SMART Remediation

Unlocking Environmental Insights: Next Generation Sequencing Applications in Groundwater Remediation, Bioaugmentation, and eDNA Analysis

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SiREM
SMART Ottawa

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Unlocking Environmental Insights: Next Generation Sequencing Applications in Groundwater Remediation, Bioaugmentation, and eDNA Analysis

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Presented by: Ximena Druar
SMART Remediation
Ottawa, ON
8-Feb-2024

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SiREM's Products and Services

Remediation Testing

treatability studies

Bioaugmentation

KB-1®

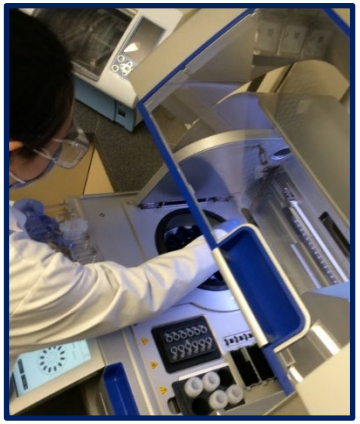
Dxo-88

DGG PLUS™

KB-1 plus®

Molecular Testing

genetrac®



CSIA

Isodetect Environmental Monitoring

Passive Sampling

SP3™

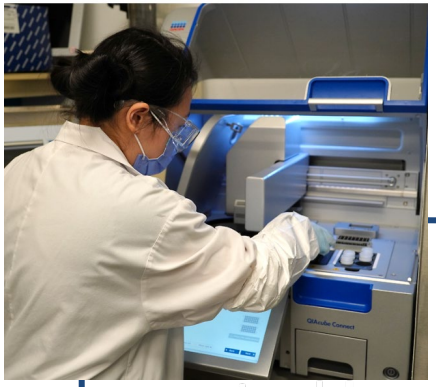
PFASsive™

WATERLOO MEMBRANE SAMPLER™

Molecular Biological Testing

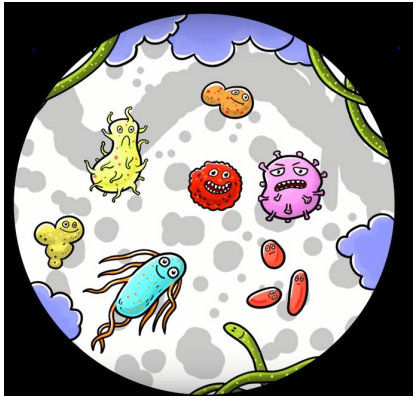
Samples

DNA Extraction



qPCR

Next Generation Sequencing (NGS)



Microbial Community Profiles

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Certificate of Analysis: Gene-Trac® NitroGen™
Ammonia Monooxygenase A Assay

Custom ... its
I84

SiREM Reference: S-8258
Report Date: 4-Oct-21
Data Files: QS3A-amoA-QPCR-0102

Table 1d: Test Results

Sample ID	Ammonia Monooxygenase A *amoA* (archaeal)		Ammonia Monooxygenase A *amoA* (bacterial)	
	Percent [2]	Gene Copies/Liter	Percent [2]	Gene Copies/Liter
MW-2-20210803	0.01 - 0.03 %	3×10^5	NA	1×10^4 U
MW-1-20210803	0.006 - 0.02 %	5×10^4	NA	1×10^4 U
INJ1-20210803	0.002 - 0.007 %	1×10^5	NA	1×10^4 U

See final page for notes.

Quantify Specific Gene targets

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A Few Ways to Look at Your Bugs

genetrac®

Quantitative PCR

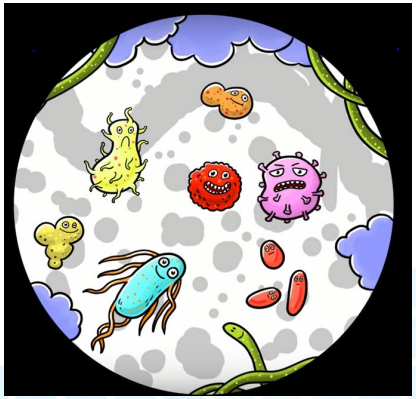
Quantify specific pre-selected targets:

