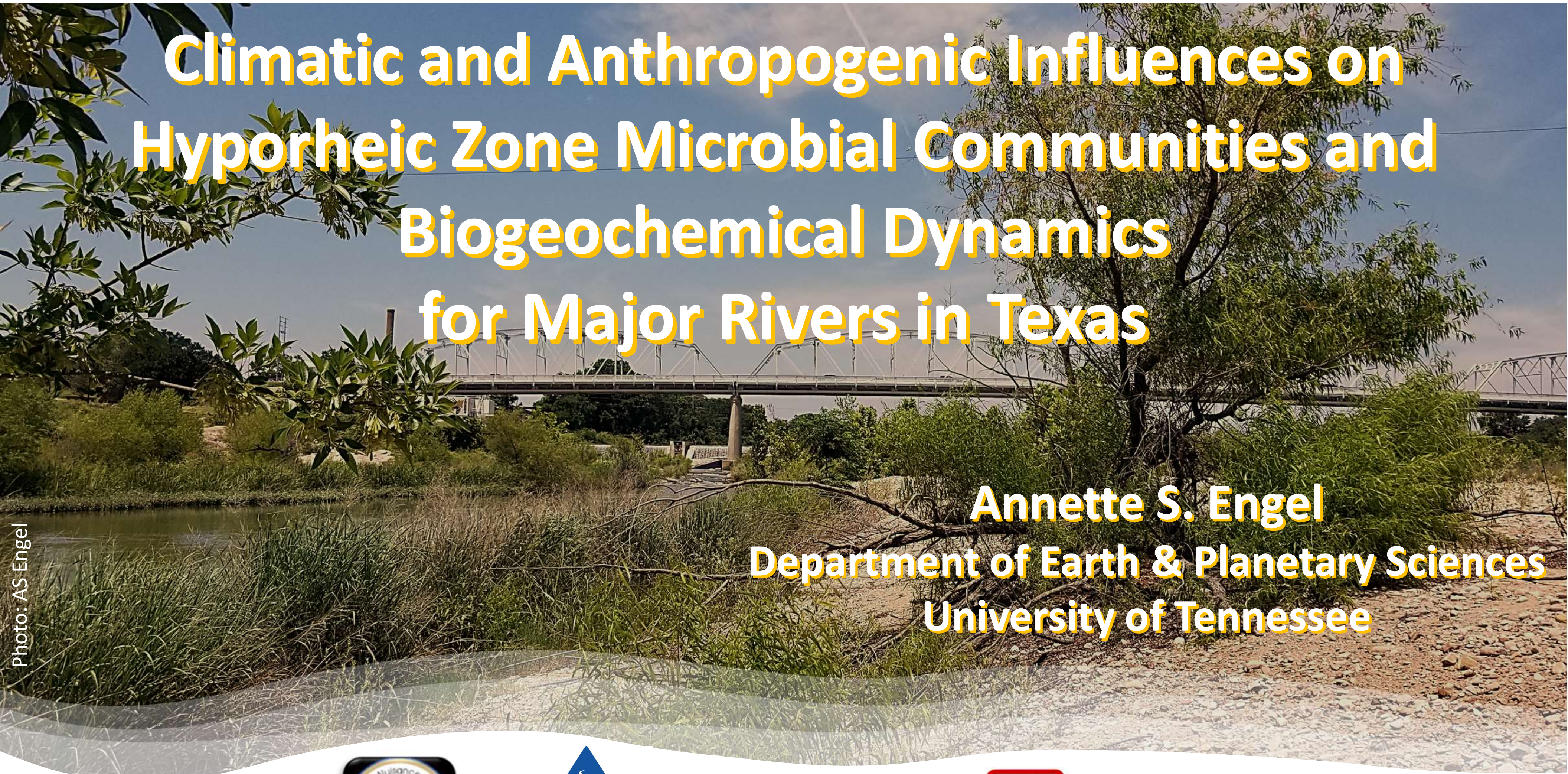

Photo: AS Engel

Climatic and Anthropogenic Influences on Hyporheic Zone Microbial Communities and Biogeochemical Dynamics for Major Rivers in Texas

Annette S. Engel
Department of Earth & Planetary Sciences
University of Tennessee

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ENGINEER RESEARCH & DEVELOPMENT CENTER

ANSRP
Aquatic Nuisance Species Research Program

TRIAGE

TEXAS STATE BIOLOGY

US Army Corps of Engineers

THE UNIVERSITY OF TENNESSEE KNOXVILLE

Research objectives – hyporheic zone

1) Reveal relationships between HZ invertebrate communities and antecedent river hydrology
★ 2) Describe relationships between HZ invertebrate communities and geochemical, geologic, and microbial properties, communities, and processes
3) Uncover environmental and physical controls on, and drivers of, genetic diversity in stygobionts in HZ
4) Assess importance of alluvial systems as corridors for movement and occupancy by stygobionts
★ 5) Quantify relationships between HZ properties and biological communities (microbial, stygobiont, and benthic)
6) Develop models linking physical properties of the HZ with hydrologic variables across climate gradients

Dr. Benjamin Schwartz

Photo: AS Engel

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Summary

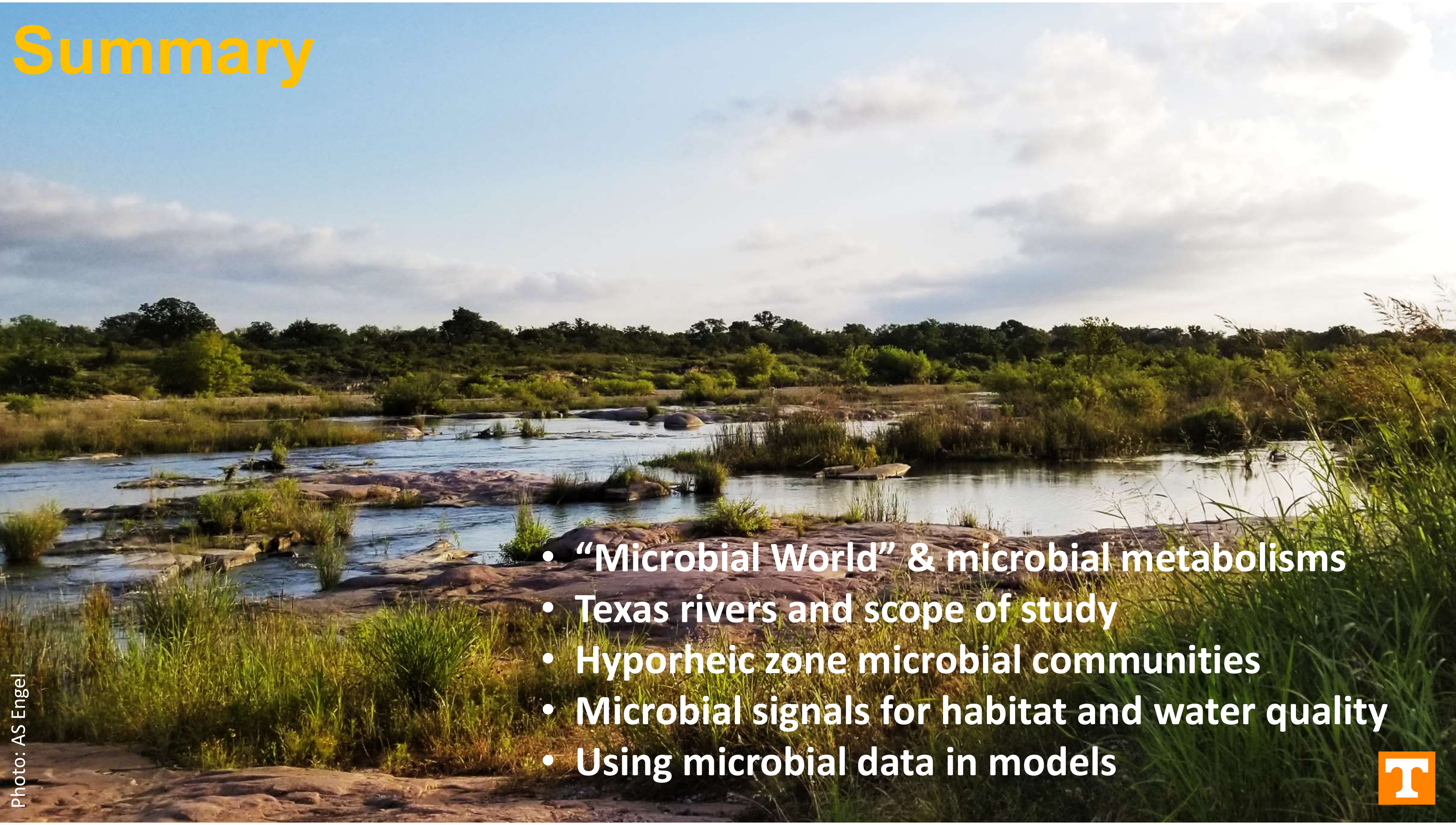


Photo: AS Engel

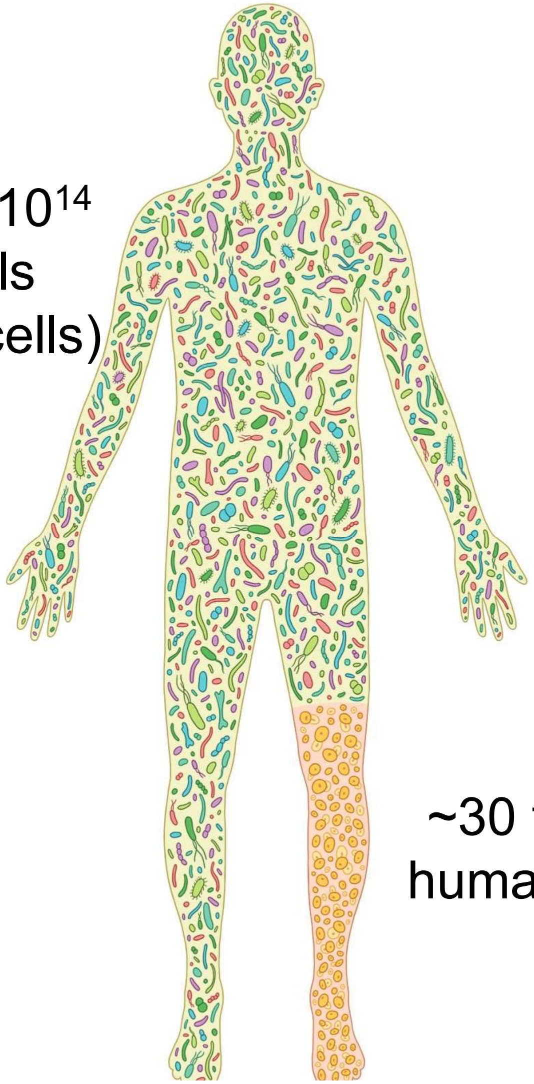
- "Microbial World" & microbial metabolisms
- Texas rivers and scope of study
- Hyporheic zone microbial communities
- Microbial signals for habitat and water quality
- Using microbial data in models

Microbes are everywhere – 10^{30} cells

1,000,000,000,000,000,000,000,000,000,000

nonillion

Microbes are here….

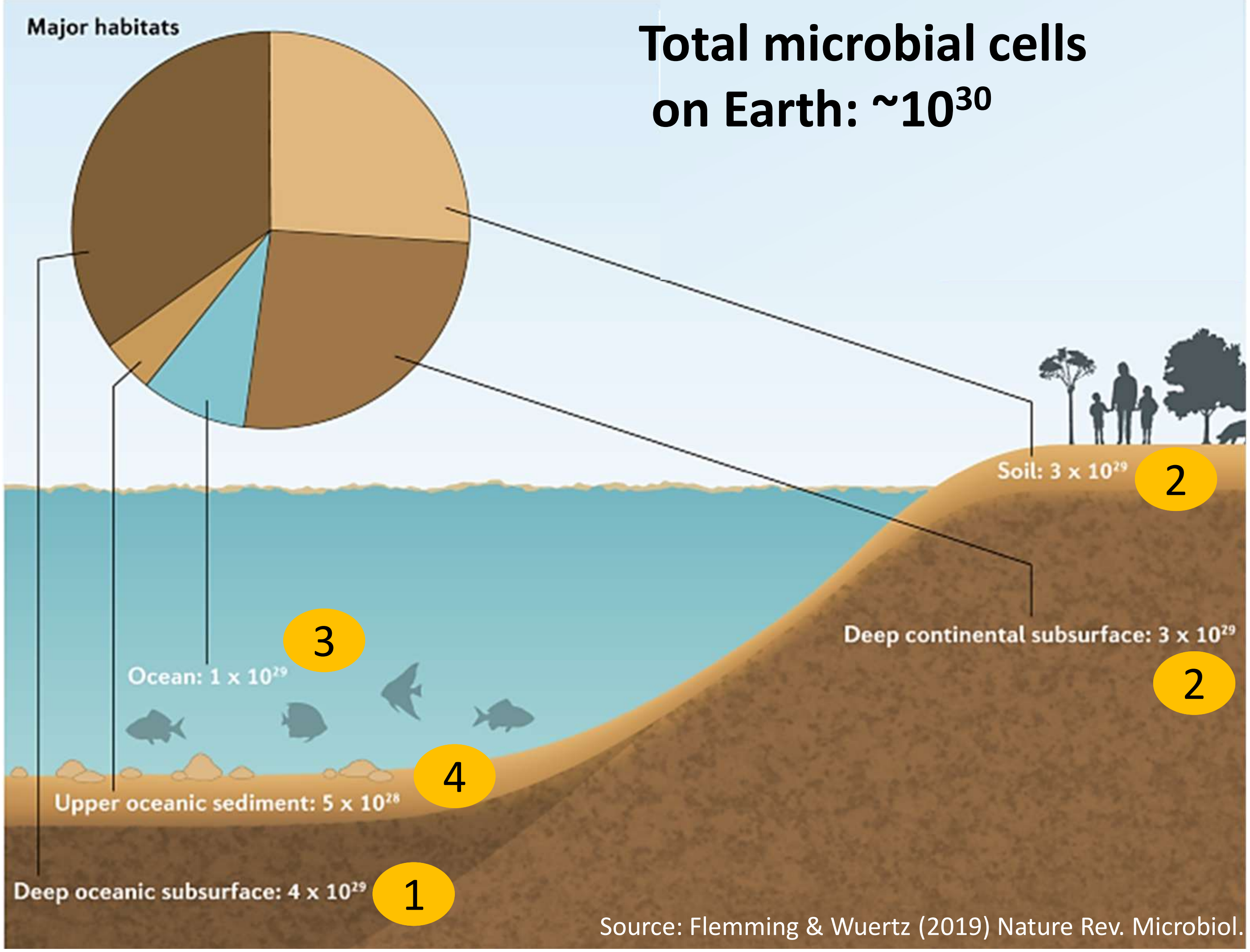


~100 trillion or 10^{14} microbial cells (70-90% of all cells)

~30 trillion human cells

~10^{23} microbial cells in all humans today

Everywhere…



Total microbial cells on Earth: ~10^{30}

Major habitats

Deep oceanic subsurface: 10^{29}
Deep continental subsurface: 10^{29}
Soil: 10^{29}
Ocean: 10^{29}
Upper oceanic sediment: 10^{28}

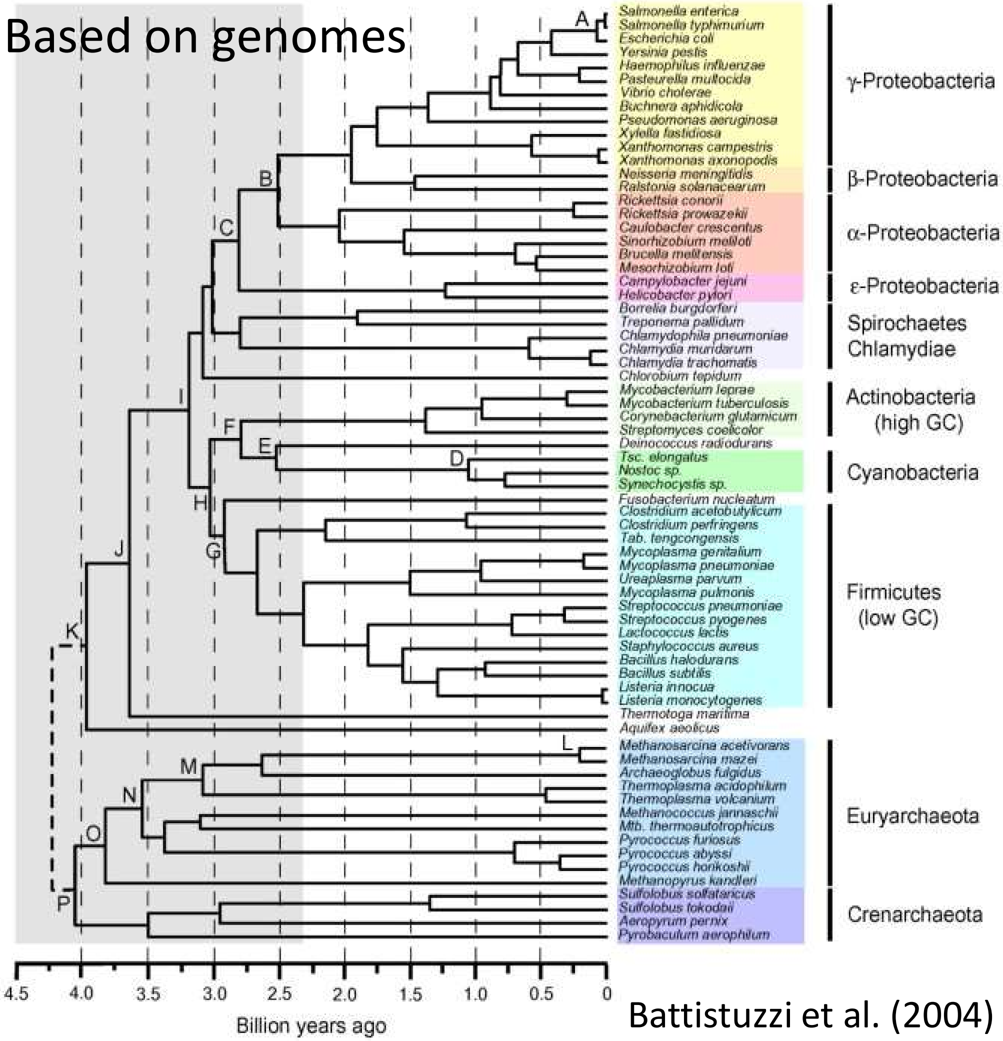
Minor habitats

Groundwater: 10^{27}
Phyllosphere: 10^{26}
Cattle: 10^{24}
Termites: 10^{23}
Pigs: 10^{23}
Humans: 10^{23}
Sea surface layer: 10^{23}
Atmosphere: 10^{22}

