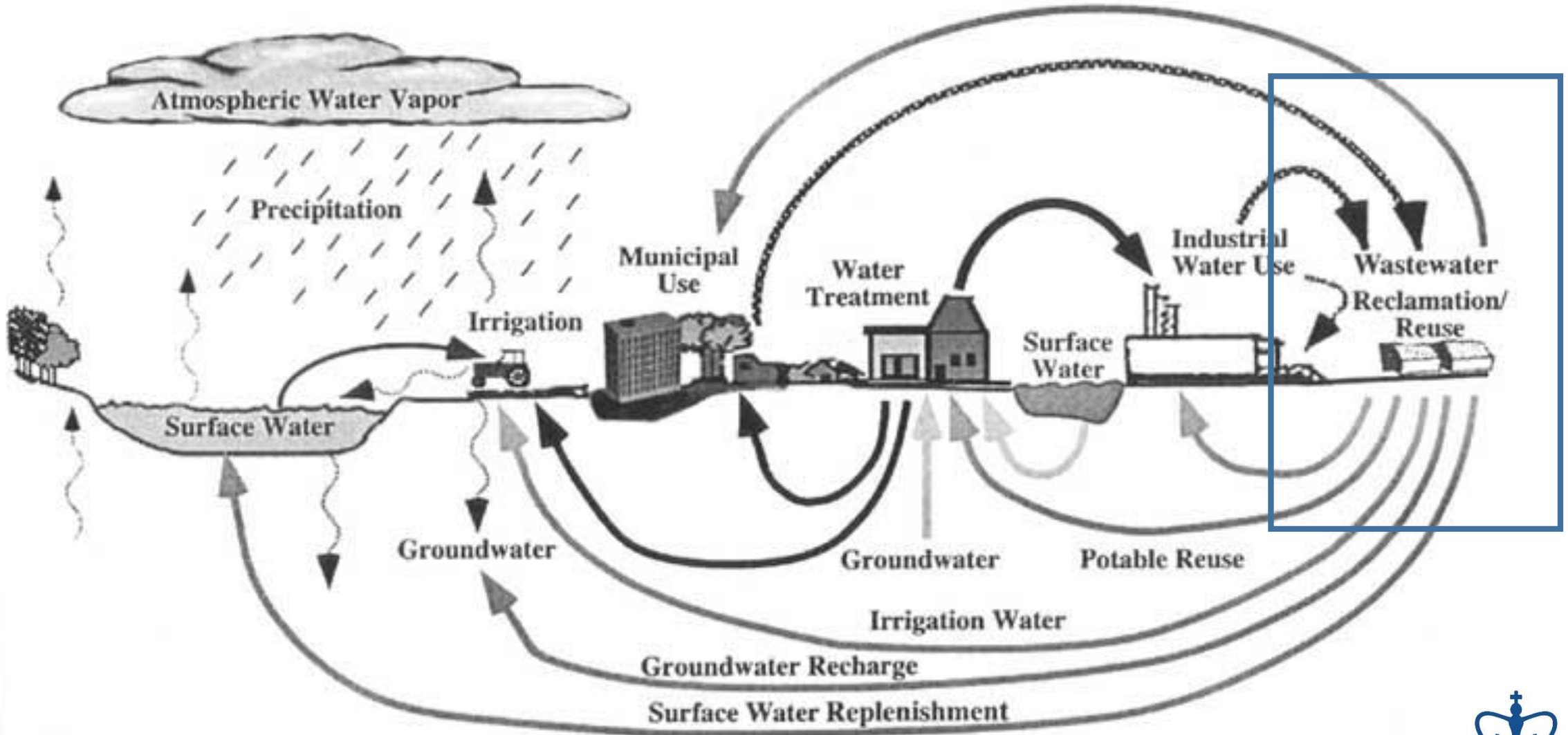
Ion World Tour | NYC | September 2015



COLUMBIA | ENGINEERING

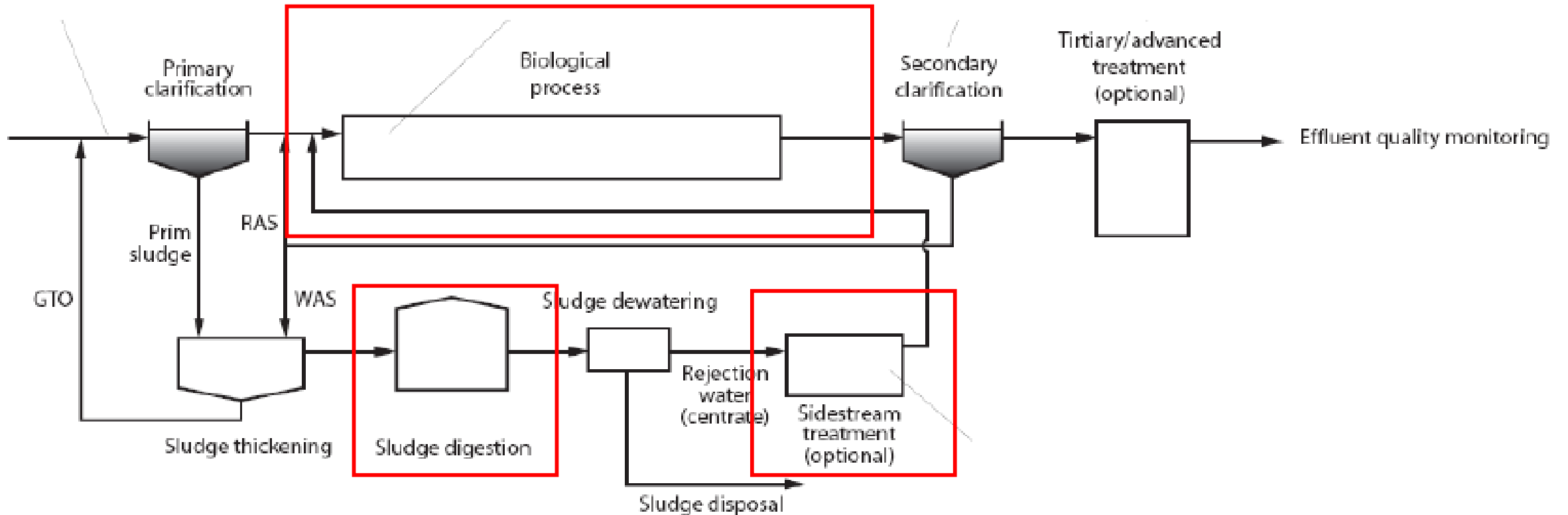
The Fu Foundation School of Engineering and Applied Science

What is the Engineered Water Cycle?