- Microbial, e.g., *Dhc*, *Dhb*, *Dhg*
- Functional genes e.g., *tceA*, *bvcA*, *vcrA*

Digital PCR

Next Generation Sequencing

Characterize the entire microbial community



If Microbes Were Cars in a Lot…

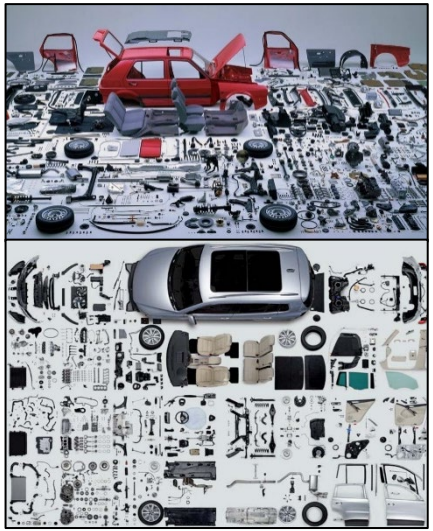
Quantitative PCR Tests

"There are 27 VWs"

Next Generation Sequencing (16S rRNA Amplicons)

"There are 27 VW, 14 Honda, 30 Toyota, 2 Ford, 6 Chevrolet…"

Metagenome Sequencing



Learn every car make (microbe) and all the individual parts of the cars (genes)

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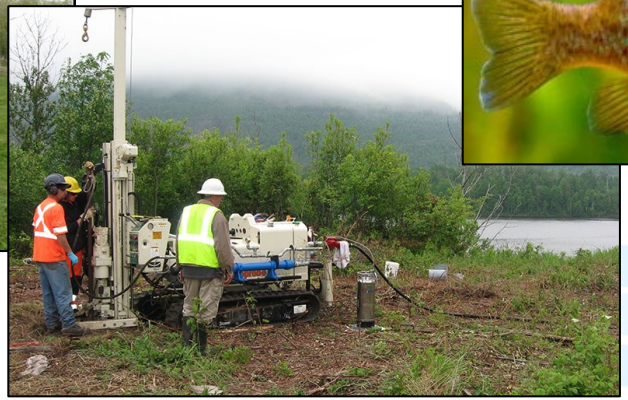
Some uses of NGS

Characterization of:

- Microbial communities in bioremediation
- Microbial cultures used in bioaugmentation
- Plant associated bacteria in phytoremediation
- Water associated biota using eDNA



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Using NGS Over Time in Remediation Projects

