

Microbial Source Tracking in the Santa Ana River

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Background

Highly populated, urbanized areas are at risk of fecal contamination in surface waters

- Fecal-oral route of transmission is common for many waterborne diseases and is a major public health concern
- Potential dangers of contaminated water sources constitute the need for monitoring programs
- Challenges balancing water use and water quality

Santa Ana Watershed provides ideal study model due to its proximity to dense urban areas and diverse uses as a community water resource

Background

There are influxes of bacterial contamination within the Santa Ana Watershed.

- Several studies have attempted to identify possible contamination sources (SAWPA, 2017).

Possible sources include recreation, wastewater effluent, industrial and agricultural runoff, and presence of homeless encampments.

- The extent of interaction between this disenfranchised community and the occupied parts of the Santa Ana River is unknown.

Study Goals

- To determine fecal contamination "hotspots" in the Santa Ana River
- To develop a microbial community approach to MST

Microbial Source Tracking (MST): DNA

Principle

*Certain microorganisms are **unique** to the gut microbiome of a **specific host***

qPCR is used to analyze **gene targets** specific to individual microorganism, thus, linking the **source of pollution**.

qPCR also **quantifies** the amount of host-specific genetic material found in a sample.

PCR-based detection strategies can be **limited** in **differentiating live and dead cell** targets

MST Targets

- Human
- Swine
- Canine
- Bovine
- Equine

Primers

Assay Name	Source	Frag; Anneal T	Target/Gene	Primer Sequence (5'-3')	Primer Name	Citation
UniBac	Total Bacteria/Archaea	180bp; 61.5°C 180bp; 61.5°C	16S rRNA	AAACTCAAAGKAA TTGACGG	926F	(De Gregoris et al 2011)
				CTCACRRCACGAG CTGAC	1062R	
HoF597	Equine	354bp; 58°C	16S rRNA	CCAGCCGTA ATAGTCGG	HoF597F	(Dick et al., 2005)
		354bp; 62°C		CACATG TTCCTC CGCTCGTA	Bac708 R	
Bac3	Bovine	NA; 62°C	16S rRNA	CTAATGGAAAATG GATGGTATCT	Bac3F	(Shanks et al., 2010)
				GCCGCCAGCTCA AATAG	Bac3R	
PF163F/ Bac708R	Swine	NA; 53°C	16s rRNA	GCGGATTAATAC CGTATGA	PF163F	(Dick et al., 2005)
				CAATCGGAGTTC TTCGTG	Bac708R	(Bernhard and Field, 2000)
HF183/B acR287	Human	N/A	16s rRNA	ATCATGAGTTCAC ATGTCCG	HF183	(Green et al., 2014)
				CTTCCTCTCAGAAC CCCTATCC	BacR28	
DG3	Canine	N/A	GTF2I	TTTTAGCCCCTTG TTTCG	Dg3-fw	(Green et al., 2014)
			GTFI	GACCATGAACACC ATCAAGTGAA	Dg3-rv	

Microbial Source Tracking (MST): DNA

Benefits

Unlike current EPA water quality monitoring standards, MST not only detects fecal contamination but links possible fecal source.

MST can offer results within hours.

Limits

Standardized MST testing method needed

DNA **persists** in the environment

Inability to **distinguish between diverse activities within the same source**

Microbial Community Analysis (MCA)

Principle

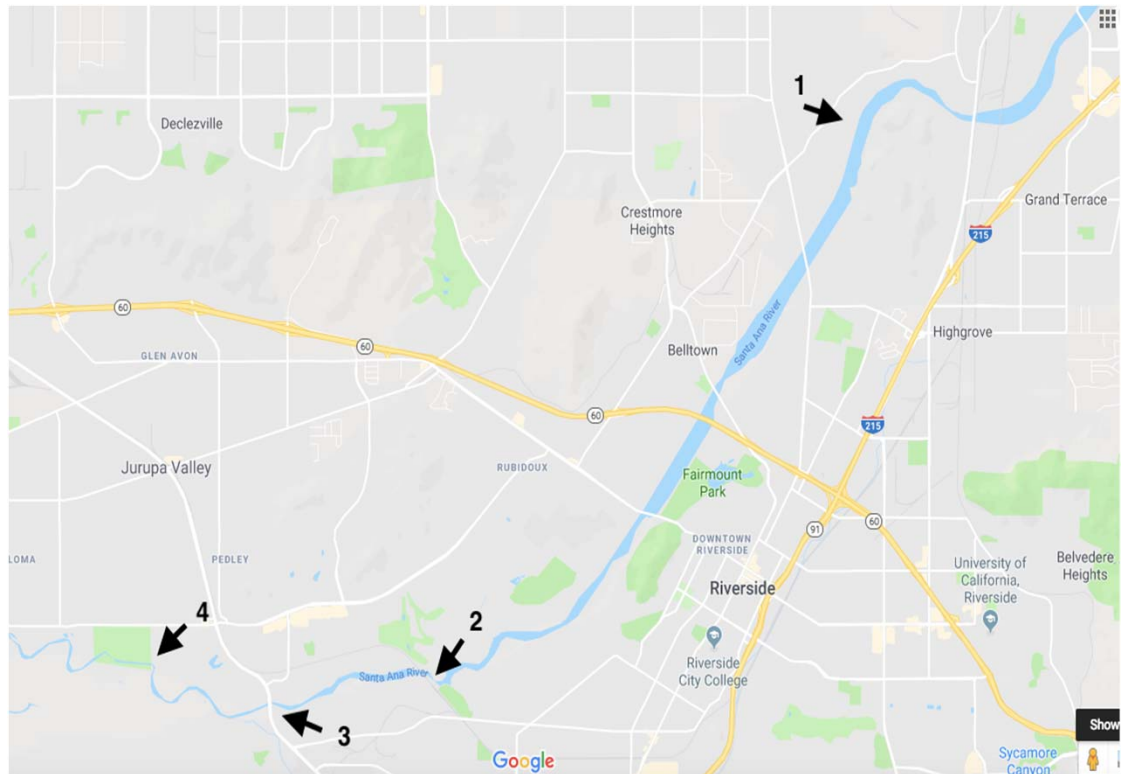
*Different locations have a **signature microbial community***