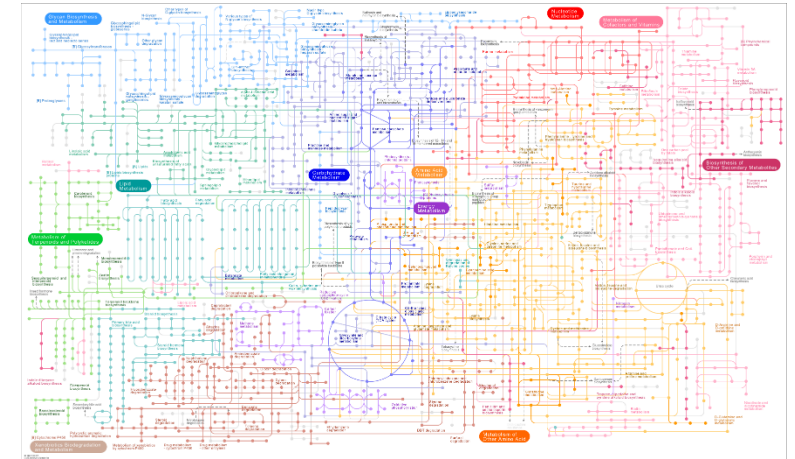
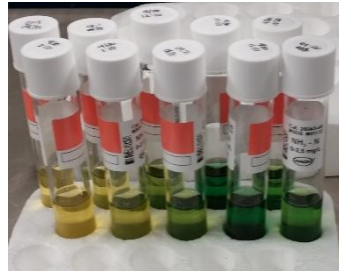
What is the Engineered Water Cycle?

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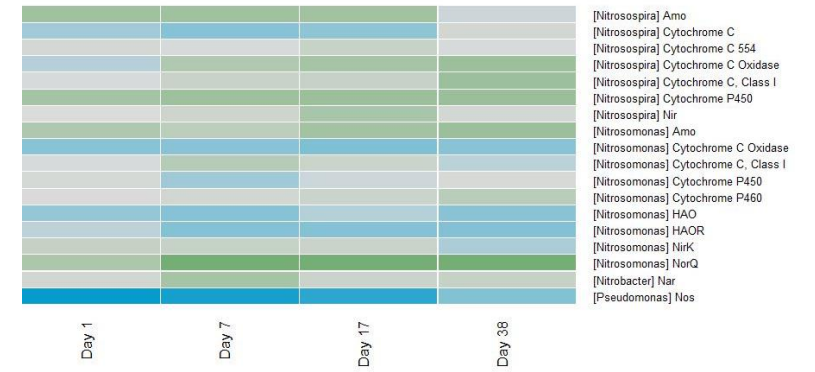




M. Annavajhala, June 2013



M. Annavajhala and A. C. Brotto, April 2015



M. Annavajhala and A. C. Brotto, April 2015



A. C. Brotto

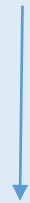


Which microorganisms
are present in the
sample?



16S Amplicon Sequencing
(Metagenomics)

Which metabolic &
functional pathways are
active in the sample?



Shotgun DNA
Sequencing
(Metagenomics),
RNA-Seq
(Metatranscriptomics)

How are genes being
differentially expressed
across samples?

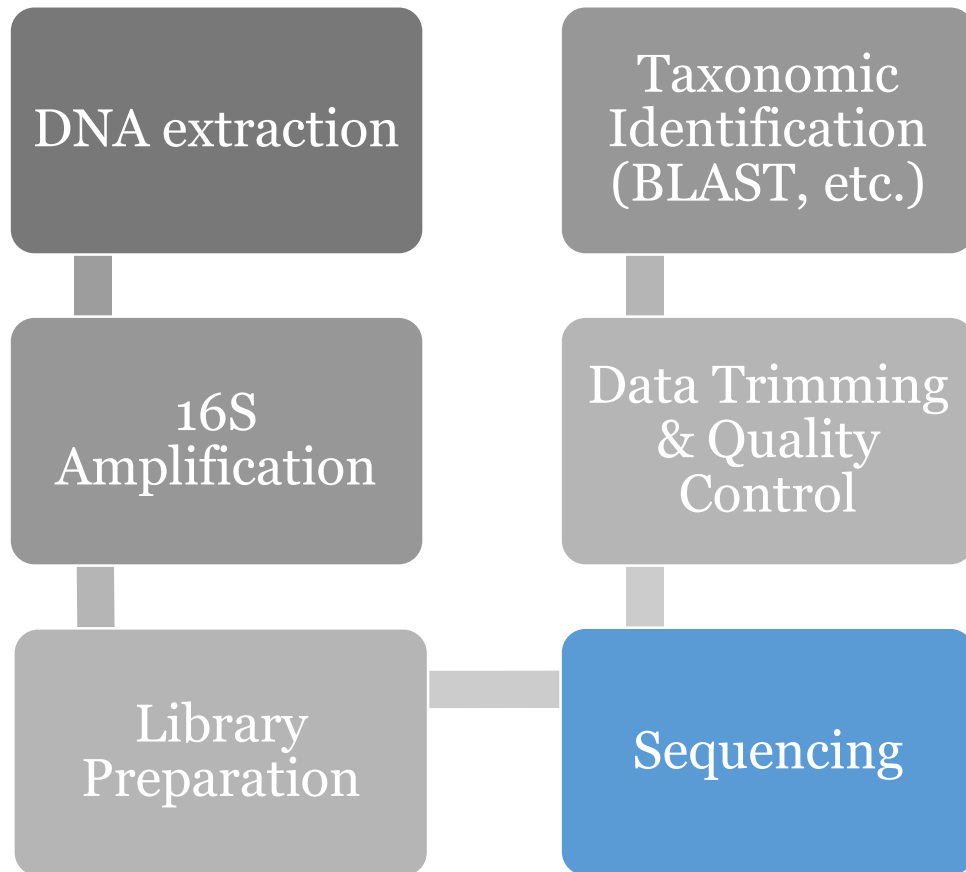


RNA-Seq
(Metatranscriptomics)