Source: Flemming & Wuertz (2019) Nature Rev. Microbiol. https://doi.org/10.1038/s41579-019-0158-9

Microbes have been around a long time

Based on genomes



Battistuzzi et al. (2004)

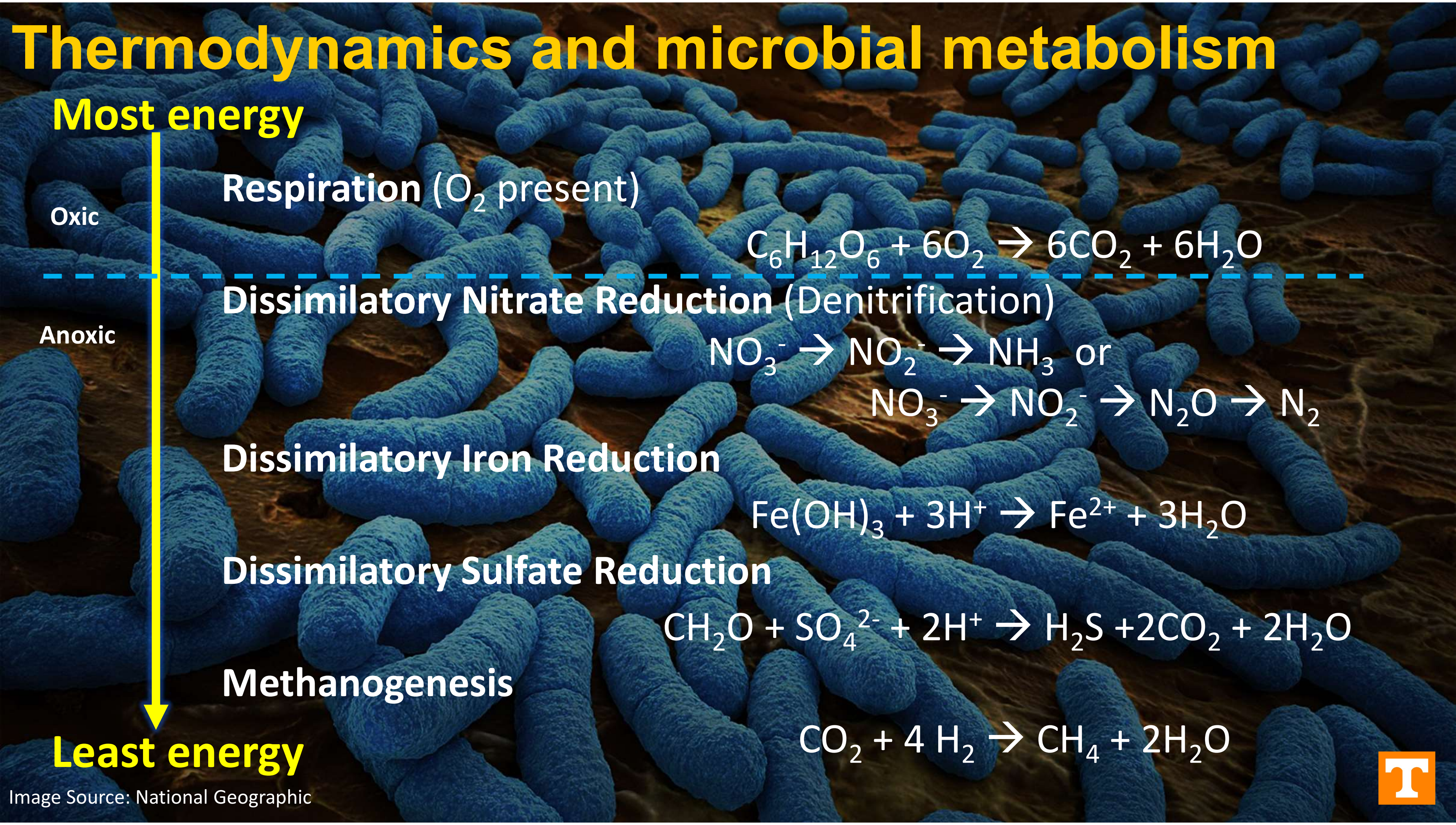
- Oxygenation of Earth's atmosphere ~2.4–2.0 Ga
- Restructured Earth's surface, water bodies, & distribution of redox-sensitive minerals
- Played an important role in animal evolution

Most of microbial evolution took place before Pangea broke up (175 million yr ago)

Microbes need… Carbon + Energy (donor) → Electron acceptor

- Carbon
  - **Inorganic carbon** (CO_2, HCO_3^-): Carbon from fixing inorganic C to organic C molecules (-*autotrophy*)
  - **Organic carbon**: obtain energy and carbon from organic compounds (-*organotrophy*; *heterotrophy*) – also depends on how get energy (electron acceptors)
- Energy
  - **Light**: pigments to harvest light energy (ATP made at the expense of sunlight) - *phototrophy*
  - **Inorganic compounds**: extract energy from chemical transformations (e.g., from *electron donors* to electron acceptors: $H_2S \rightarrow SO_4^{2-}$; $Fe^{2+} \rightarrow Fe^{3+}$) – *chemolithoautotrophy*
- Electron acceptors
  - Aerobe: requires O_2 (for respiration; terminal *electron acceptor use*)
  - Anaerobe: does not require O_2 (use alternative electron acceptors to “respire”)
  - Facultative: O_2 or no O_2 (depends on metabolism, carbon sources)
- Environmental conditions (not only tolerate conditions but require them to grow)
  - **pH**: acidophile, alkalophile, neutrophile
  - **Temperature**: thermophile, psycrophile
  - **Pressure**: barophile… etc.

Thermodynamics and microbial metabolism



Most energy

Oxic

Respiration (O_2 present)

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

Anoxic

Dissimilatory Nitrate Reduction (Denitrification)

$NO_3^- \rightarrow NO_2^- \rightarrow NH_3$ or

$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$

Dissimilatory Iron Reduction

$Fe(OH)_3 + 3H^+ \rightarrow Fe^{2+} + 3H_2O$

Dissimilatory Sulfate Reduction

$CH_2O + SO_4^{2-} + 2H^+ \rightarrow H_2S + 2CO_2 + 2H_2O$

Methanogenesis

$CO_2 + 4\,H_2 \rightarrow CH_4 + 2H_2O$

Least energy

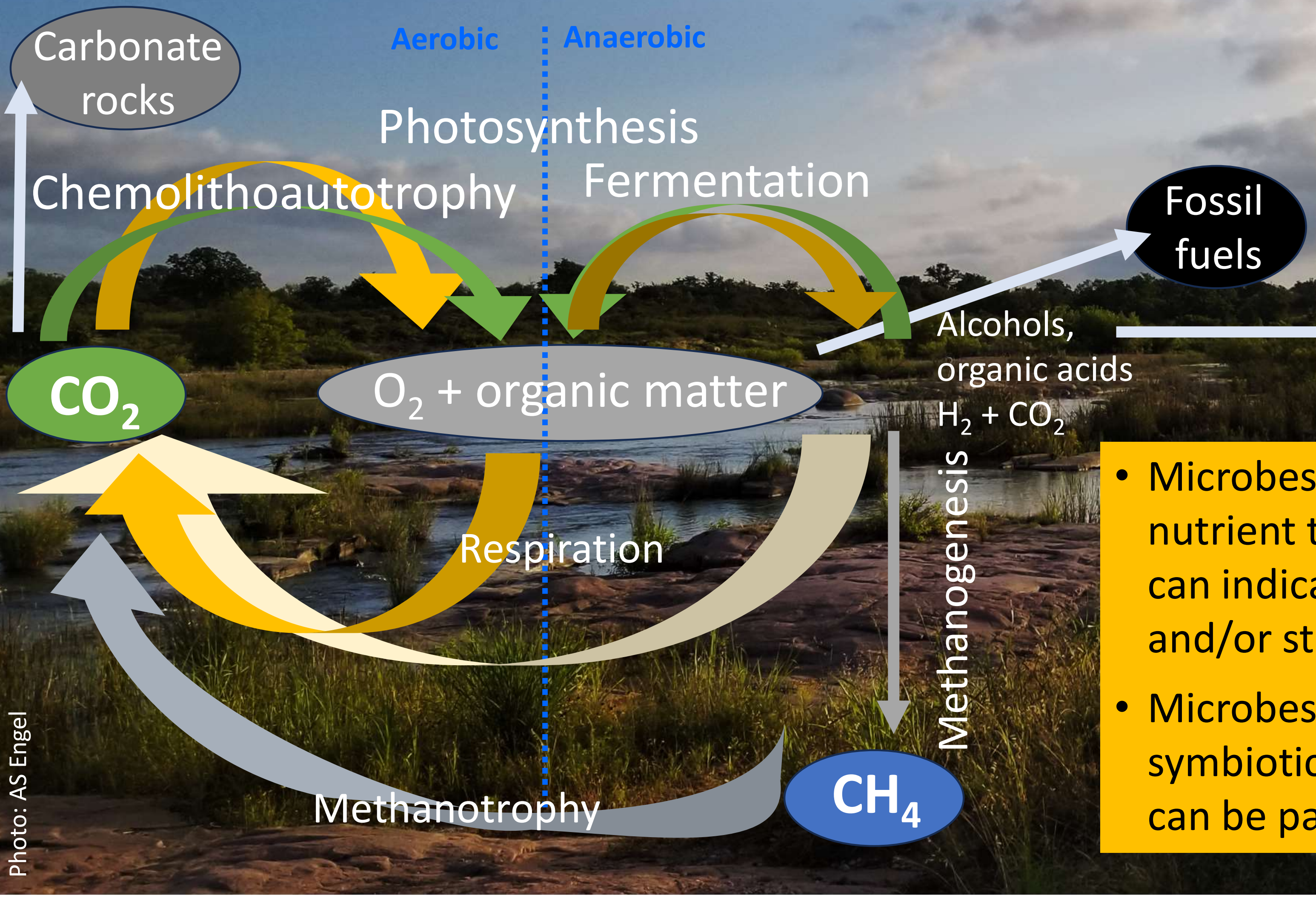
Image Source: National Geographic

Microbes can…

- **Concentrate** – locally accumulate inorganic minerals (e.g., $CaCO_3$ deposits, or FeS_2 formation from sulfate reduction)
- **Disperse** – cause solubilization, mobilization, and dispersion (e.g., organic carbon, Fe(III) oxide reduction)
- **Produce** – use some compounds (CO_2, H_2S) and make new ones (e.g., acids H_2CO_3 or H_2SO_4)
- **Fractionate** – preferentially use one component in a mixture, which results in elemental and isotopic fractionation

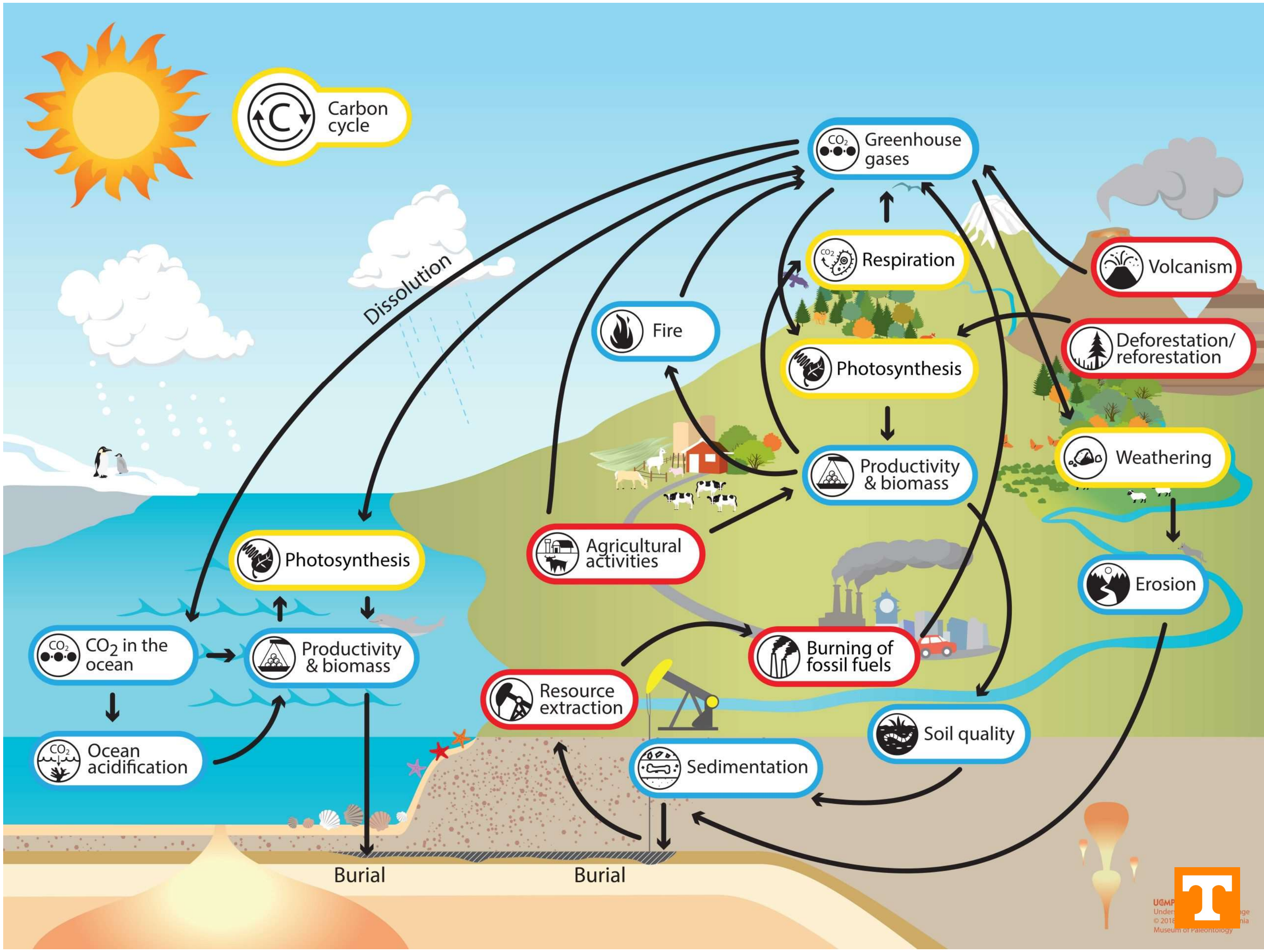
Source: American Academy of Microbiology colloquium, 2000