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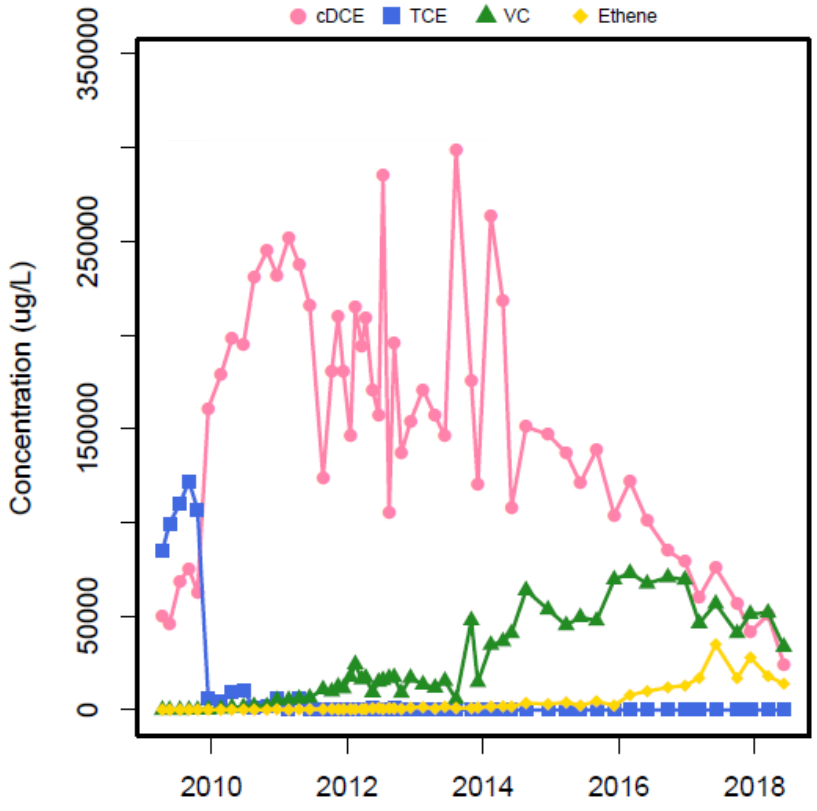
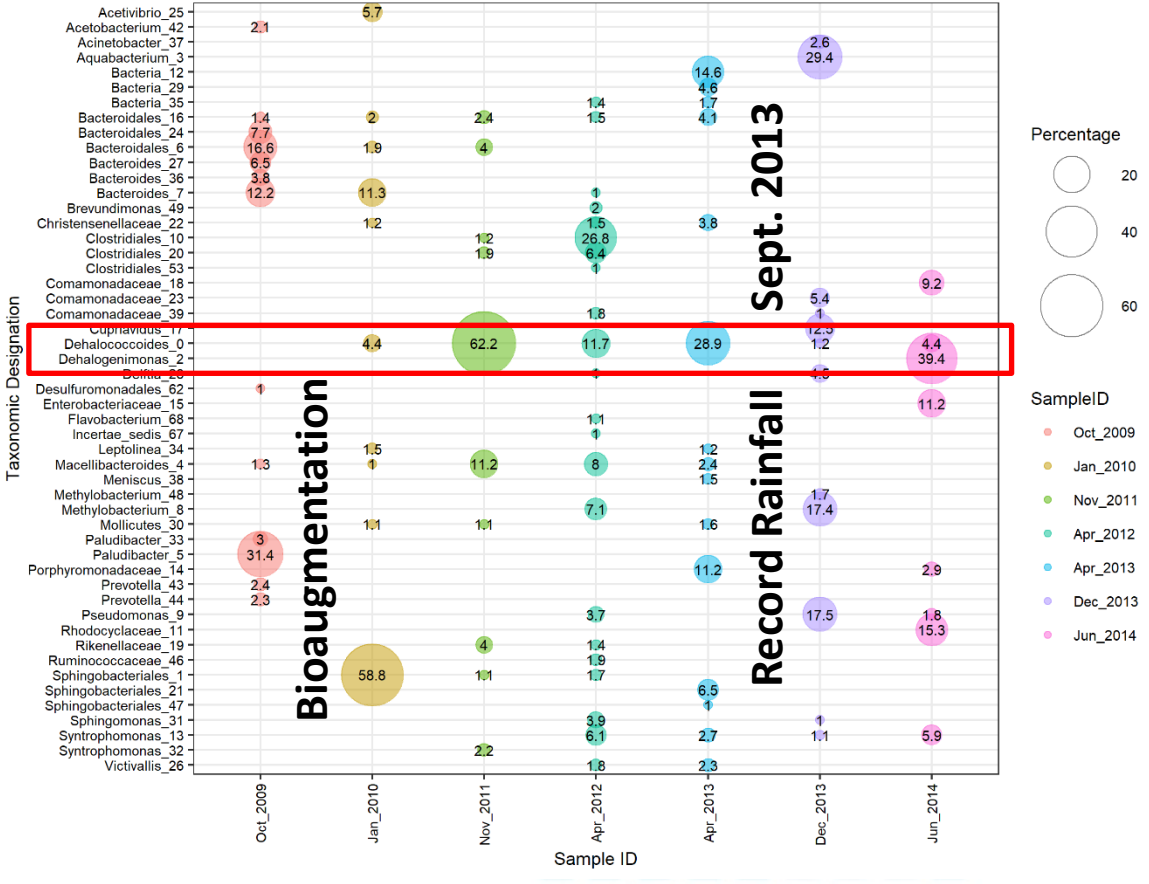
Oregon Site Dechlorination



- Bioaugmentation site KB-1 (Dhc)
- >99% TCE mass removal after one year
- Residual cDCE and VC are declining
- Long term Dhc monitoring and NGS study