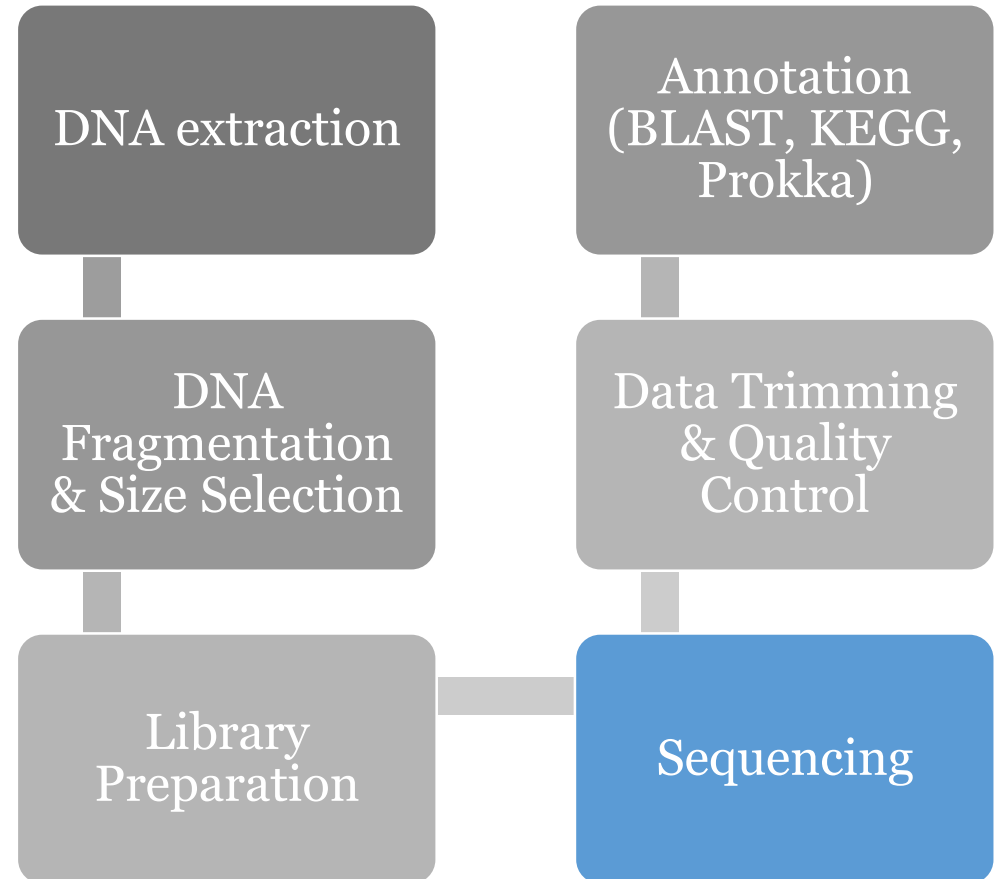


(Meta)genomics

Identifying the Community
(16S Amplicon Sequencing)

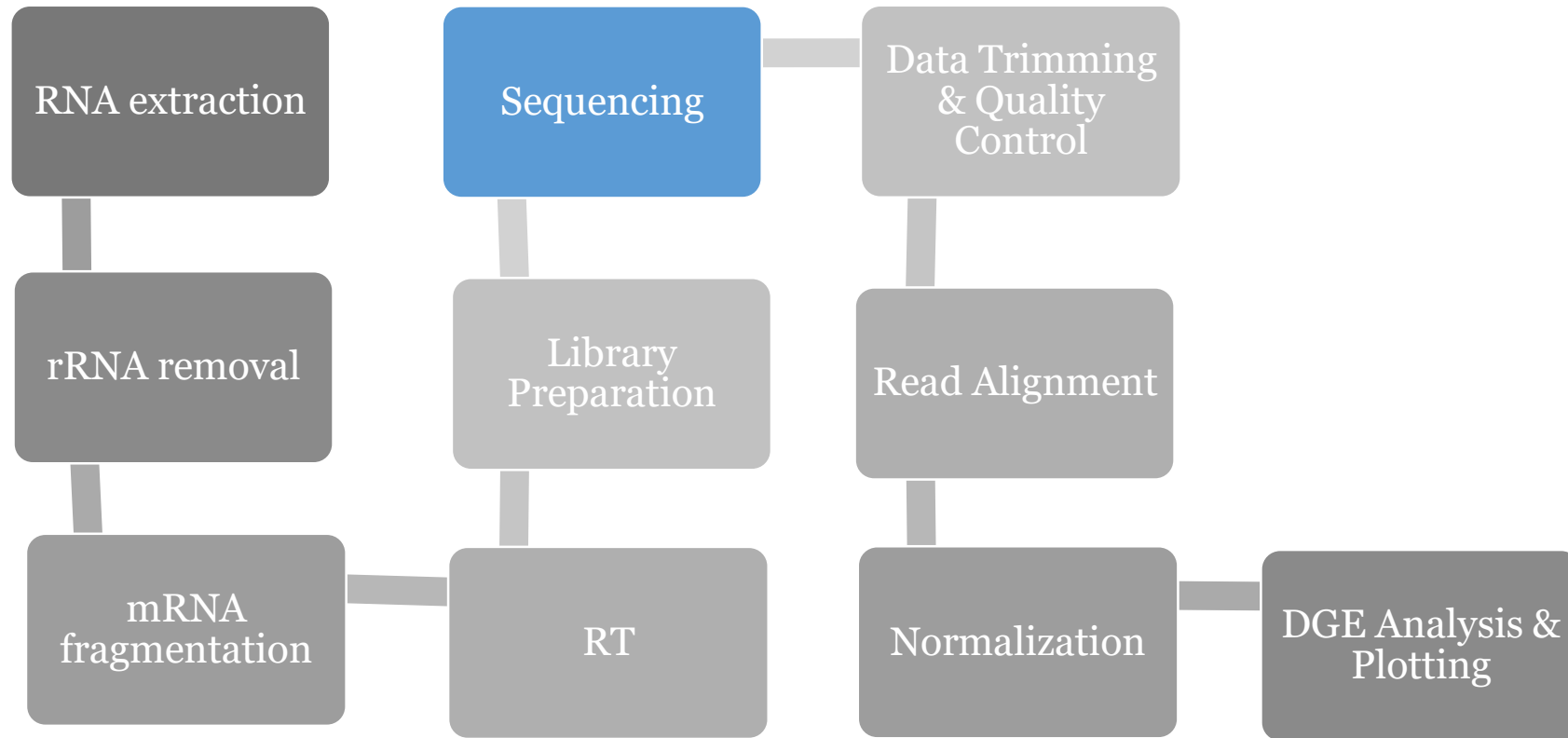


Identifying Functional Pathways
(Shotgun Sequencing)



(Meta)transcriptomics (RNA-Seq)

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Library Preparation & Sequencing Kits

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16S
Amplicon
Sequencing

In-house developed fusion primers (target: 16S V4 region)

Shotgun
Metagenomics

NEB Next Fragmentation Kit
Ion Xpress Plus Fragment Library Kit
Ion Xpress Barcode Adaptors