Microbes control the carbon cycle



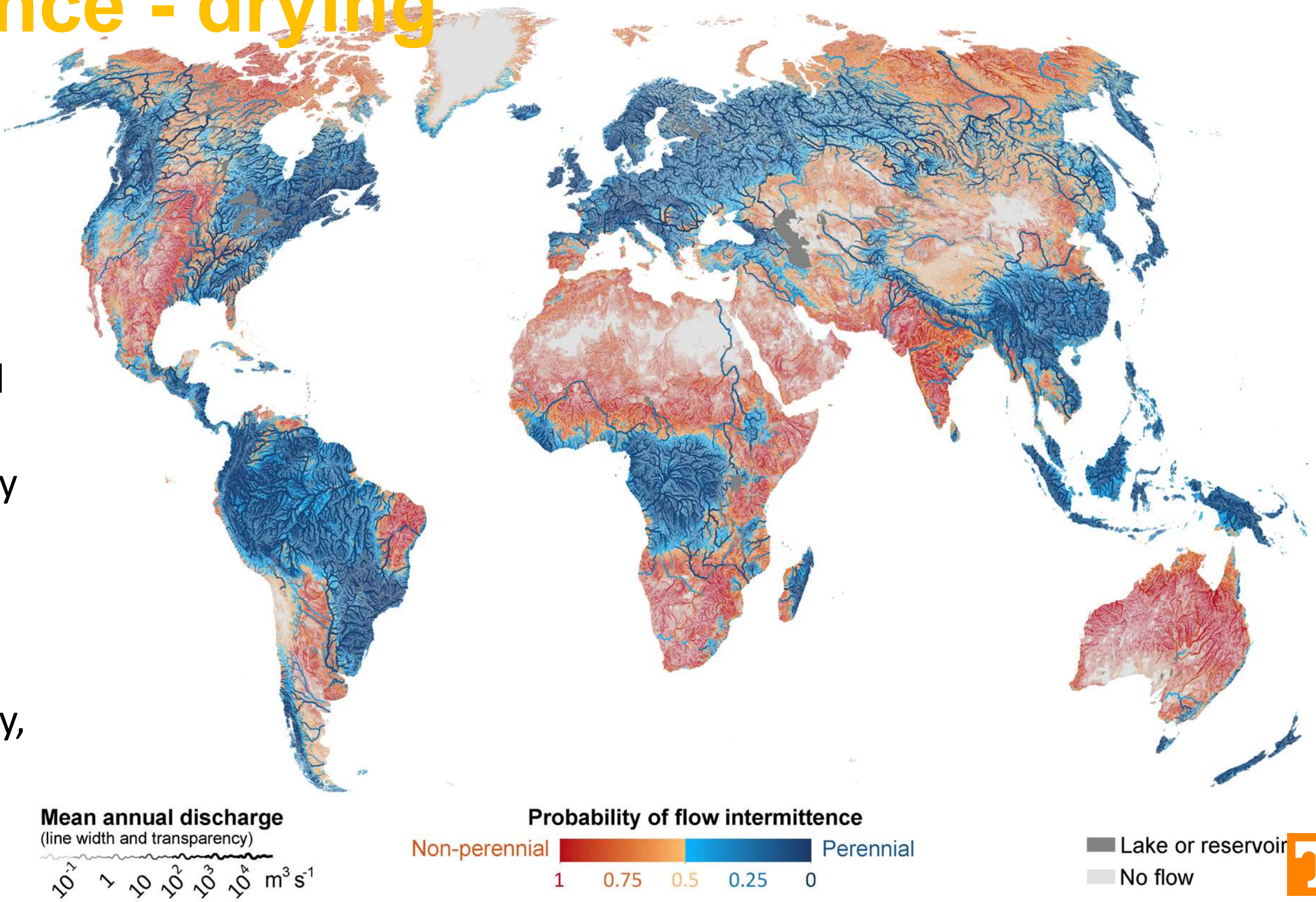
- Microbes control and regulate nutrient transformations and can indicate ecosystem health and/or stress
- Microbes become food, form symbiotic associations, and/or can be pathogens

Microbes play a key role in regulating climate



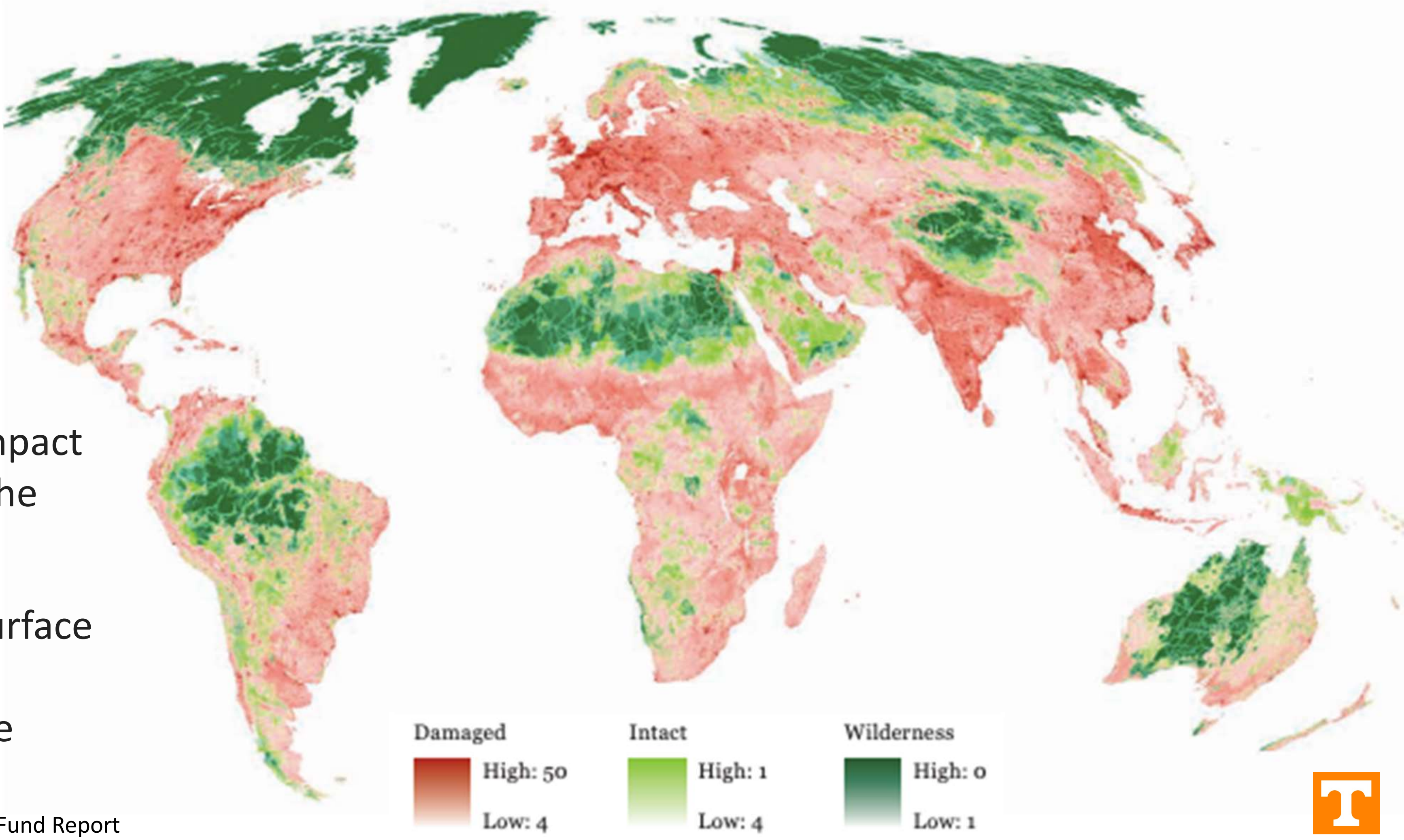
Disturbance - drying

- Flow changes, deviation from perennial flow
- 51% - 60% of 64 million km rivers around the world stop flowing periodically or dry during a year
- Drying (and wetting) affects chemistry, biology, hydrogeology



Source: Messager et al. (2021) *Nature* 594, 391–397
DOI: 10.1038/s41586-021-03565-5

Disturbance - natural vs anthropogenic



- Relating to or involving the impact of humans on the environment
- >58% of land surface under intense human pressure

Source: 2020 World Wildlife Fund Report

Climate change affects Texas rivers

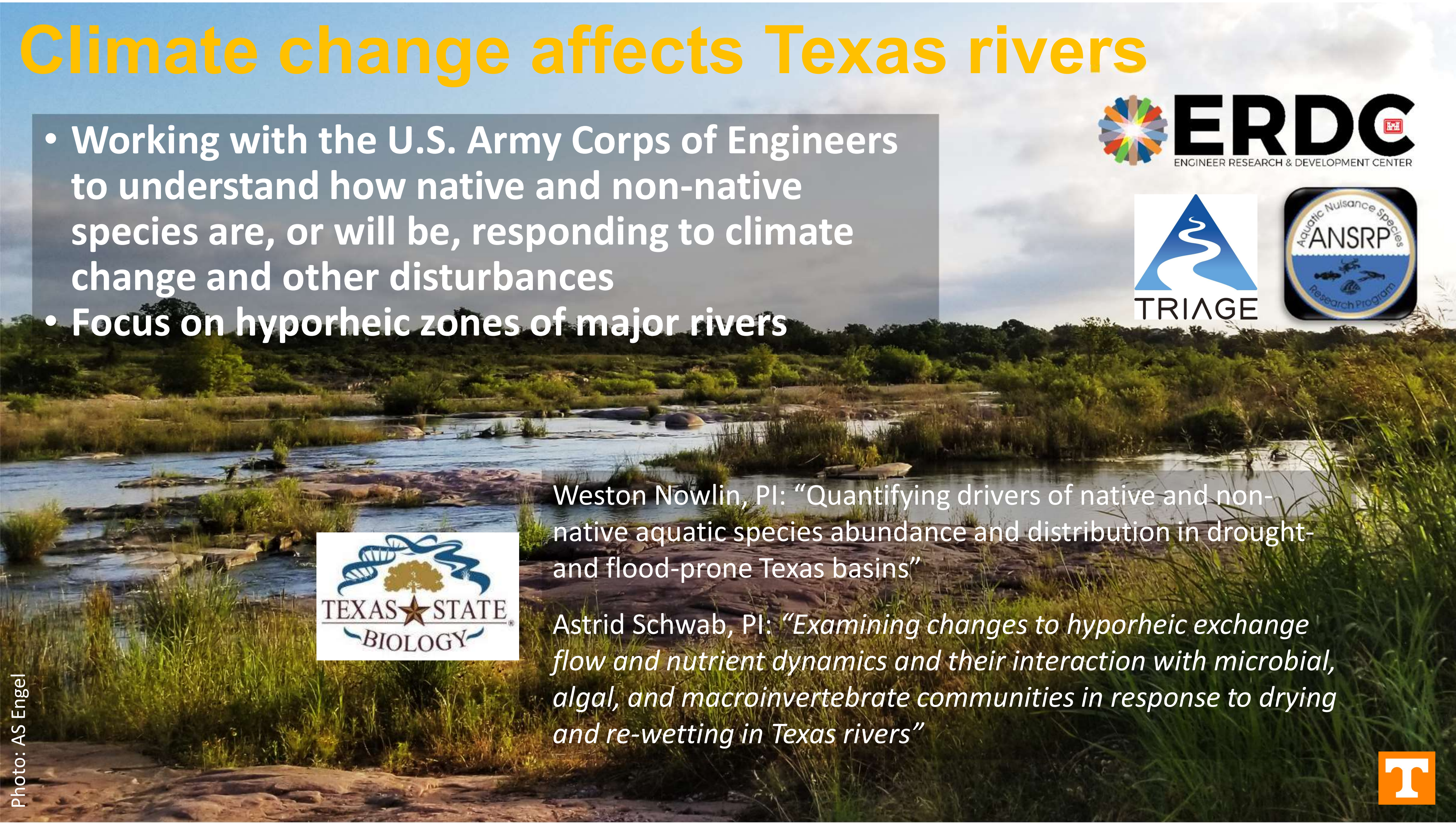


Photo: AS Engel

- Working with the U.S. Army Corps of Engineers to understand how native and non-native species are, or will be, responding to climate change and other disturbances
- Focus on hyporheic zones of major rivers

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Research Program

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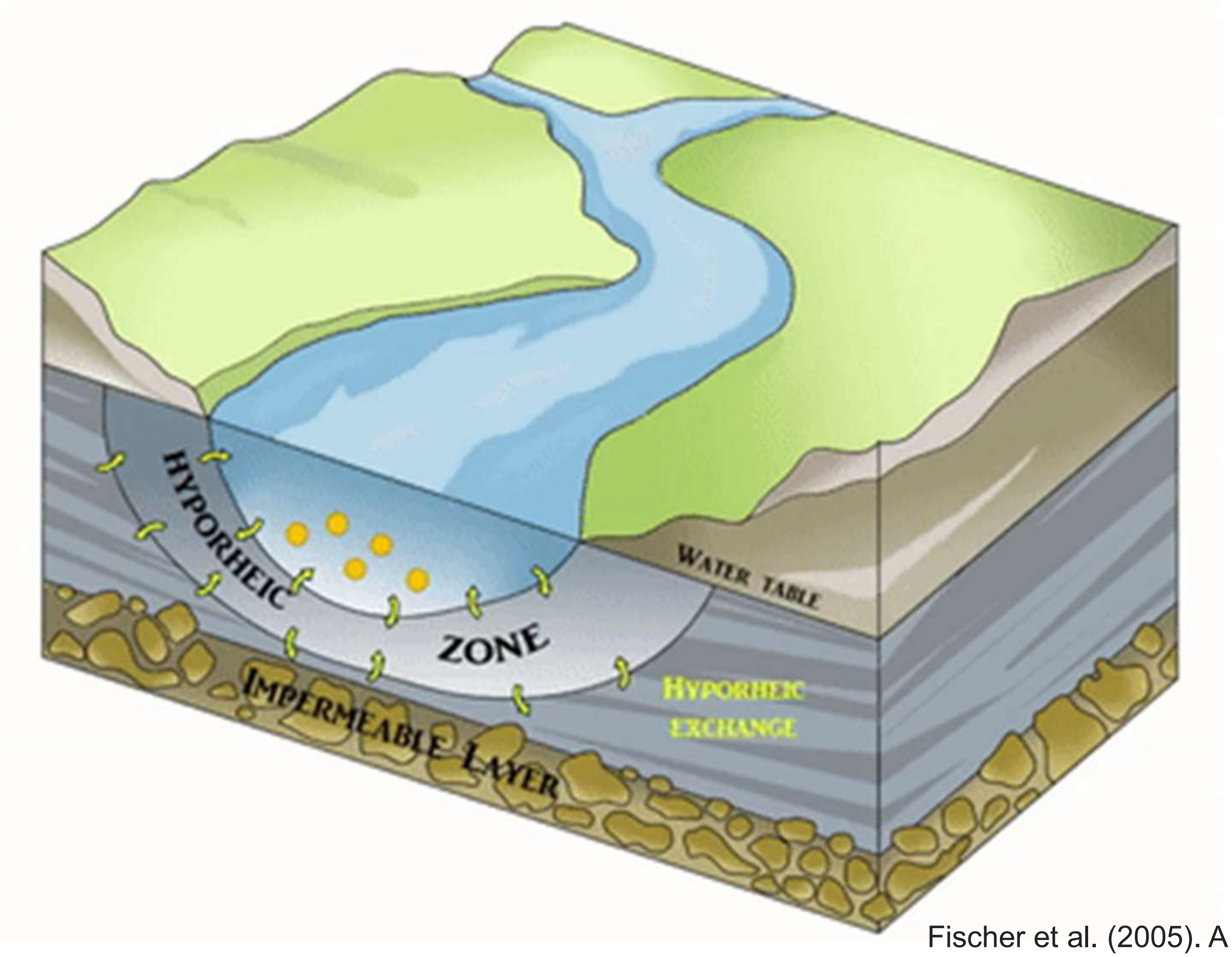
BIOLOGY

Weston Nowlin, PI: “Quantifying drivers of native and non-native aquatic species abundance and distribution in drought- and flood-prone Texas basins”

Astrid Schwab, PI: *“Examining changes to hyporheic exchange flow and nutrient dynamics and their interaction with microbial, algal, and macroinvertebrate communities in response to drying and re-wetting in Texas rivers”*

Hyporheic zone



Source: WikiCommons

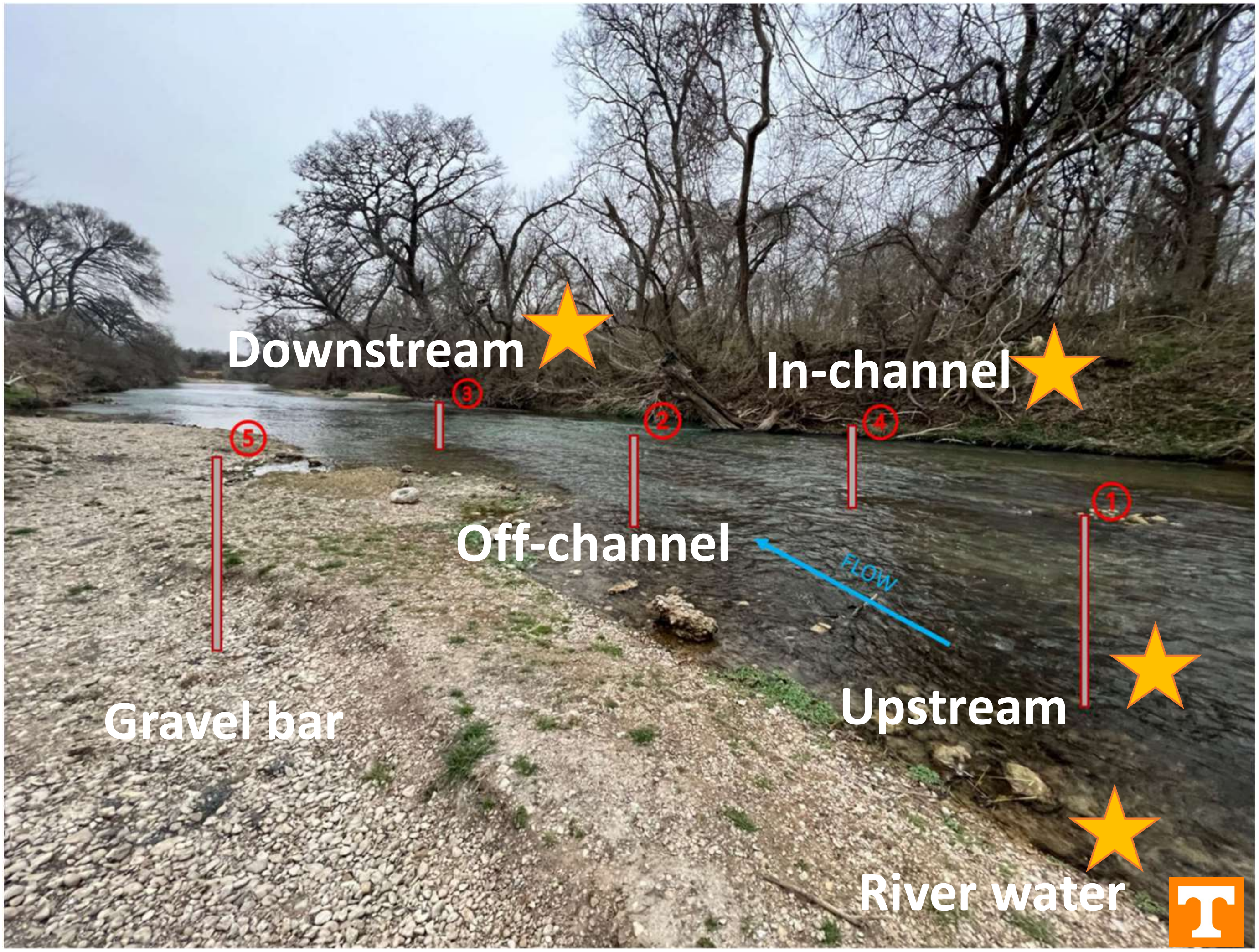
- Recognized over past 70 years, but research challenging
- Ecotone between terrestrial (dry) and aquatic (wet) habitats
- Exchange and mixing of gases and nutrients between surface runoff and groundwater, through gaining or losing conditions
- Important ecosystem services include nutrient turnover, organic carbon transformation, fine particle filtering, aquatic life refugia, reservoir of biodiversity
- A “river’s liver”

Fischer et al. (2005). A river’s liver–microbial processes within the hyporheic zone of a large lowland river. Biogeochemistry 76, 349–371.