Oregon Site NGS

- *Dehalococcoides* (Dhc) increases after bioaugmentation
- *Dehalococcoides* (Dhc) decline after April 2013 Why?
- September 2013 was the wettest on record in Oregon, 17 cm rain = surface water infiltration
- After 2014 *Dehalogenimonas* dominated



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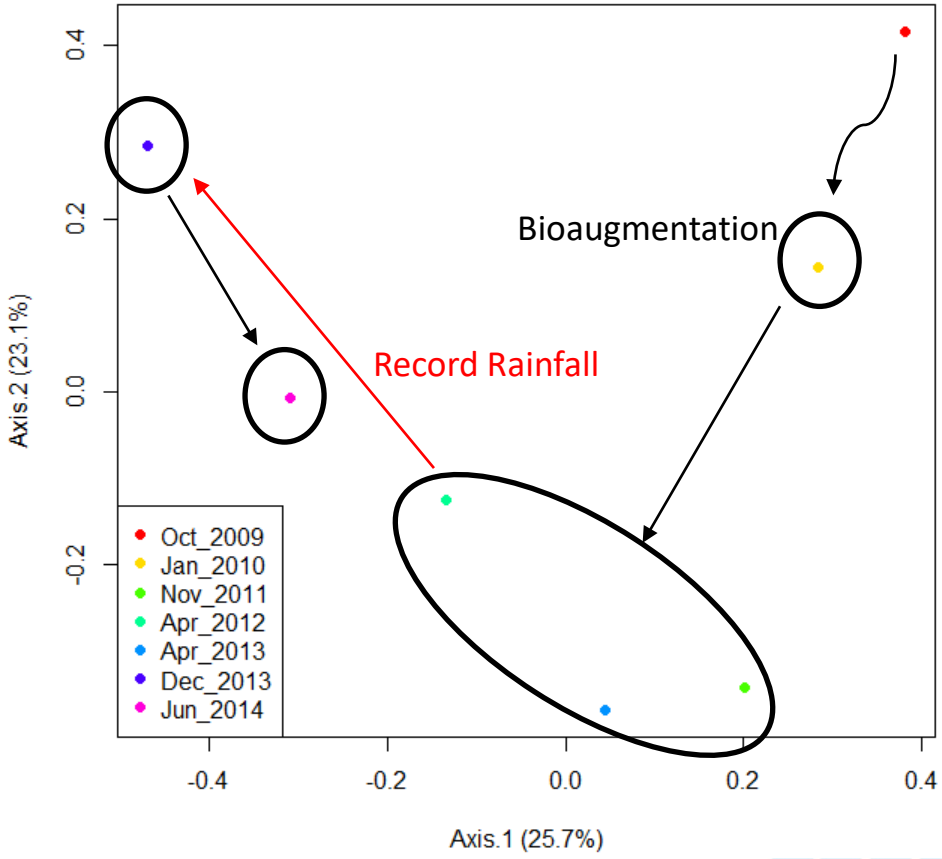
Oregon Site NGS



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Bioaugmentation Culture Characterization

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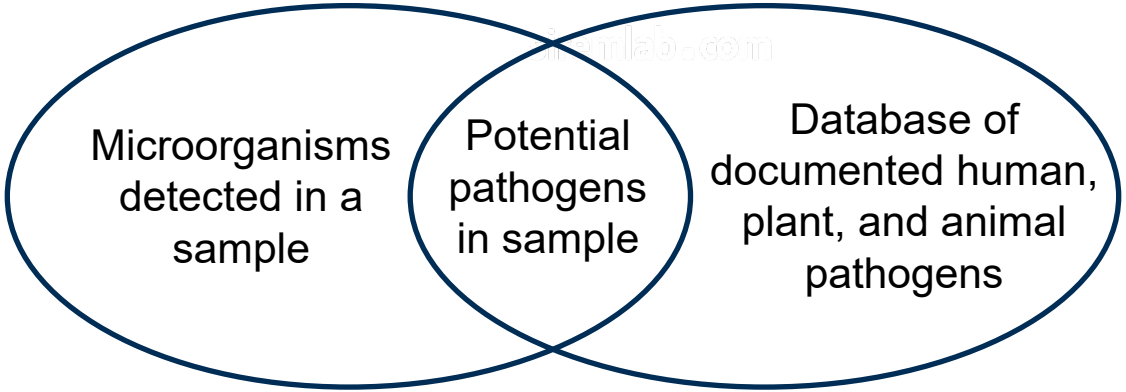
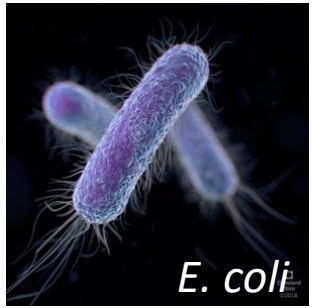
Are pathogens present?