RNA-Seq/
Meta-
transcriptomics

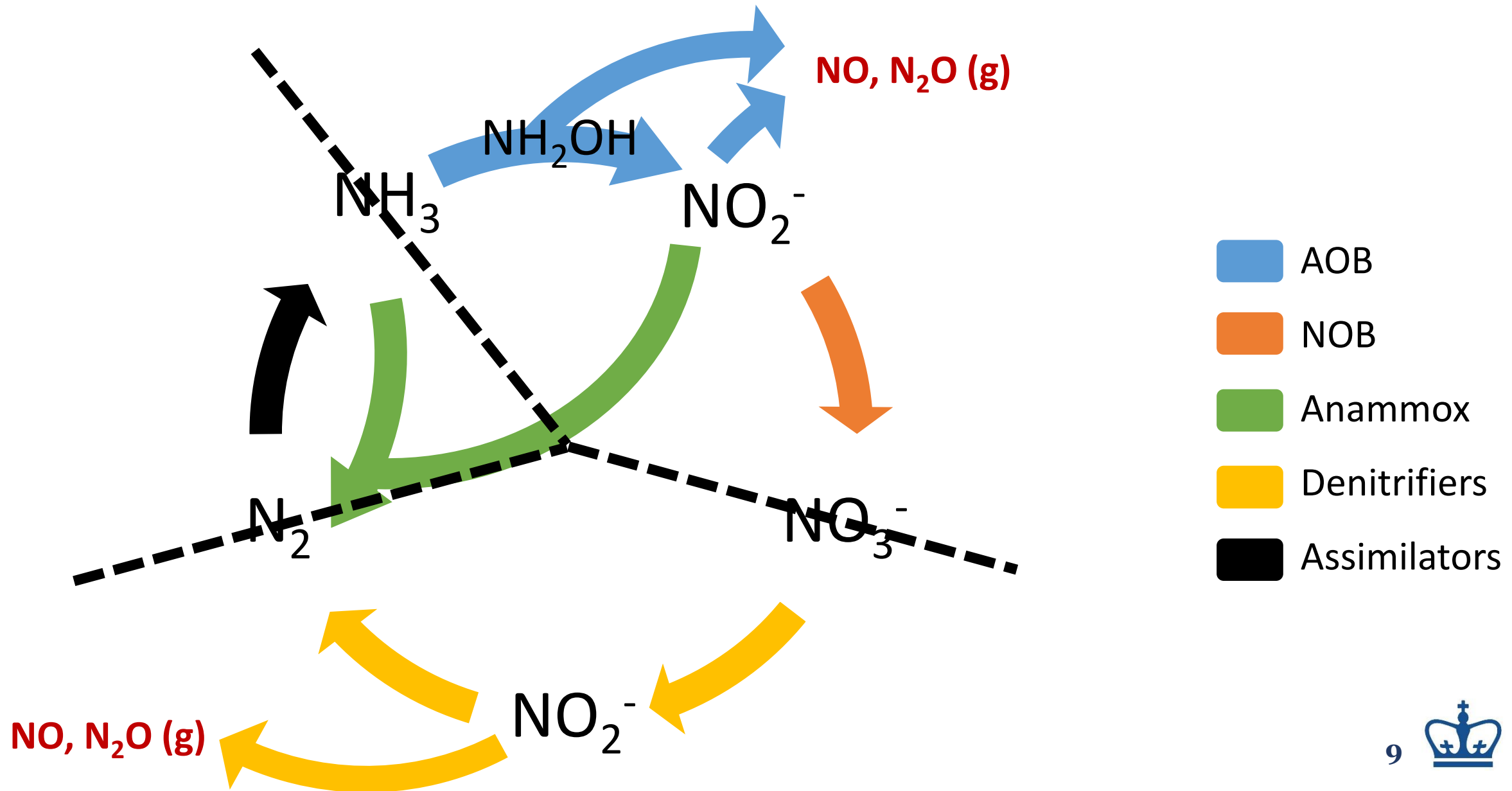
Ion RNA-Seq Kit v2
Ion Xpress RNA Barcode Adaptors

Ion OT2 &
PGM

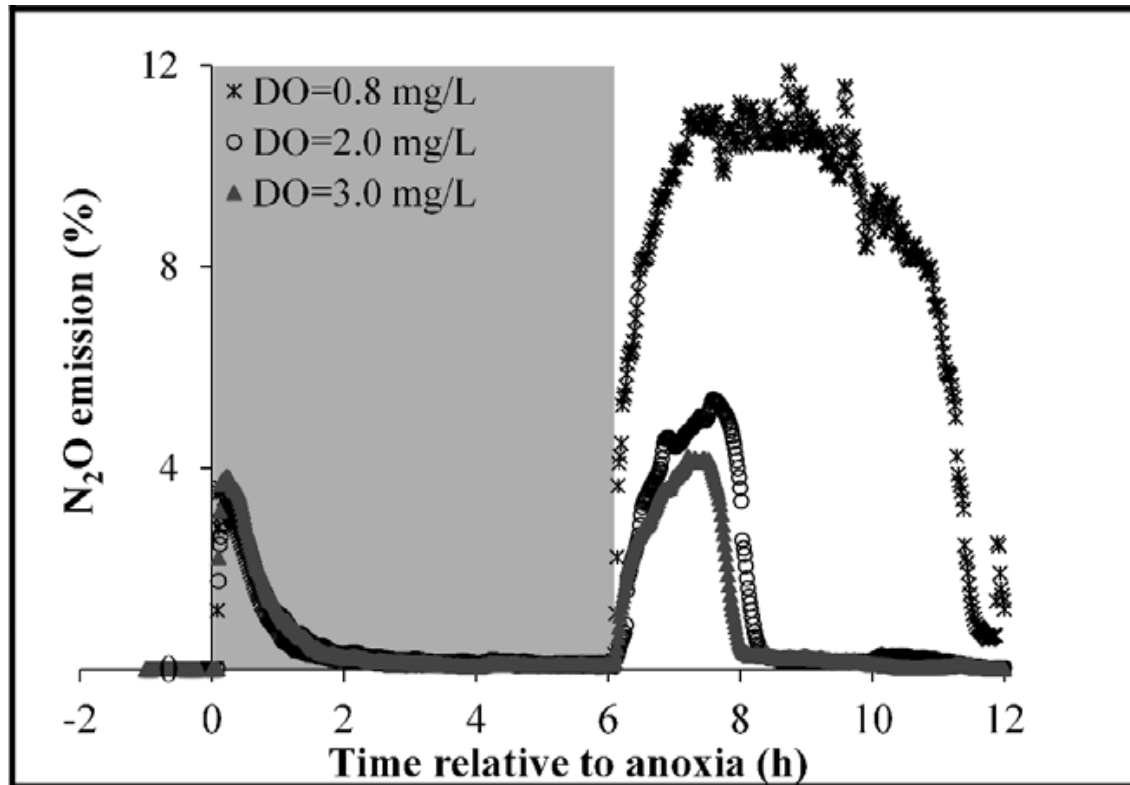
KAPA Library Quantification Kit
Ion Quantitation Kit
Ion PGM 400 OT2 Kit
Ion PGM 400 Sequencing Kit
Ion PGM Hi-Q OT2 Kit
Ion PGM Hi-Q Sequencing Kit
Ion 316v2, 318v2 Semiconductor Chips