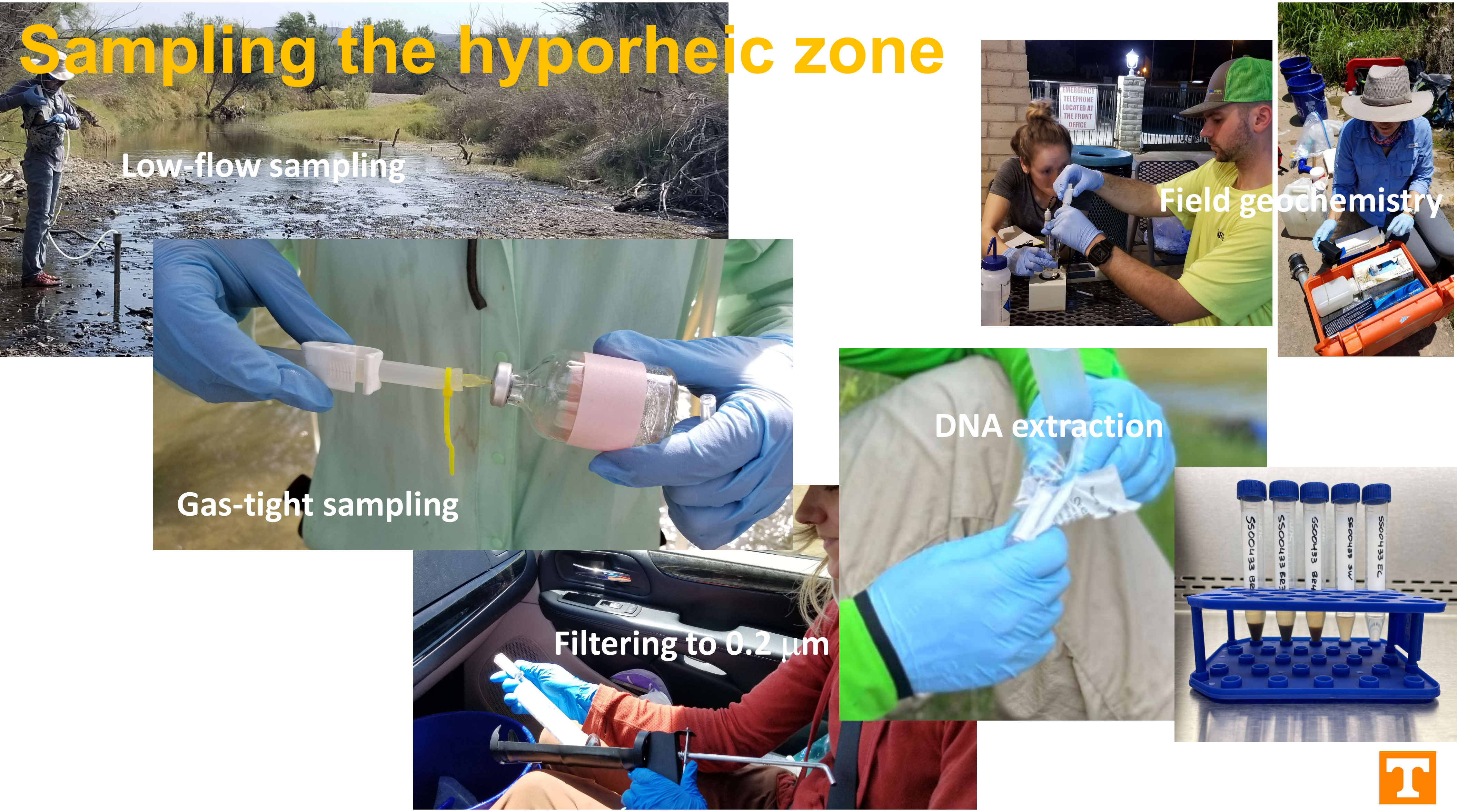
Sampling

Sampling the hyporheic zone


Using a Bou Rouch sampler
to collect sediment
& invertebrates



Sampling the hyporheic zone



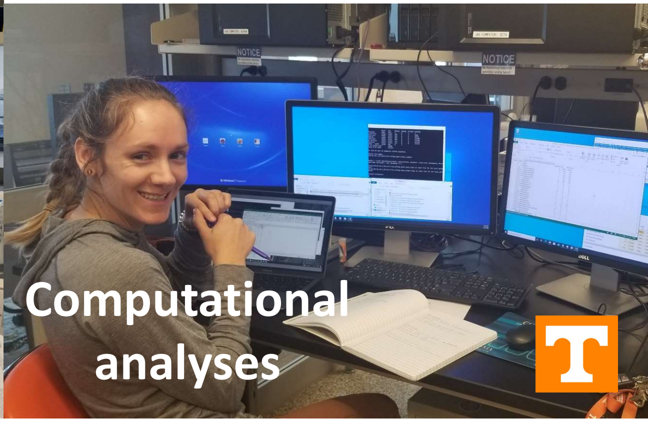
Laboratory analyses - geochemistry


TOC & TN analyses


Lipids – GC/MS


Major cations & anions
(Ion chromatography)


Trace metals – ICP-OES

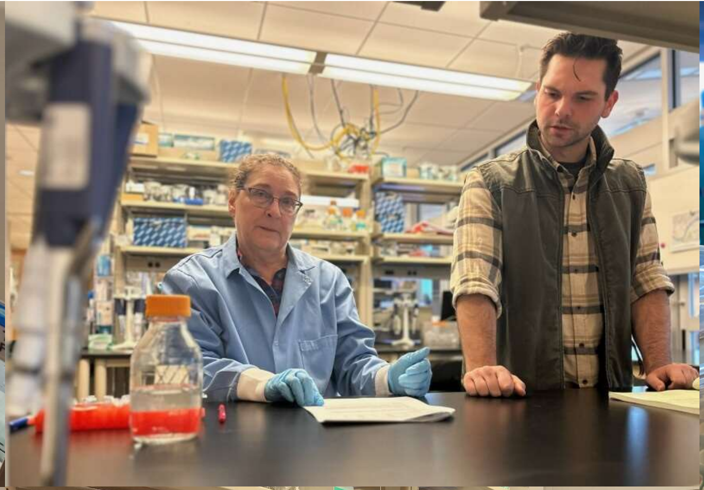

Computational analyses

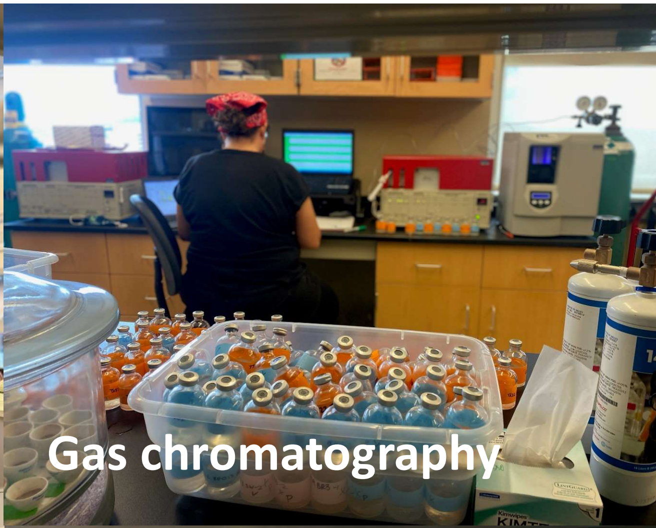
Research Staff
Audrey Paterson (Lab Manager)
Susan Pfiffner (Research Professor)

Graduate Students
Ethan Sweet (thesis research)
Hannah Rigoni (field, lab help)

Undergraduate students
Anna Carter

Laboratory analyses - genetics

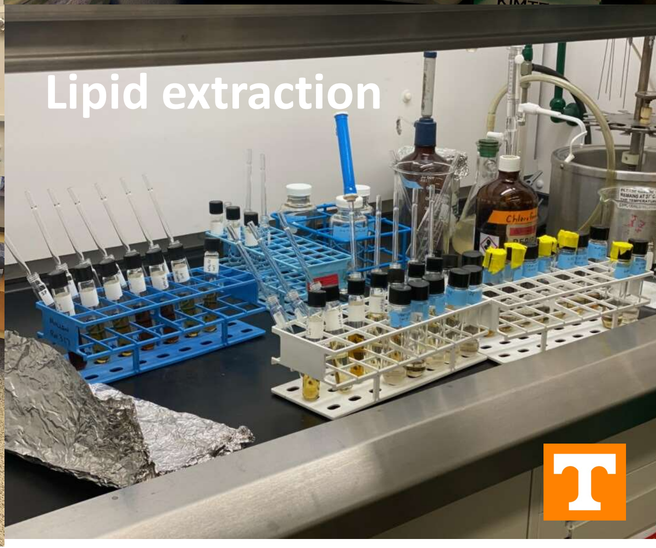




Gas chromatography

DNA extraction

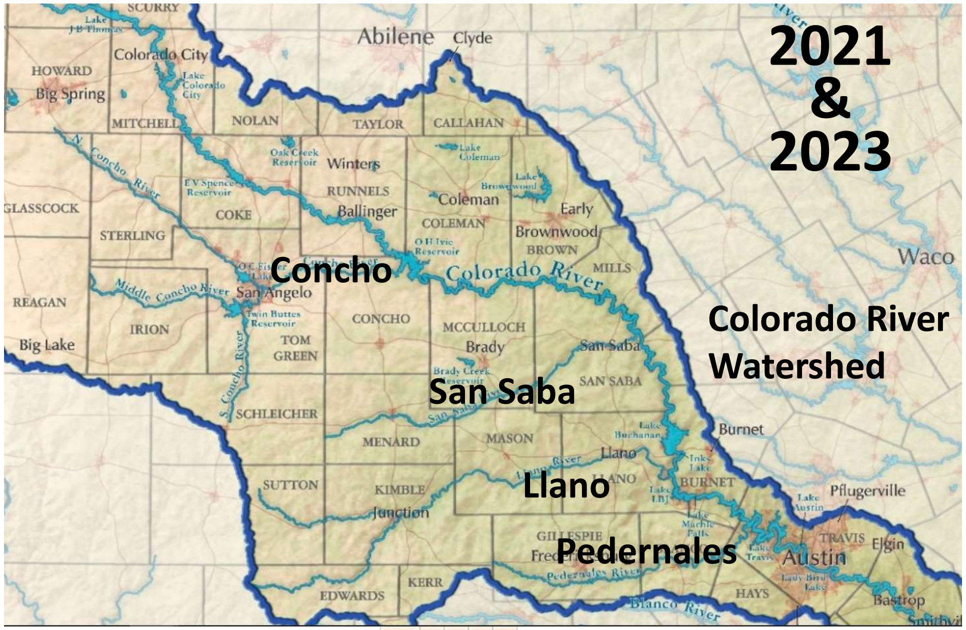
DNA extraction

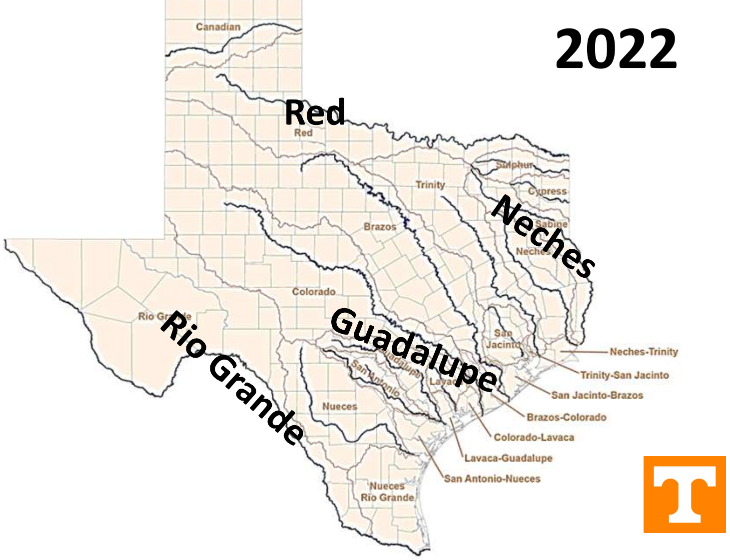


Lipid extraction

Sampling efforts

- Sampled 9 different rivers for total of 110 separate sample sites
- Permeability, hydraulic conductivity (vertical + horizontal) at most sites
- 529 water samples, with 30+ analytes per sample
- 386 microbial samples, with 35+ analytes including DNA sequences and lipid analyses
- 419 HZ invertebrate samples (>50,000 invertebrates for 2021 samples alone)
- 40+ million microbial sequences just from 2021

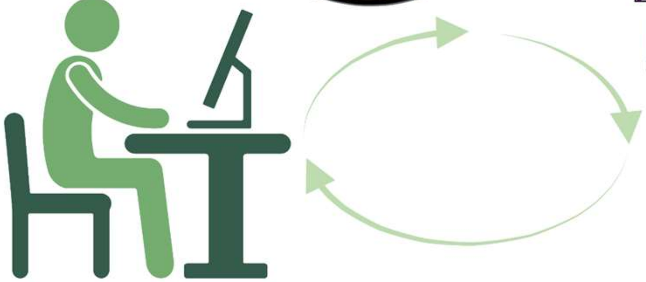


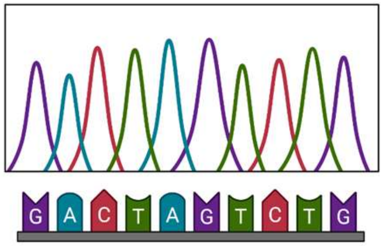


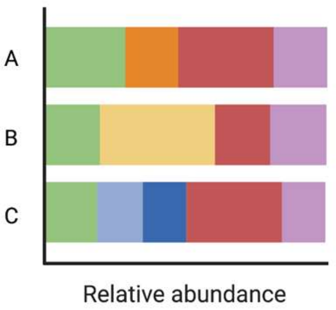
Microbial data analysis pipeline

Who's there? What are they doing?