UNIVERSITY OF TORONTO

- Pathogens are microorganisms that can cause disease
- NGS can be used to detect the DNA signature of pathogens in a sample





Using NGS to Characterize Cultures

- NGS data is used to ensure our bioaugmentation cultures meet Canada's New Substances Notification (NSN) Regulations
- KB-1, DGG-Plus in 2022, KB-1 Plus
- We confirmed that there was no significant evidence of pathogens

Canada

KB-1®

KB-1 plus®

DGG™ PLUS

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Looking for Plant Associated Microbes

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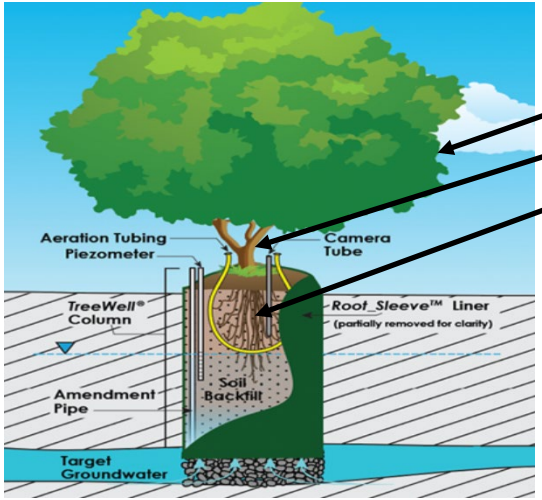
Using NGS with Phytoremediation

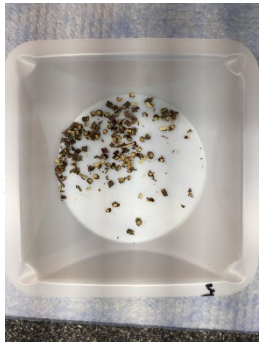
- In general, plant-associated microbes aid in plant growth, help acquire nutrients and moisture from soil, confer resistance to stresses, and some fix N_2
- **Phytoremediation Sites**
  - Petroleum Hydrocarbon/BTEX Site in Oklahoma
  - TCE/TCA Site in Pennsylvania
- **Study Goal** - Characterize plant-associated microbes that may play a major role in contaminant degradation in phyto systems e.g., PHCs and TCE

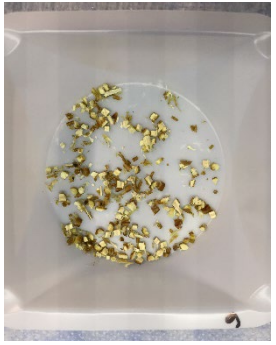
Tree Samples

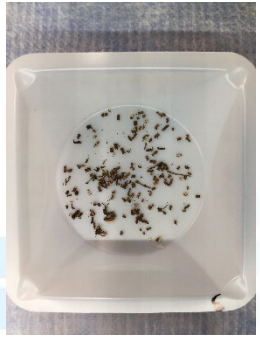


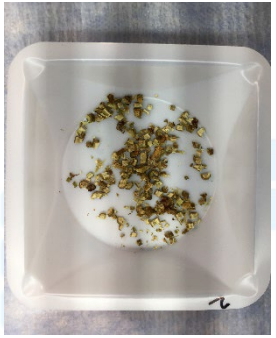
Collect samples at three key locations:

- Stems
- Tree Cores
- Roots









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Are contaminant degraders present in tree tissue?