Biological Nitrogen Removal & GHG Production



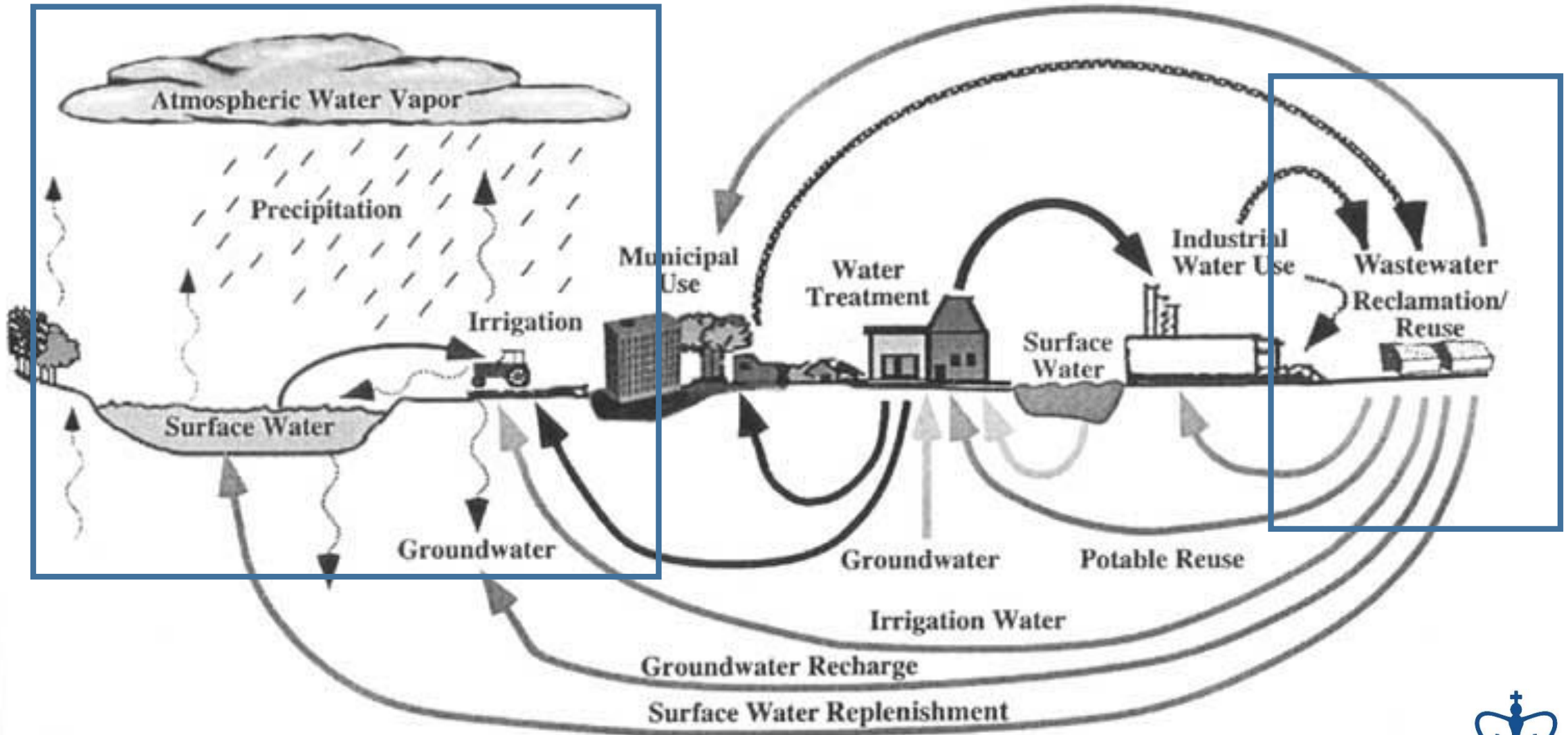
Oxygen Cycling: Goals



- Identify pathways of NO, N₂O production in full nitrification lab-scale reactor under transiently oxic conditions
- Connect chemical & gaseous data to NGS findings
- Find parallels/interactions between N, C, and other cycles
- Track changes in pathways/gene expression over time



What is the Engineered Water Cycle?