MOTHUR
(v. 1.48.0)

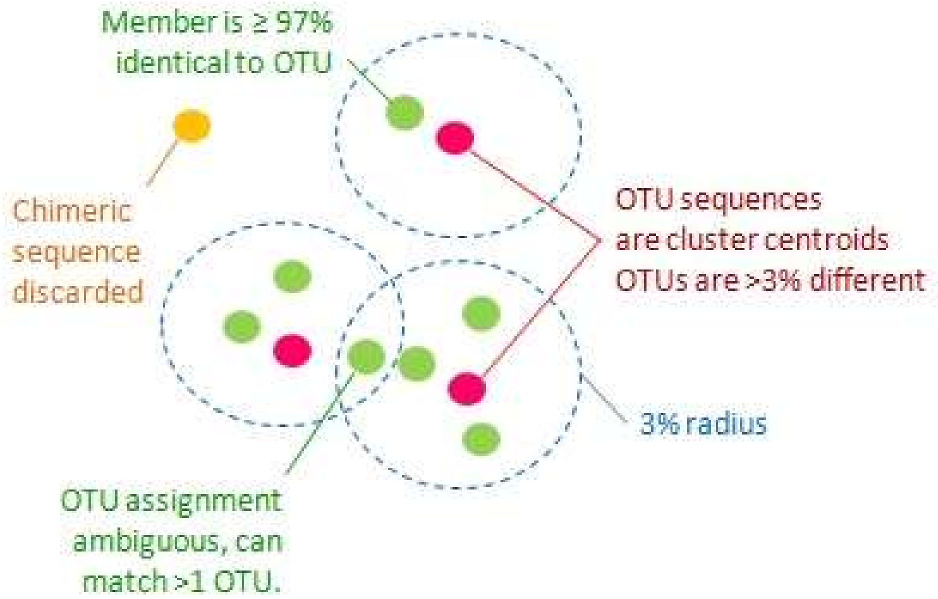




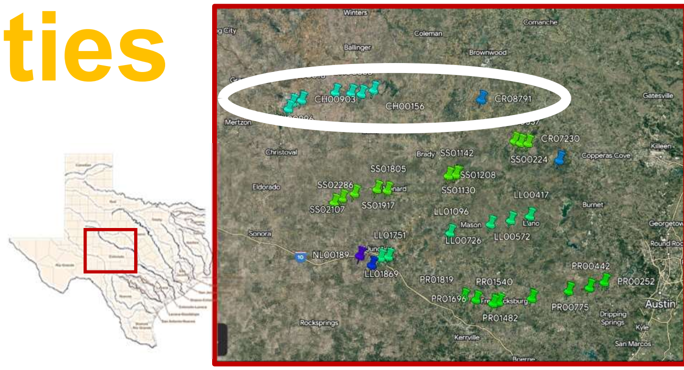


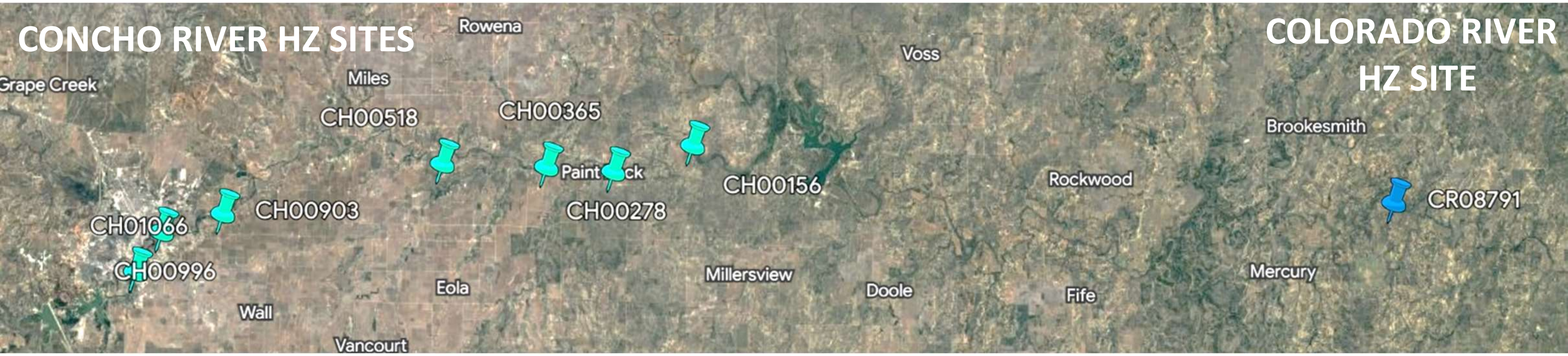
Relative Abundances

Operational Taxonomic Units (OTUs)



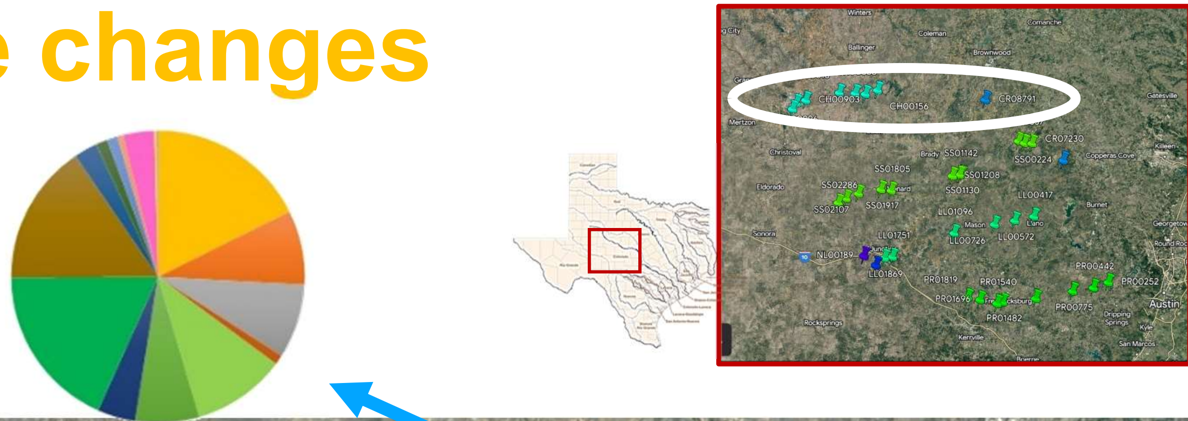
Compare river vs HZ communities

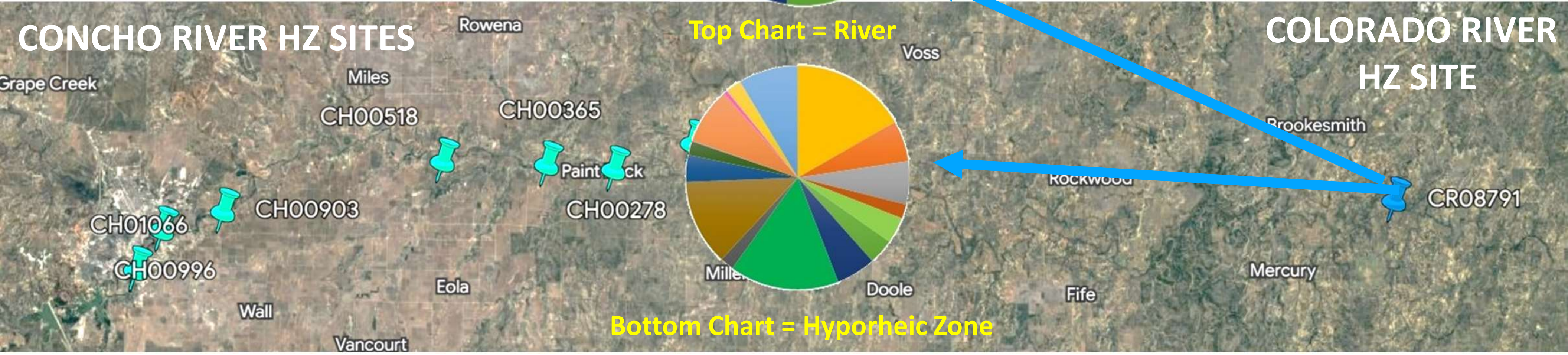




Bottom map: GoogleEarth, Landsat

Relative abundance changes





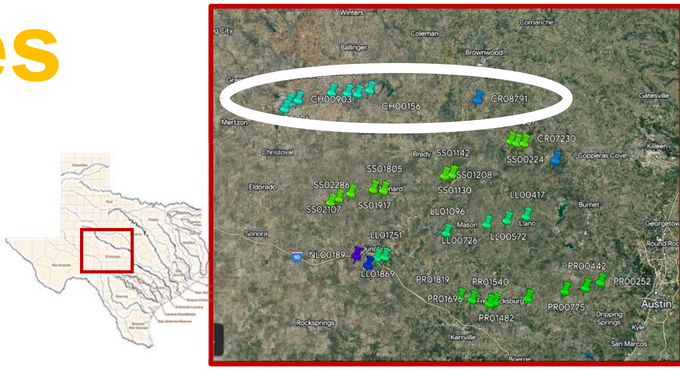
Bottom map: GoogleEarth, Landsat

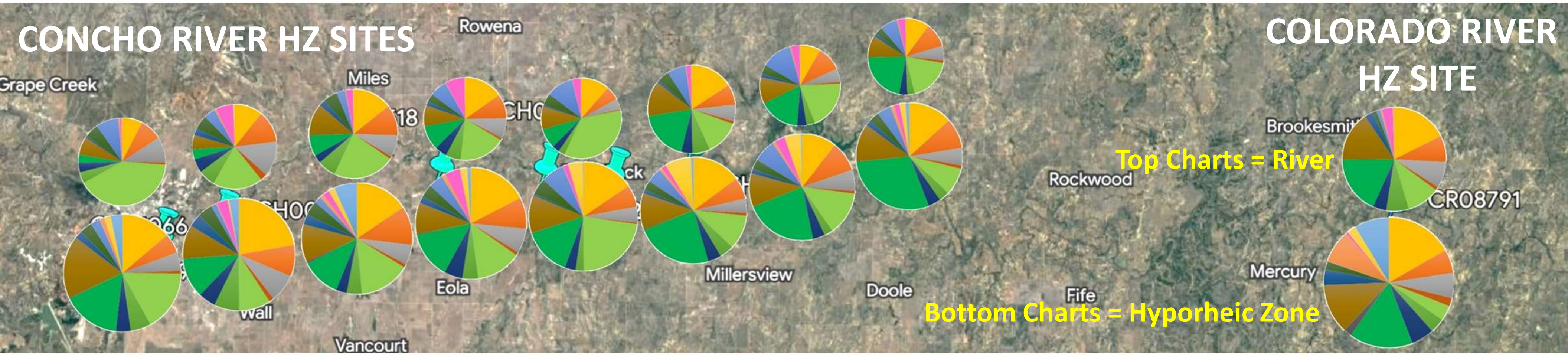
Major Microbial Families
>1% relative abundances

- 🟨 Candidatus Nanopelagicaceae
- 🟧 Burkholderiaceae
- ⬜ Comamonadaceae
- 🟫 Unc. Burkholderiales
- 🟩 Synechococcaceae
- 🟩 Chitinophagaceae
- 🟦 Oxalobacteraceae
- 🟩 Pelagibacteraceae
- ⬛ Moraxellaceae
- 🟫 Demequinaceae
- 🟦 Zoogloeaceae
- 🟩 Microbacteriaceae
- 🟦 Merismopediaceae
- 🟧 Gemmataceae
- 🟪 Microcystaceae
- 🟨 Pseudomonadaceae
- 🟦 Anaeromyxobacteraceae

Distinct river & HZ communities

- River groups dominated by phototrophs, bacterioplankton
- HZ microbial groups differ, dominated by chemoorganotrophs, capable of breaking down complex C, as well as S & N cyclers





Bottom map: GoogleEarth, Landsat

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🟩 Microbacteriaceae
🟦 Merismopediaceae
🟧 Gemmataceae
🟪 Microcystaceae
🟨 Pseudomonadaceae
🟦 Anaeromyxobacteraceae

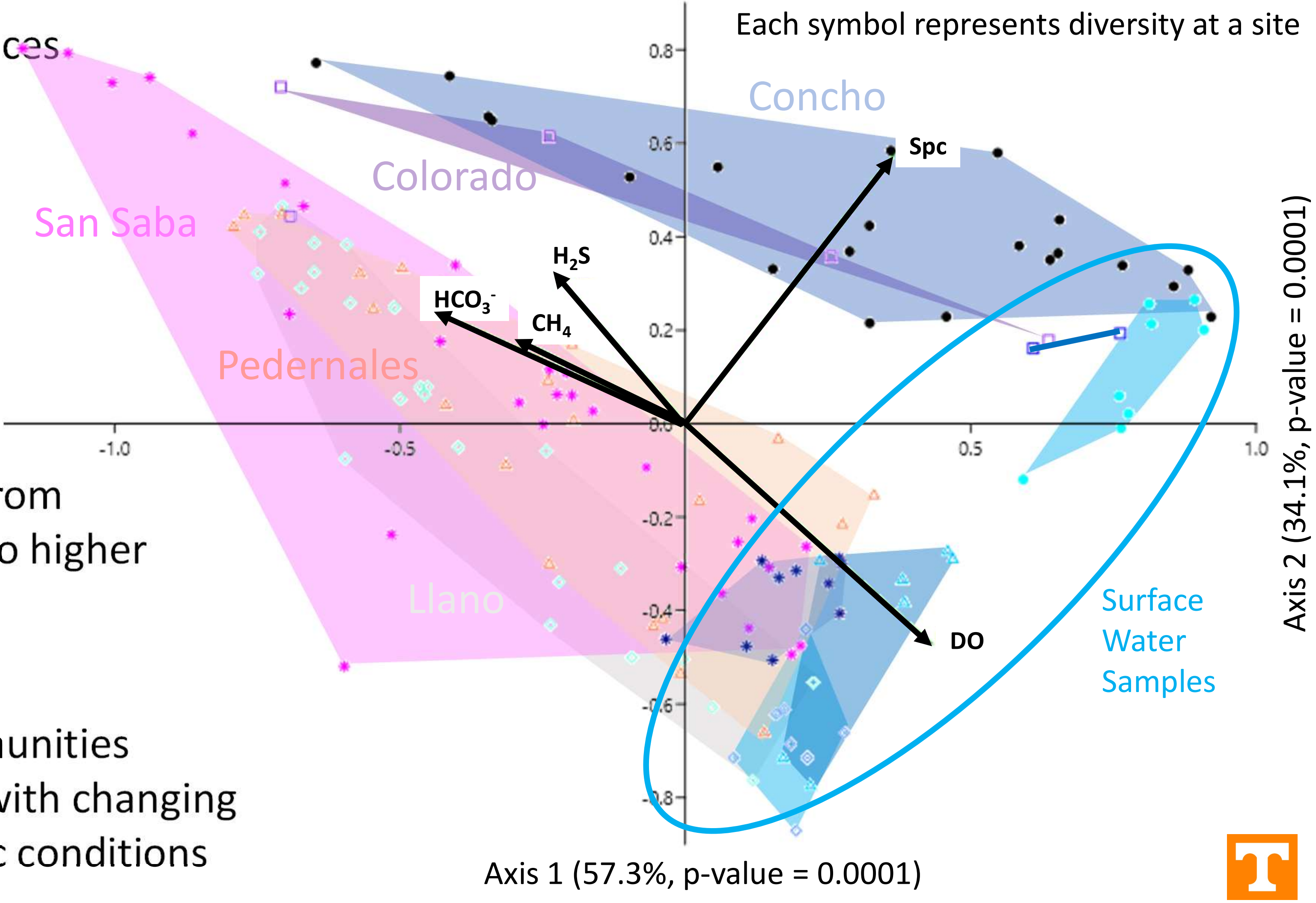
Controls on communities

- ~40 Million DNA sequences
- Distinct communities based on specific conductance
- River communities controlled by DO (higher levels than HZ)
- HZ communities differ from river communities due to higher concentrations of redox variables (sulfide, CH_4)
- Major shifts in HZ communities and river communities with changing geochemical & lithologic conditions

Canonical Correspondence Analysis

Family-level, >0.5% relative abundances

Each symbol represents diversity at a site



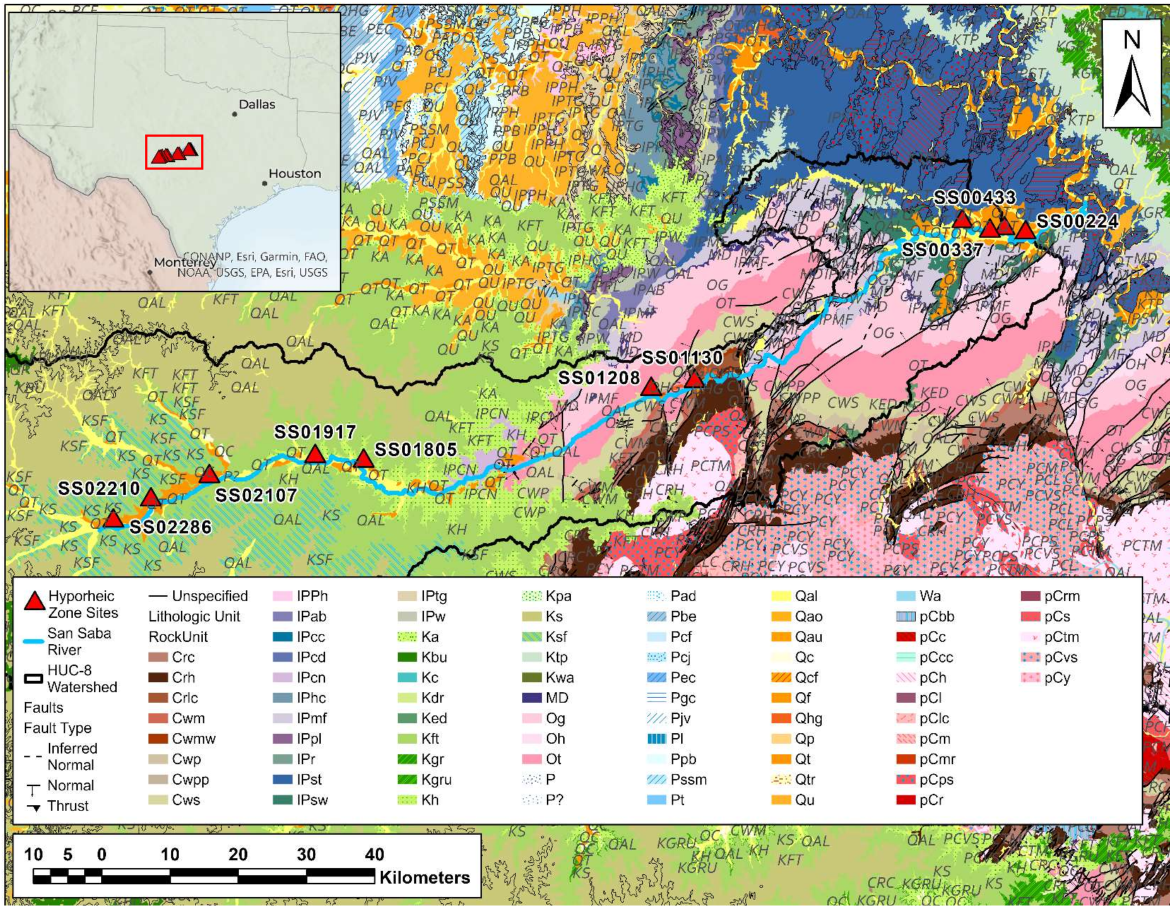
HYPORHEIC ZONE HYDRODYNAMICS, BIOGEOCHEMISTRY, AND MICROBIAL COMMUNITY DISTRIBUTIONS OF THE SAN SABA RIVER, TEXAS