Common Endophyte Genera Detected:

- *Burkholderia, Azoarcus, Rahnella, Pseudomonas, Pantoea, Enterobacter, Arthrobacter, Streptomyces, Bradyrhizobium*

Other Specific Genera or Species Detected with Biodegradation potential:

- *Dehalococcoides* spp., - known CVOC degraders
- *Pseudonocardia* spp., - include 1,4-Dioxane degraders
- *Polaromonas* spp., – aerobic degraders of Naphthalene & DCE
- *Phenylobacterium* – include obligate herbicide degraders
- *Methylibium* spp., - known MTBE degraders
- *Variovorax paradoxus* – known benzene degrader
- *Enterobacteracea* spp. – wide range of biodegradation activities
- *Pseudomonas* – wide range of biodegradation activities

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Detecting Flora and Fauna with Environmental DNA

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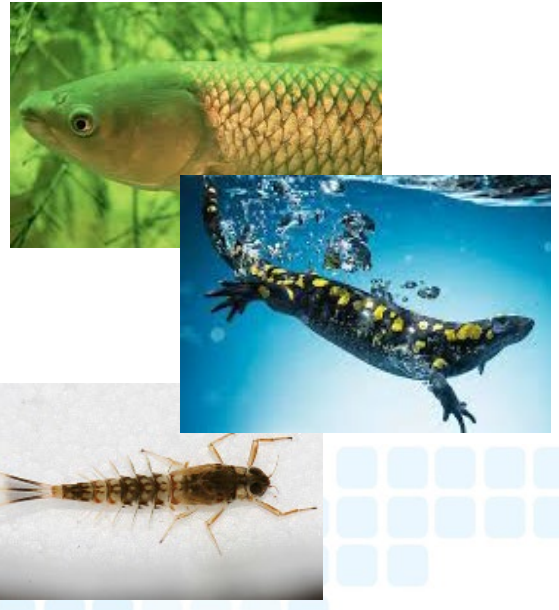
What is eDNA?

- Environmental DNA is expelled and accumulates when an organism interacts with an environment

- Analysis is cheaper & easier to perform than physical bioassessment surveys

- eDNA can be used to:
  - Determine ecological health of water bodies
  - Assess changes to ecosystems over time
  - Detect presence of endangered species
  - Track invasive species



Sequencing Gene Targets

- Gene-Trac NGS (Bacteria and Archaea 16S rRNA)

- Fish
- Macroinvertebrates
- Amphibians

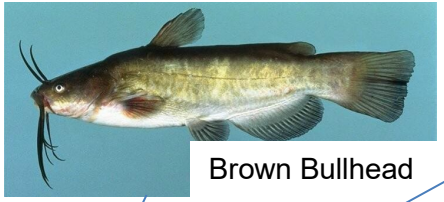
eDNA Targets
12S rRNA (fish only)
Cytochrome C Oxidase I

DNA was extracted from water and sediment

One sample, multiple organisms


Spottail Shiner

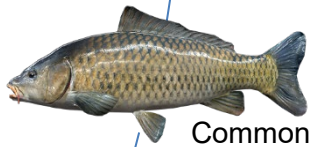

Alewife

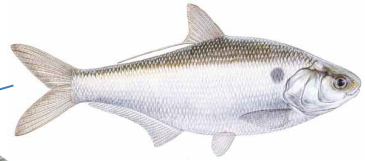

Brown Bullhead


Green Sunfish

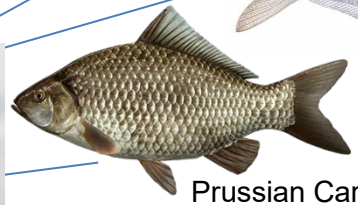

Brook Stickleback


Flathead Minnow


Common Carp

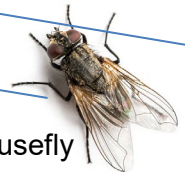

Gizzard Shad


Pumpkinseed


Prussian Carp


Frog


Salamander


Housefly


Canada Goose


Red Ear Slider


House Mouse


Human


Cattle

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Conclusions

Some of many applications of NGS:

- Track entire microbial communities during bioremediation
- Predict functions of microbial communities
- Determine whether pathogenic microorganisms are present
- Look for contaminant degrading bacteria in plants, soil, and groundwater
- Ecological surveys for organisms including fish, macroinvertebrates, and amphibians
- And many more uses are out there!

Questions

Thank you for your attention!

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