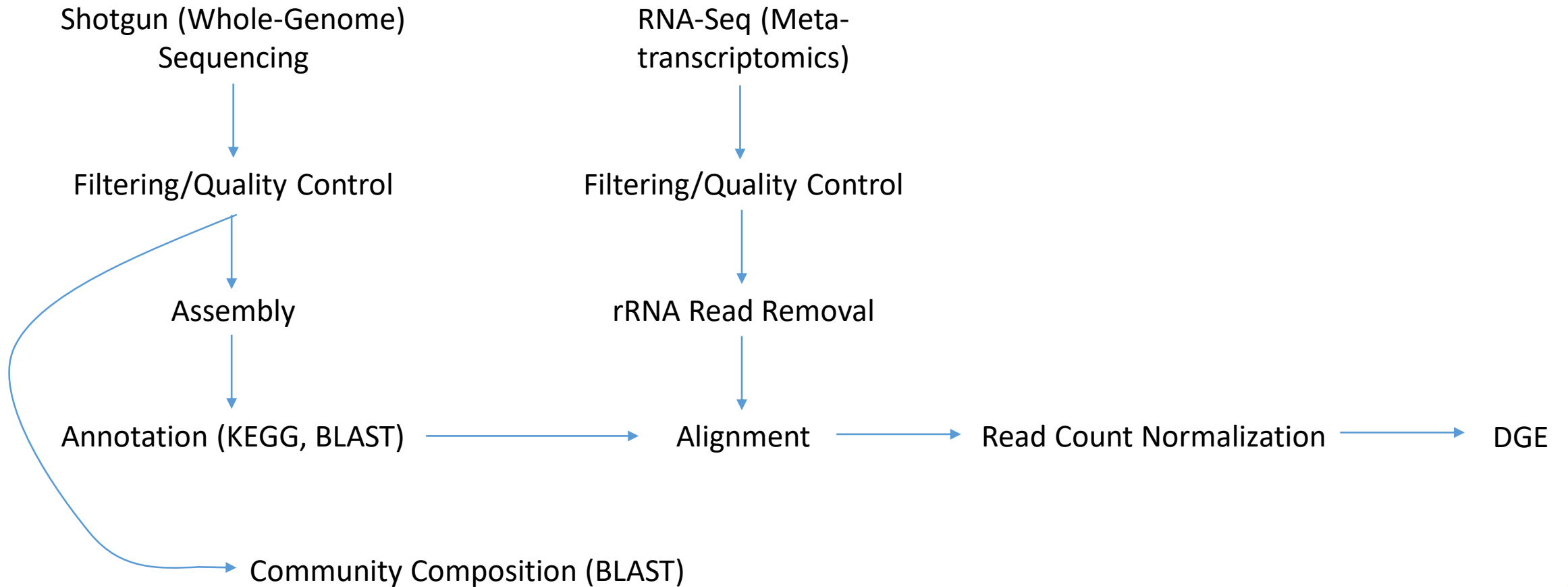
Oxygen Cycling: Experimental Design

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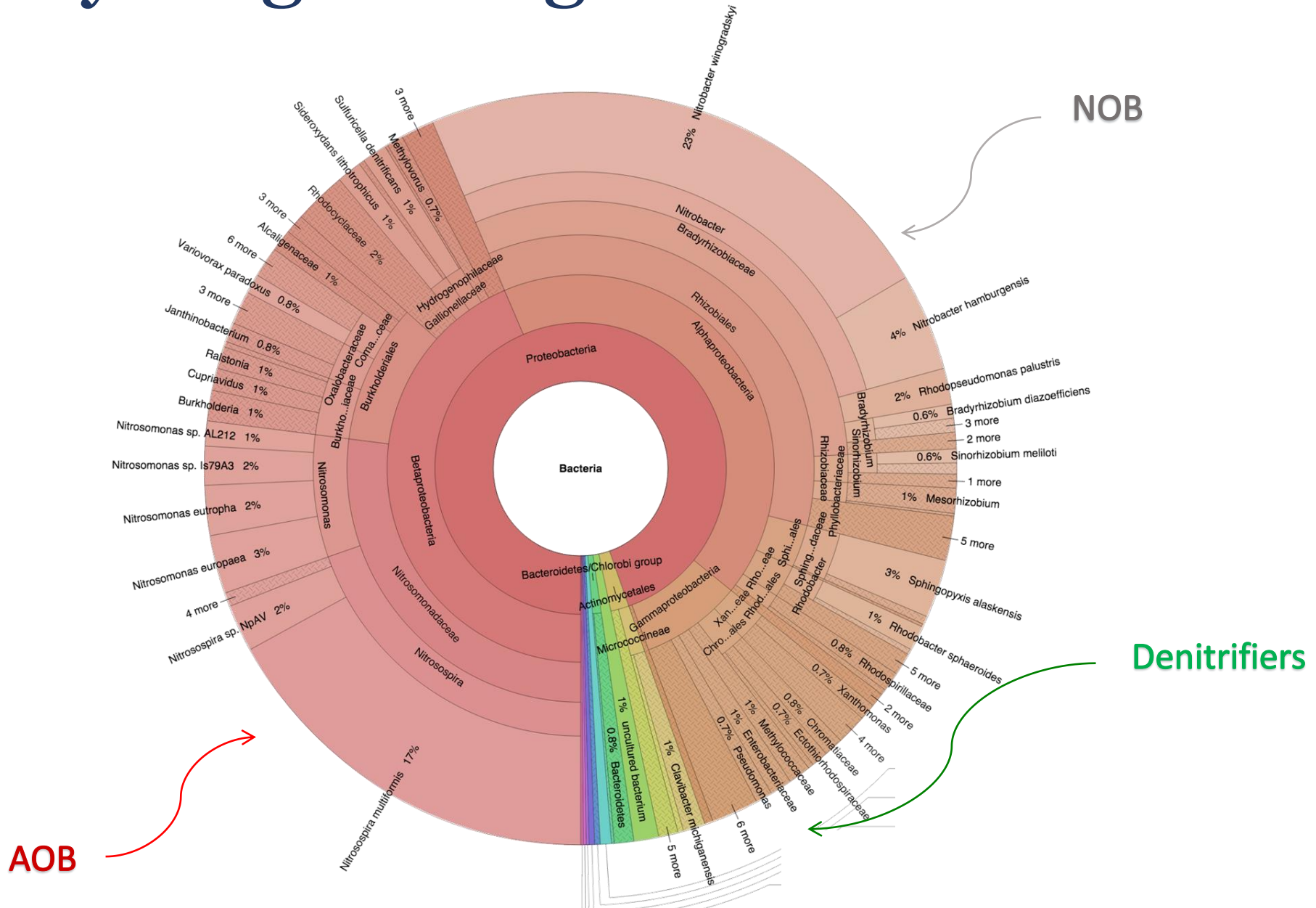
- Mimic full nitrification in lab-scale Sequencing Batch Reactor (SBR)
- Impose transient anoxic-oxic conditions
- Collect chemical and gaseous nitrogen-cycle data
- Collect biomass at specific timepoints for NGS