Ethan Sweet, MS thesis
University of Tennessee-Knoxville
Defended August 2023 (will graduate December 2023)

Research Objectives:

- Explore spatial variations in hydrology & geochemistry in HZ
- Determine patterns in hydrological & geochemical parameters
- Characterize microbial community composition & diversity

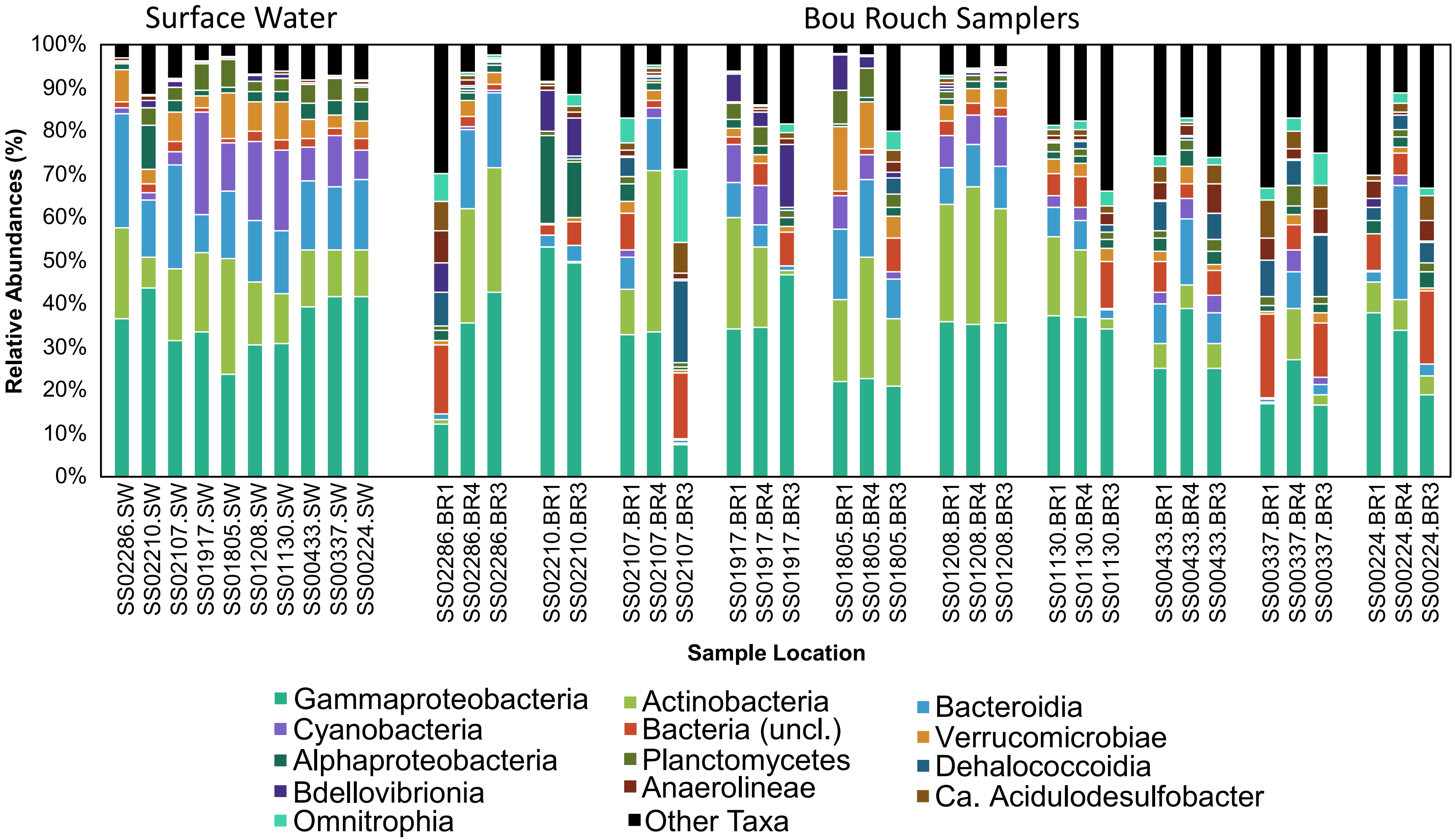
San Saba River




ETHAN SWEET, M.S. UTK

- Limited development (<2% of subbasin), minimize potential overprinting of HZ processes by anthropogenic effects (e.g., impervious runoff, urban impacts)
- Impacted by climate change; higher temperatures, more frequent spring flooding events, extreme droughts
- Drought-induced groundwater extraction affects HZ biogeochemical dynamics, impacts native species, spreads invasive species, and causes land subsidence

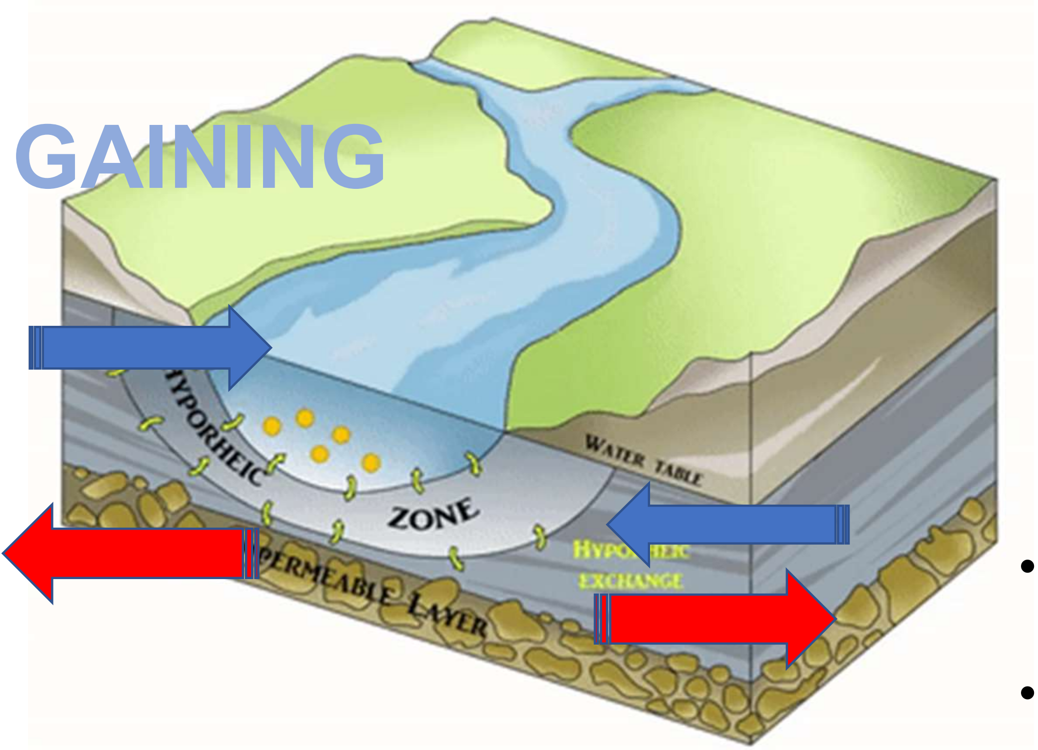
Diversity of microbial communities




ETHAN SWEET,
M.S. UTK

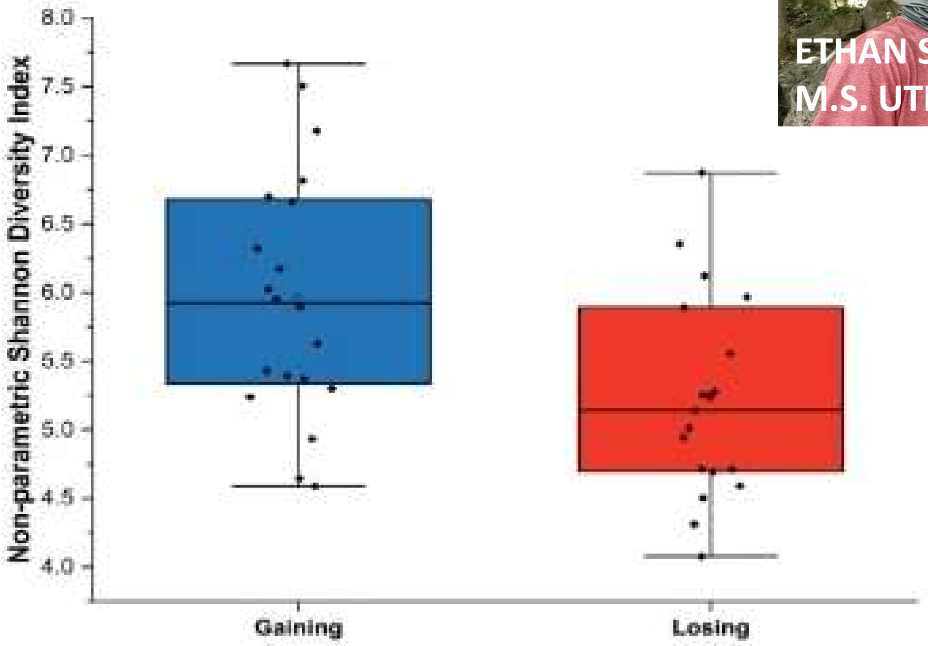
From >8M DNA sequences analyzed for Operational Taxonomic Units

Hydrodynamic controls on diversity



LOSING

Source: WikiCommons

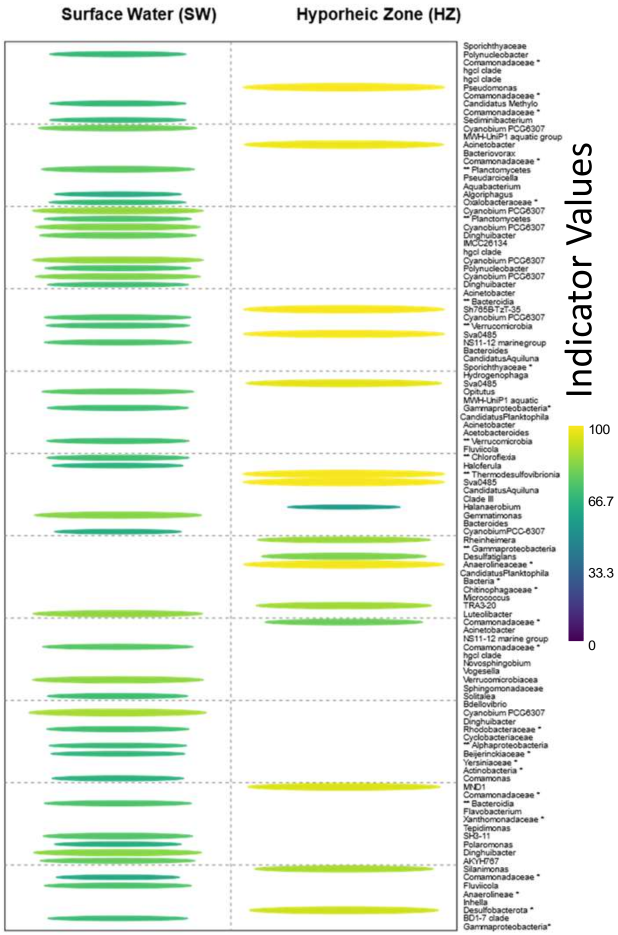


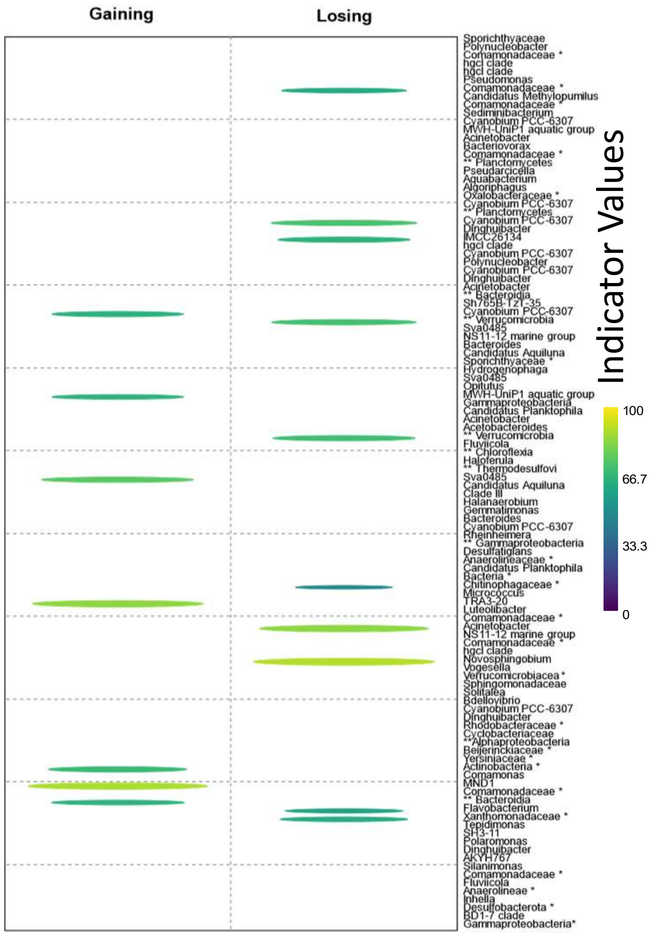
- Diversity higher in gaining reaches (groundwater added different microbes into HZ)
- Diversity lower in losing reaches (river diversity is lower and HZ communities like river)

From >8M DNA sequences analyzed for Operational Taxonomic Units and analyzed for Shannon Index

Microbial Indicator Species – river vs HZ

Indicator Species Analysis (ISA) calculated in PAST, showing indicator values (≥ 50; p-values < 0.05)





- Distinct HZ and river microbial communities; correlates to changing hydrological regime (gaining/losing)
- Geochemical indicators reflect gaining/losing conditions; sulfide and CH_4 concentrations higher in gaining reaches
- Use indicator species to model HZ exchange with river communities; monitor changes in hydroregime over time

Evidence of river & faunal health

- ■ Candidatus Nanopelagicaceae
- ■ Burkholderiaceae
- ■ Comamonadaceae
- ■ Unc. Burkholderiales
- ■ Synechococcaceae
- ■ Chitinophagaceae
- ■ Oxalobacteraceae
- ■ Pelagibacteraceae
- ■ Moraxellaceae
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- ■ Zoogloeaceae
- ■ Microbacteriaceae
- ■ Merismopediaceae
- ■ Gemmataceae
- ■ Microcystaceae
- ■ Pseudomonadaceae
- ■ Anaeromyxobacteraceae


Photo: POWIST


Photo: B. Schwartz


Photo: National Park Service

Microcystis

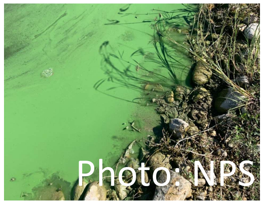
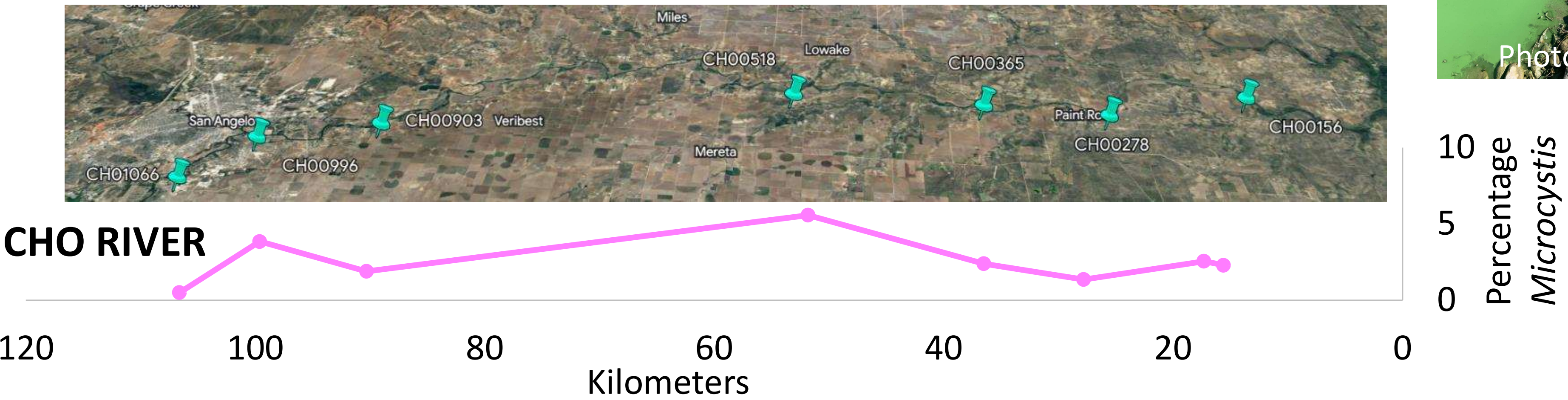
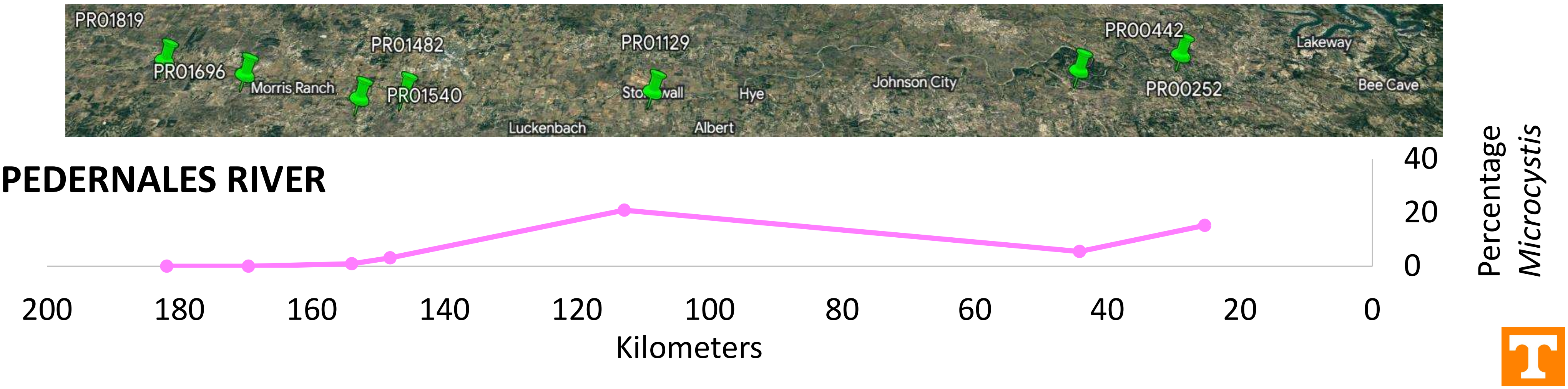