Oxygen Cycling: NGS Workflow



Oxygen Cycling: Metagenomics

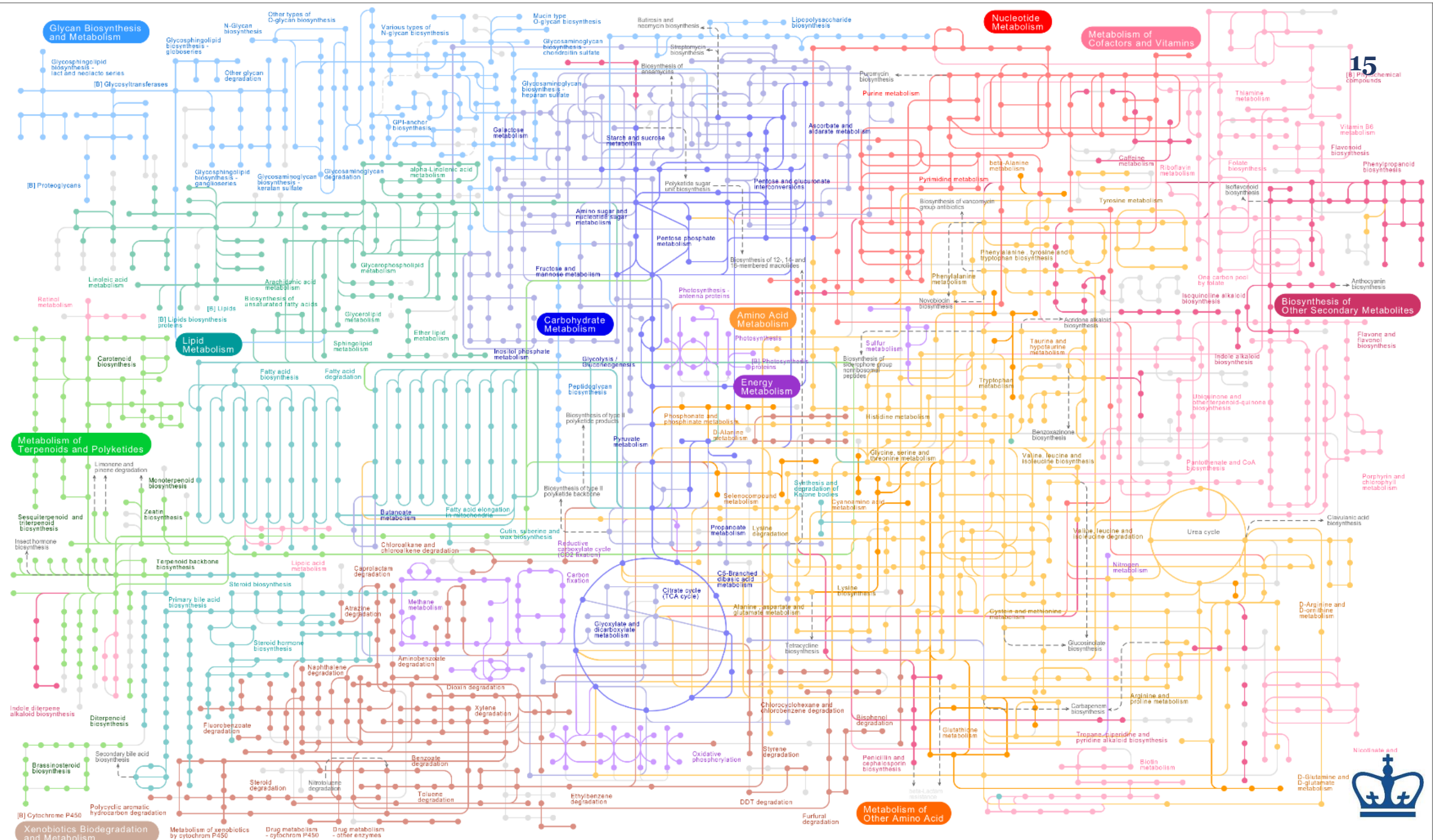


AOB

NOB

Denitrifiers

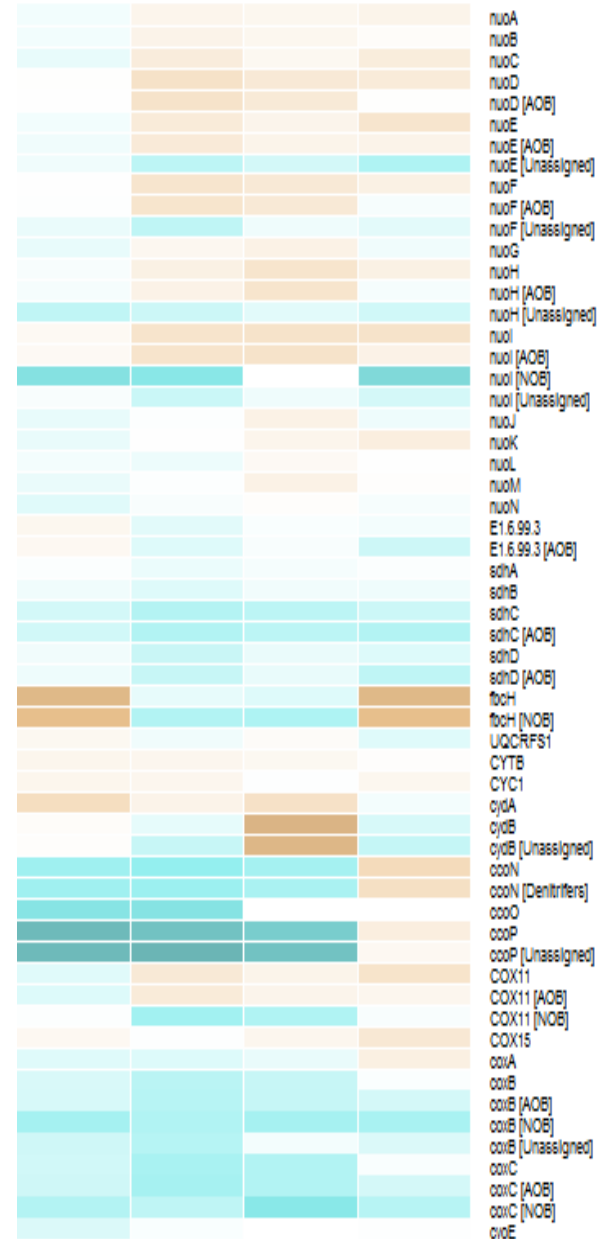
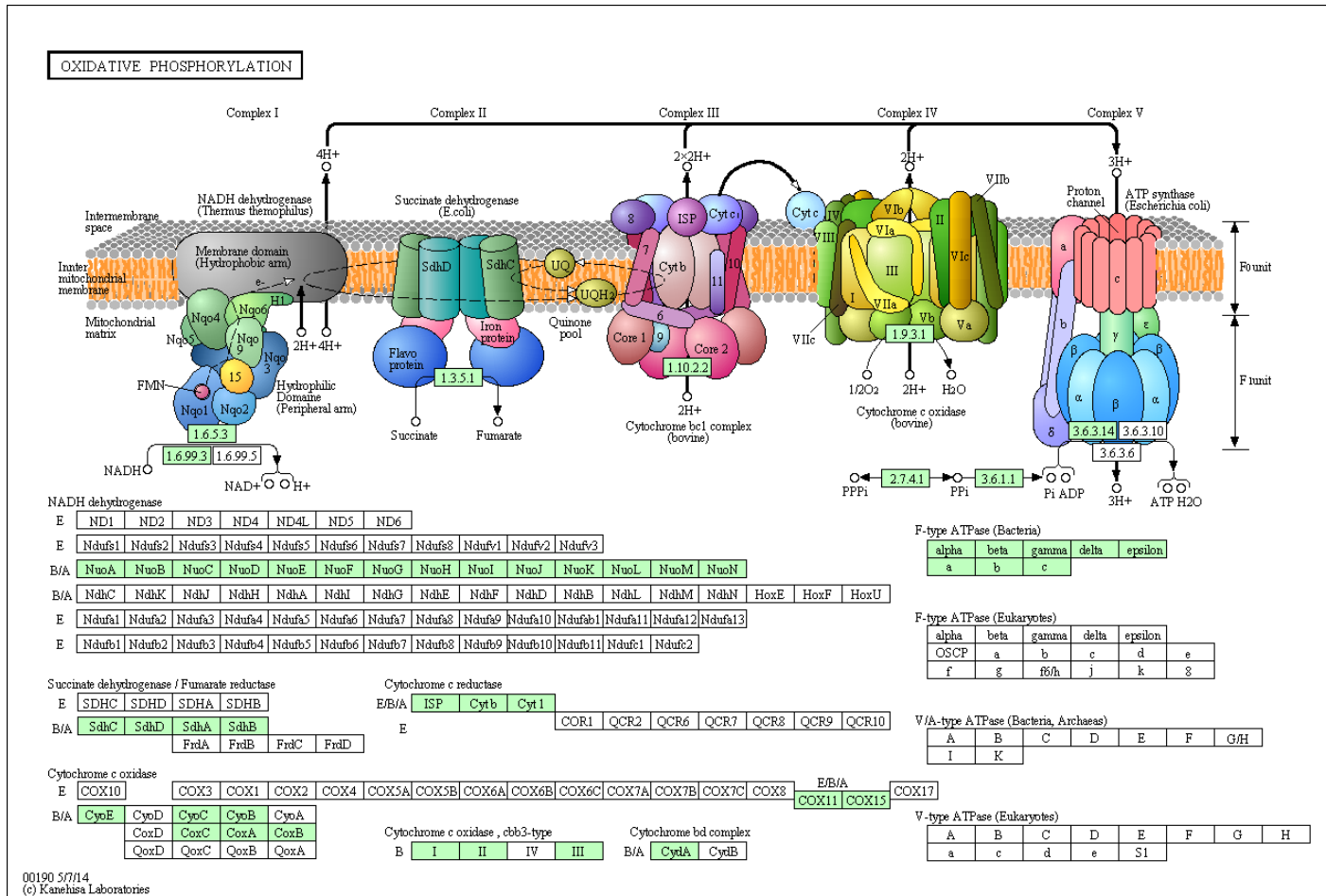




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[B] Phytochemical compounds



Metatranscriptomics & DGE



- nuoA
- nuoB
- nuoC
- nuoD
- nuoD [AOB]
- nuoE
- nuoE [AOB]
- nuoE [Unassigned]
- nuoF
- nuoF [AOB]
- nuoF [Unassigned]
- nuoG
- nuoH
- nuoH [AOB]
- nuoH [Unassigned]
- nuoI
- nuoI [AOB]
- nuoI [NOB]
- nuoI [Unassigned]
- nuoJ
- nuoK
- nuoL
- nuoM
- nuoN
- E1.6.99.3
- E1.6.99.3 [AOB]
- sdhA
- sdhB
- sdhC
- sdhC [AOB]
- sdhD
- sdhD [AOB]
- ftcH
- ftcH [NOB]
- UQCRF51
- CYTB
- CYC1
- cyoA
- cyoB
- cyoB [Unassigned]
- cooN
- cooN [Denitrifiers]
- cooO
- cooP
- cooP [Unassigned]
- COX11
- COX11 [AOB]
- COX11 [NOB]
- COX15
- coxA
- coxB
- coxB [AOB]
- coxB [NOB]
- coxB [Unassigned]
- coxC
- coxC [AOB]
- coxC [NOB]
- cyoE



Challenges & Lessons Learned

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- “Who is present/active” and “What are the system’s capabilities/functions” are much easier to answer than “Who is doing what?”
- Extremely well annotated reference sequences (whether already available or created as part of your NGS workflow) are crucial to meaningful RNA-Seq results
- Complex microbial communities make collection (coverage) & interpretation (resolution) of NGS data difficult, as expected
- Meta-omics capabilities are available, but still require troubleshooting on both library preparation and bioinformatics ends
- Experimental design should be driven by end goals; large amounts of data can lead to limits in capacity/experience/analysis

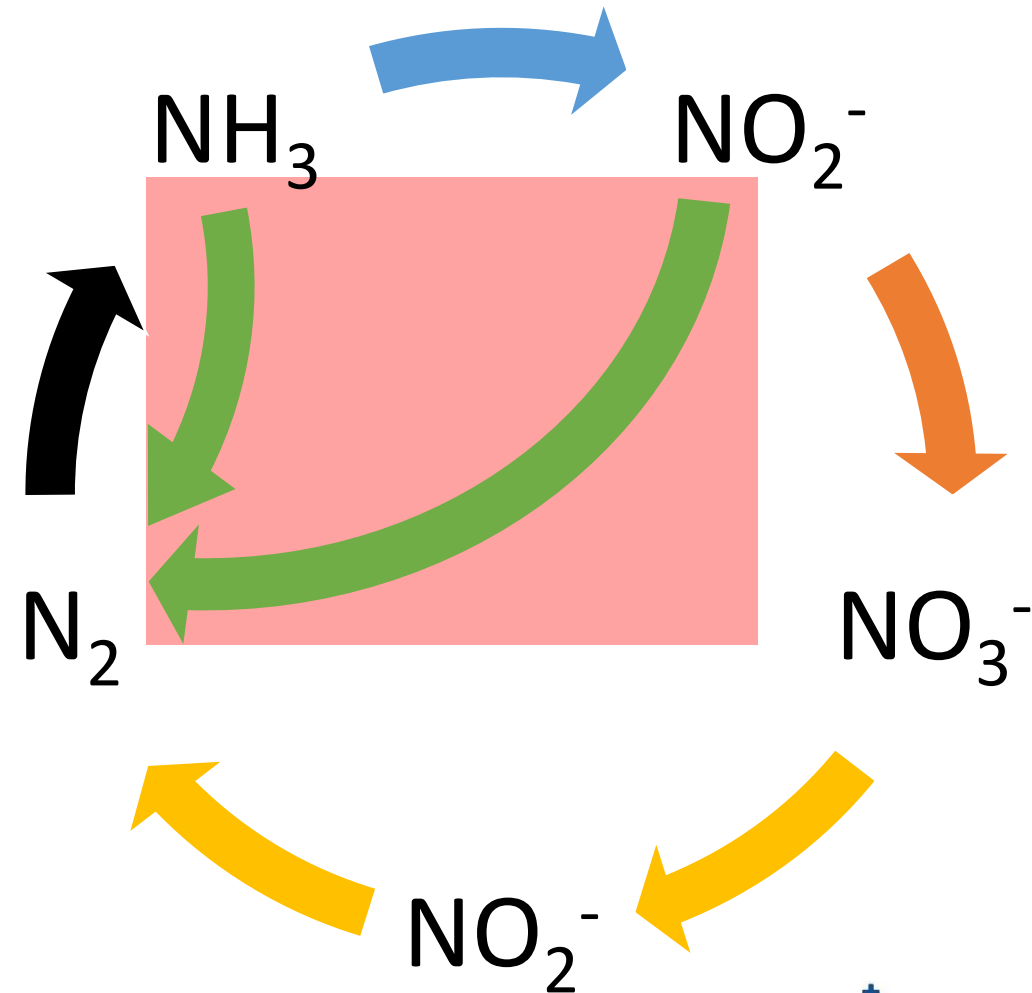


Other NGS Projects & Implications

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- Anammox work
- Pure culture studies
- Carbon Cycle & Alternate Endpoints
- Persistent Organic Pollutants

Similar questions can be answered using NGS, but with varied applications and implications



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