Photo: NPS

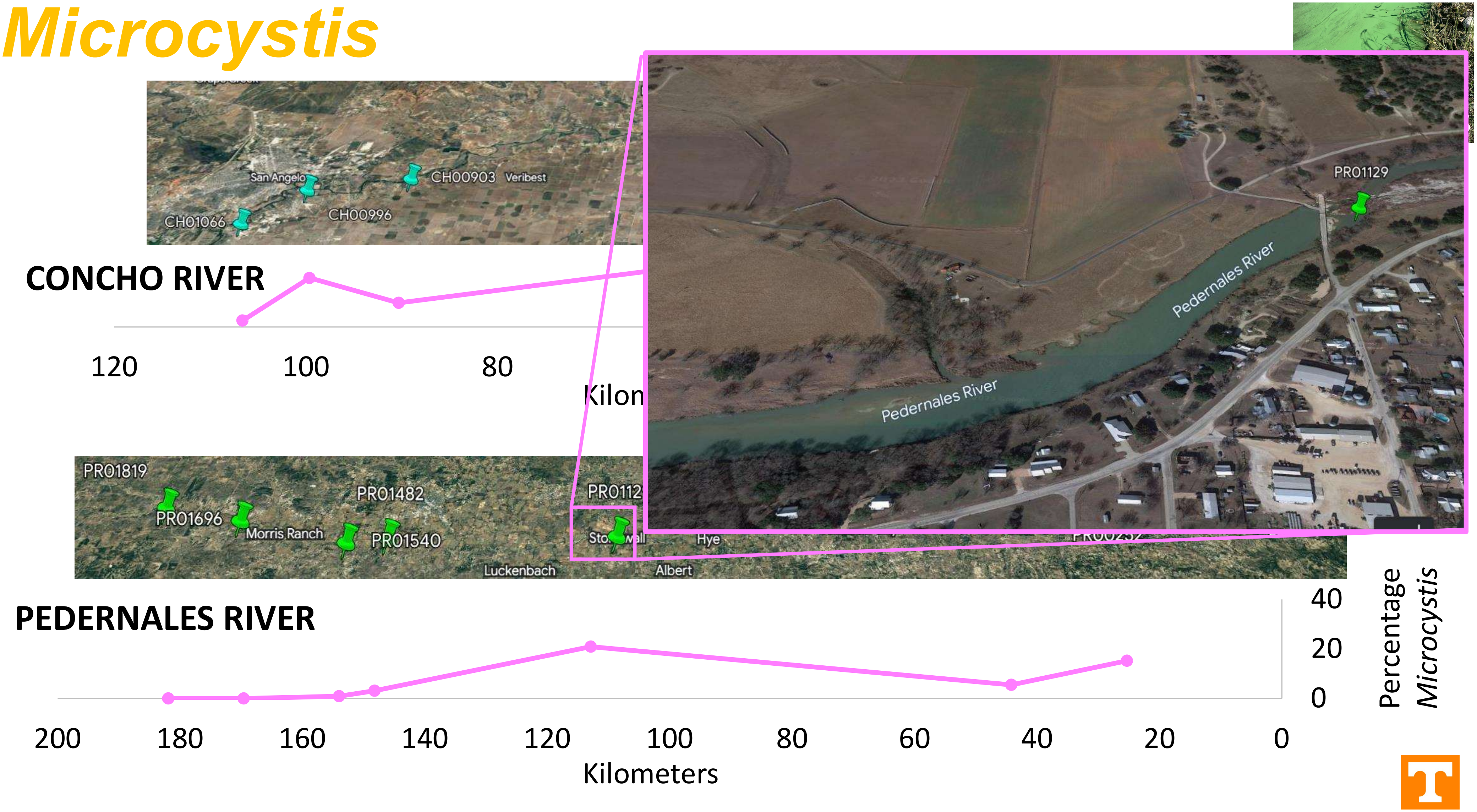
CONCHO RIVER



PEDERNALES RIVER



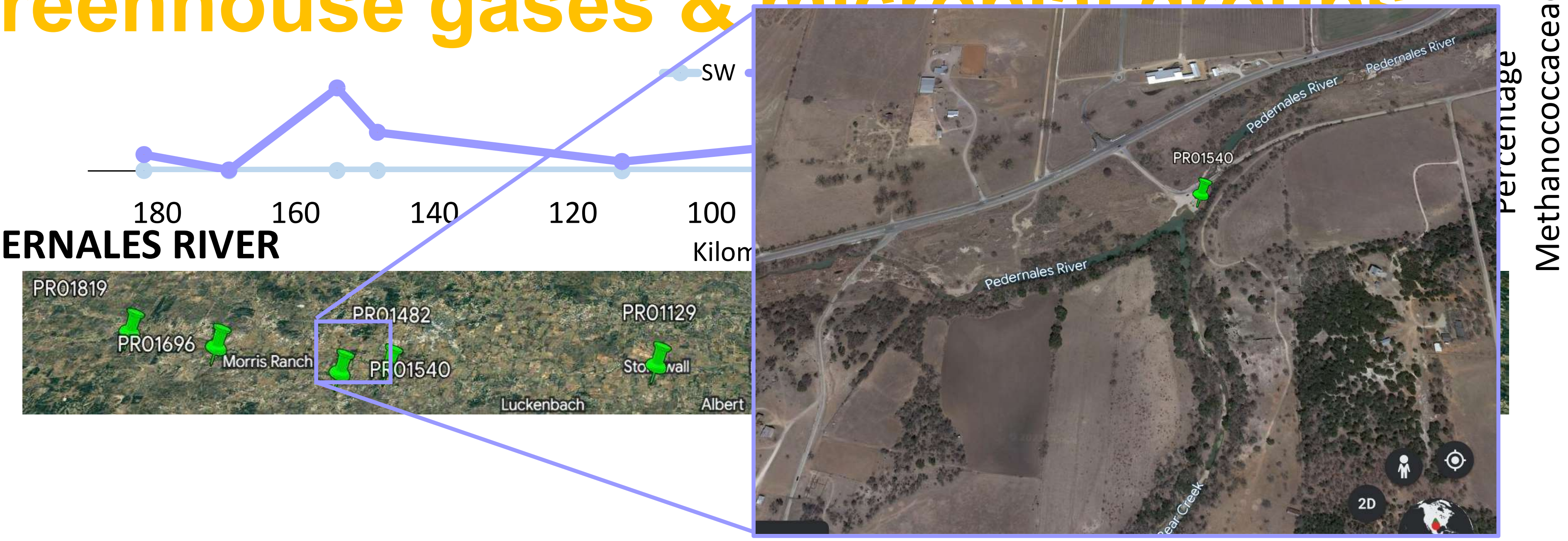
Microcystis



CONCHO RIVER

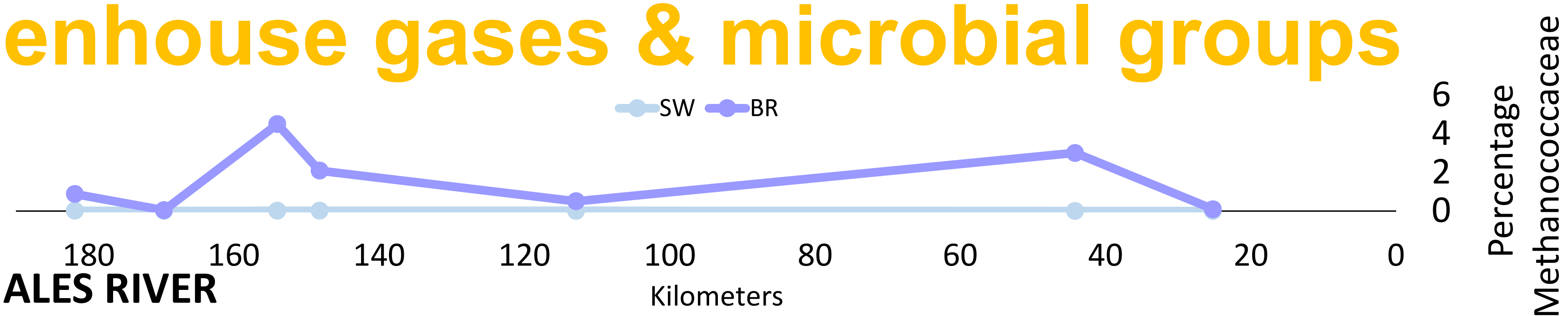
PEDERNALES RIVER

Greenhouse gases & microbial groups

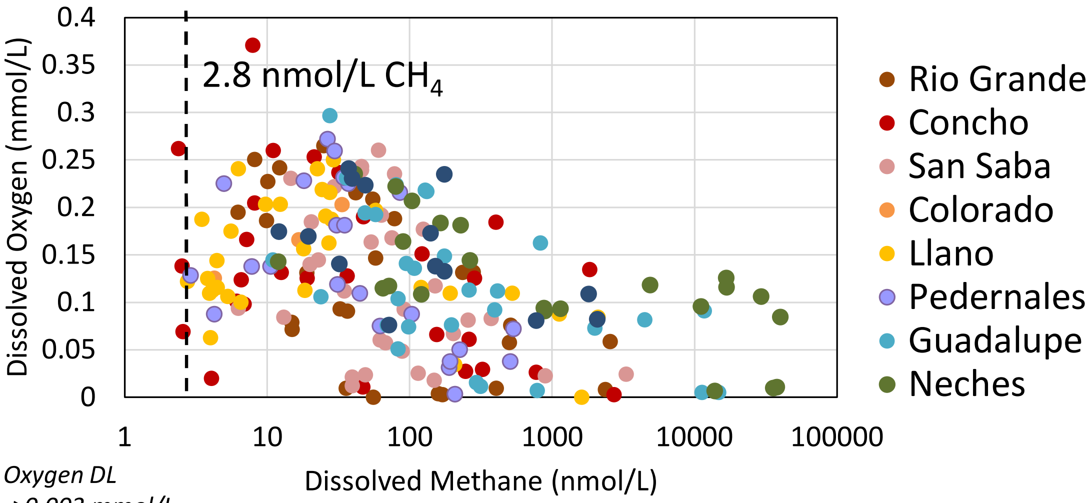


PEDERNALES RIVER

Greenhouse gases & microbial groups



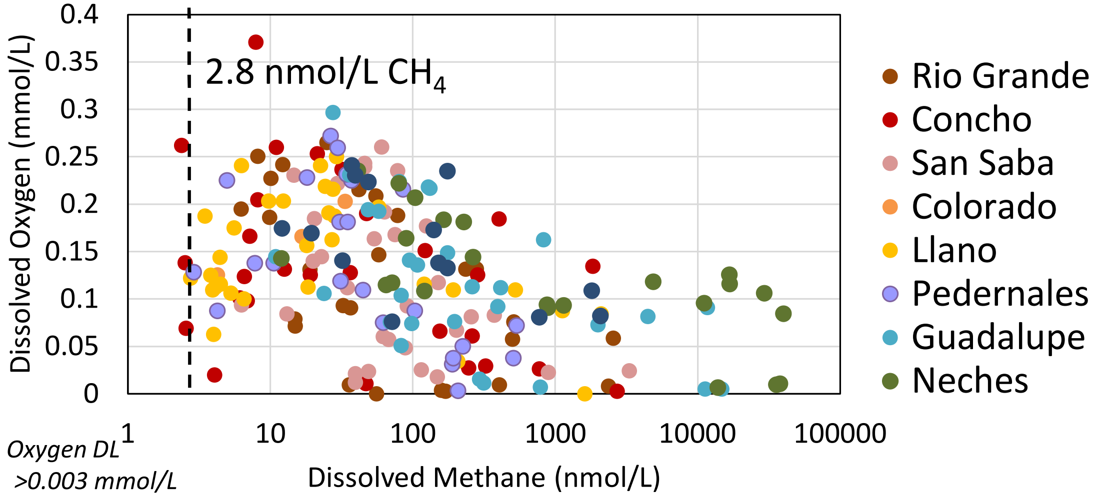
PEDERNALES RIVER

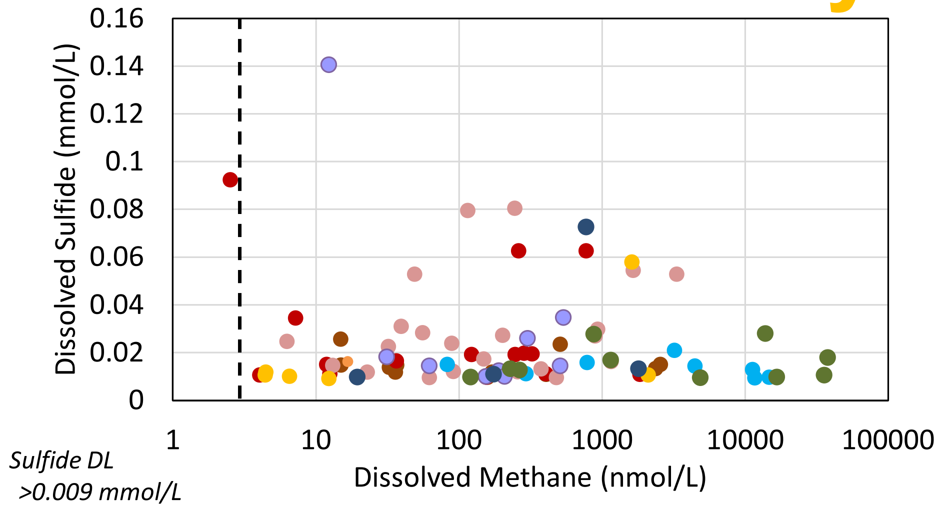


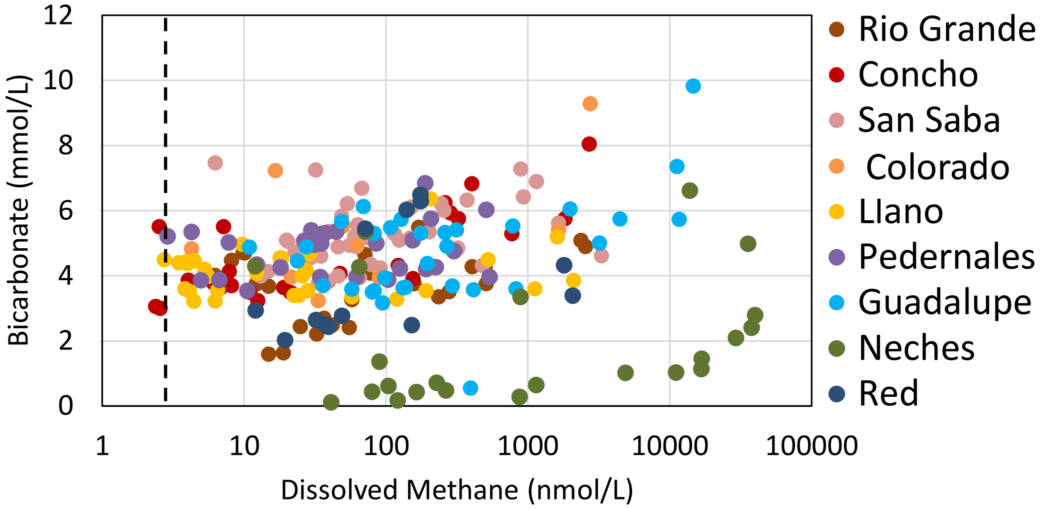
Oxygen DL
>0.003 mmol/L

- CH_4 >2.8 nmol/L in 98.3% of samples
- Few waters were totally anoxic (<0.01 mmol/L DO) but most waters were reducing
- Neches River had highest CH_4 levels

Redox conditions – indicate microbial activity

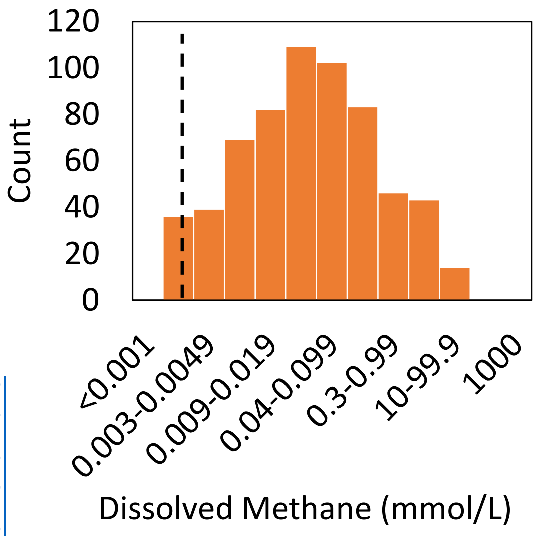


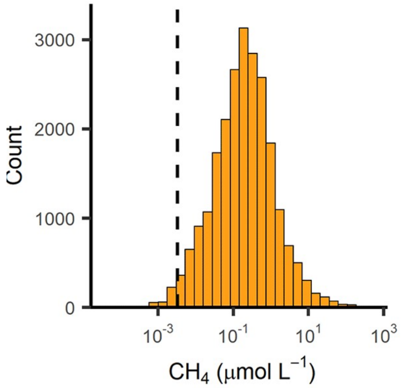




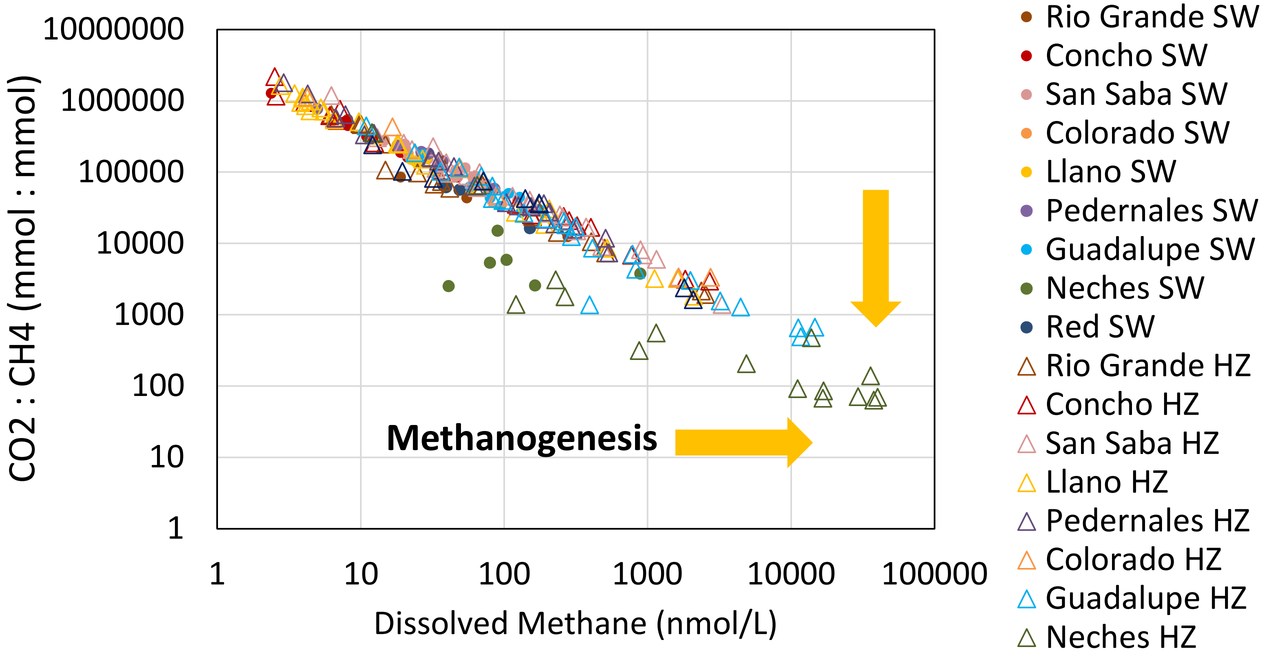
- Higher CH$_4$ concentrations when DO and sulfide concentrations are lower
- Guadalupe River, high CH$_4$ corresponds to high dissolved bicarbonate (carbonate bedrock)
- Neches River, high CH$_4$ corresponds to low dissolved bicarbonate (siliciclastics)

Rivers are sources of CH_4 (and CO_2)





Stanley et al. (2023) GRiMeDB: the global river methane database of concentrations and fluxes. Earth System Science Data 15: 2879-2926, https://doi.org/10.5194/essd-15-2879-2023



- CH_4 levels comparable to global river CH_4 database
- Neches River CH_4 levels strongly indicate methanogenesis or another CH_4 contribution

Importance of microbial data for river science

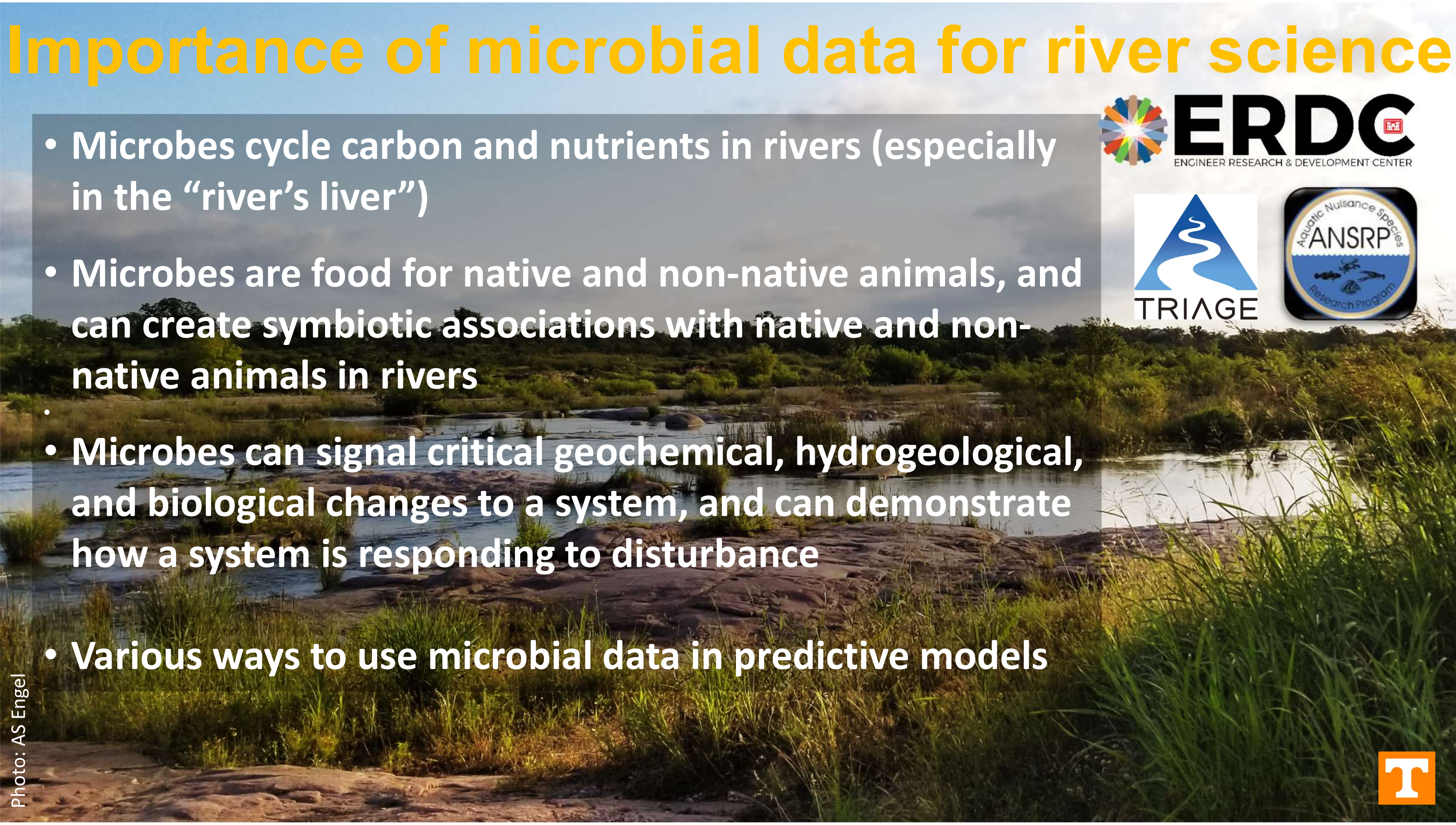


Photo: AS Engel

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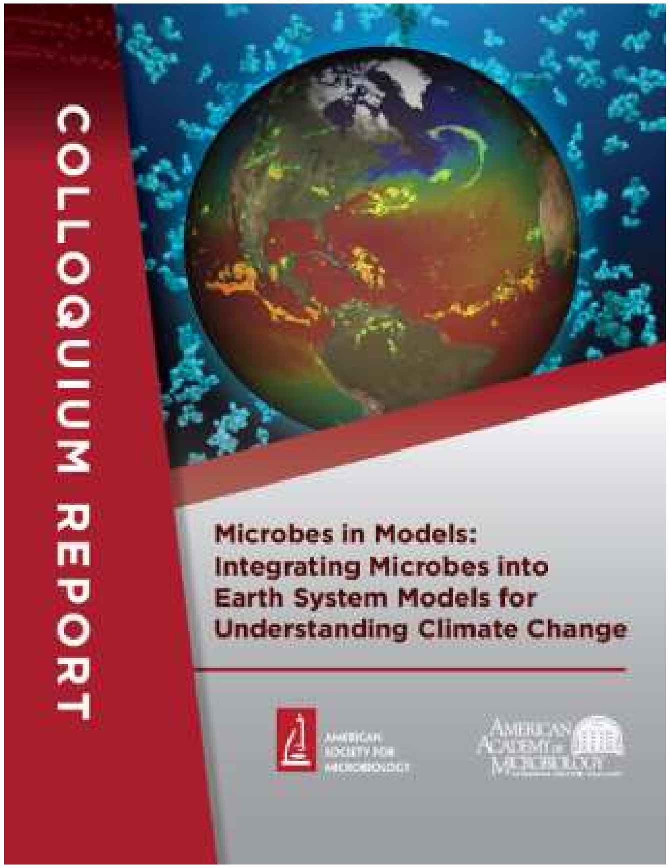
Aquatic Nuisance Species
ANSRP
Research Program

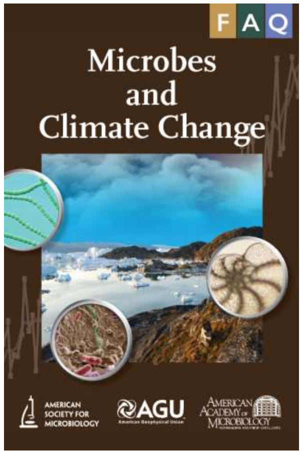
- Microbes cycle carbon and nutrients in rivers (especially in the “river’s liver”)
- Microbes are food for native and non-native animals, and can create symbiotic associations with native and non-native animals in rivers
- Microbes can signal critical geochemical, hydrogeological, and biological changes to a system, and can demonstrate how a system is responding to disturbance
- Various ways to use microbial data in predictive models

Many models approaches, and growing

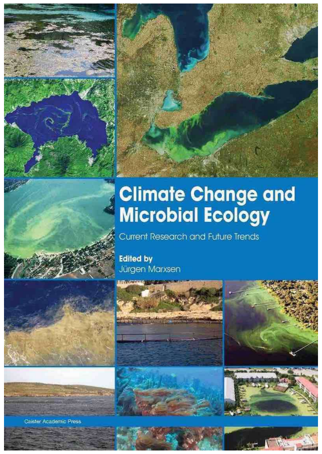

COLLOQUIUM REPORT

Microbes in Models:
Integrating Microbes into
Earth System Models for
Understanding Climate Change

June 2023 American Society for Microbiology
https://asm.org/Reports/Microbes-in-Models-Integrating-Microbes-into-Earth


FAQ

Microbes
and
Climate Change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Current Research and Future Trends
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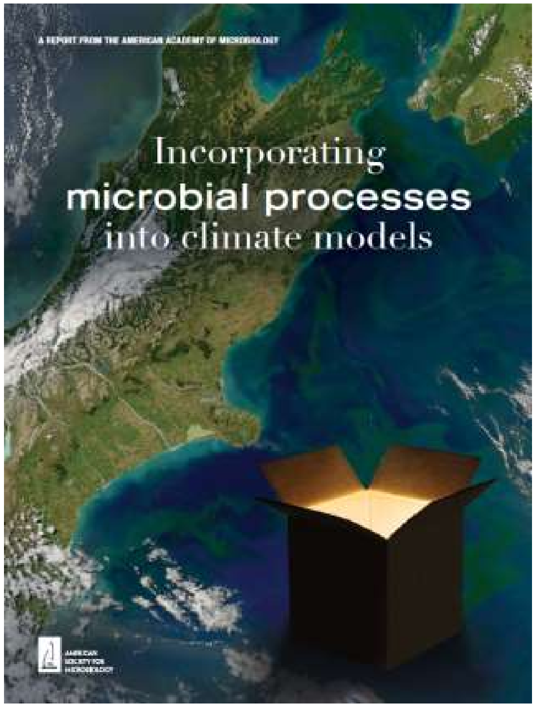
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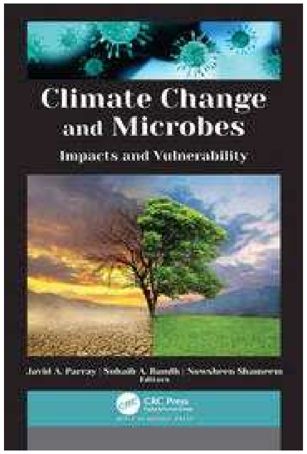
Incorporating
microbial processes
into climate models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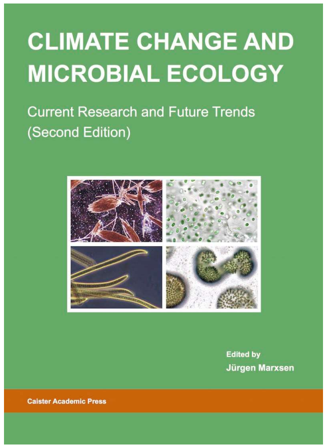
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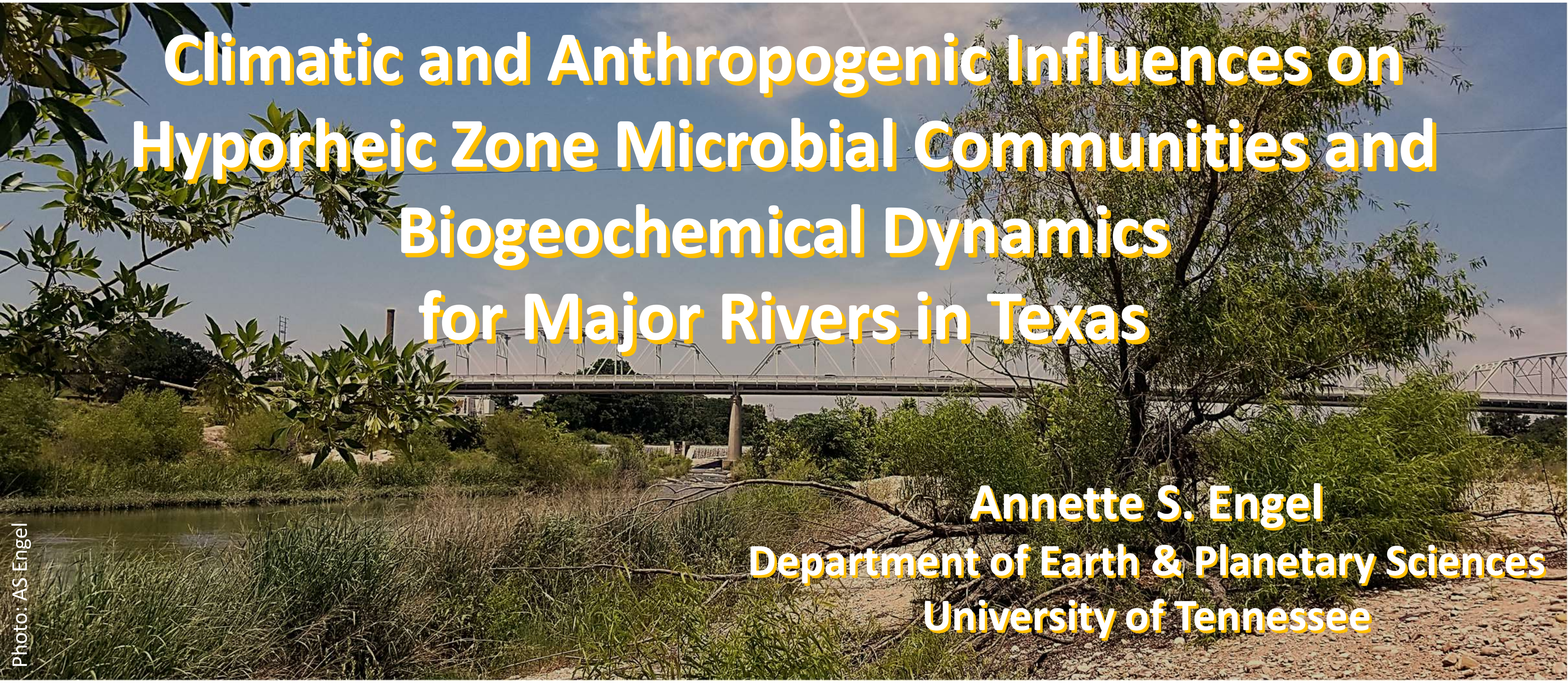

Climatic and Anthropogenic Influences on Hyporheic Zone Microbial Communities and Biogeochemical Dynamics for Major Rivers in Texas

Annette S. Engel
Department of Earth & Planetary Sciences
University of Tennessee

Photo: AS Engel

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