DNA is extracted directly from the source for **genetic sequencing** in order to identify all organisms present and their concentration.

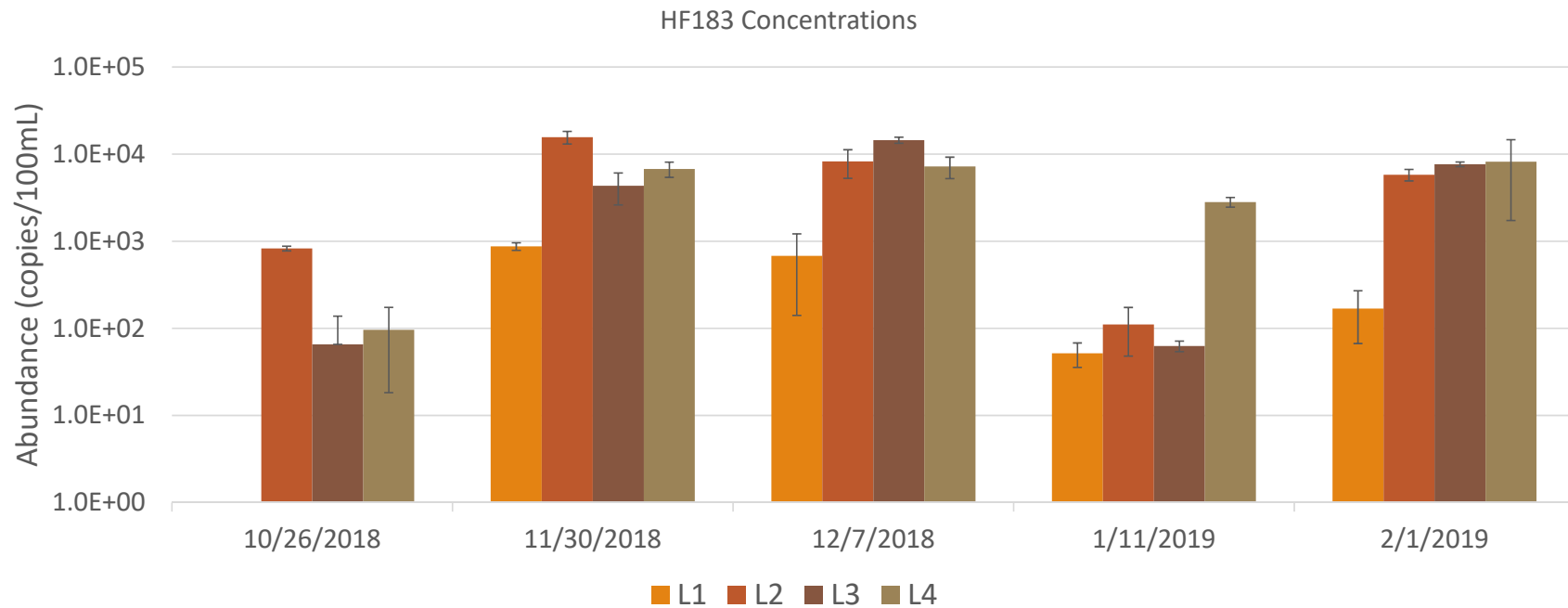
The resulting **microbial fingerprint** is related to a **specific source of pollution** (i.e. recreational, wastewater effluent, runoff).

Sampling Locations

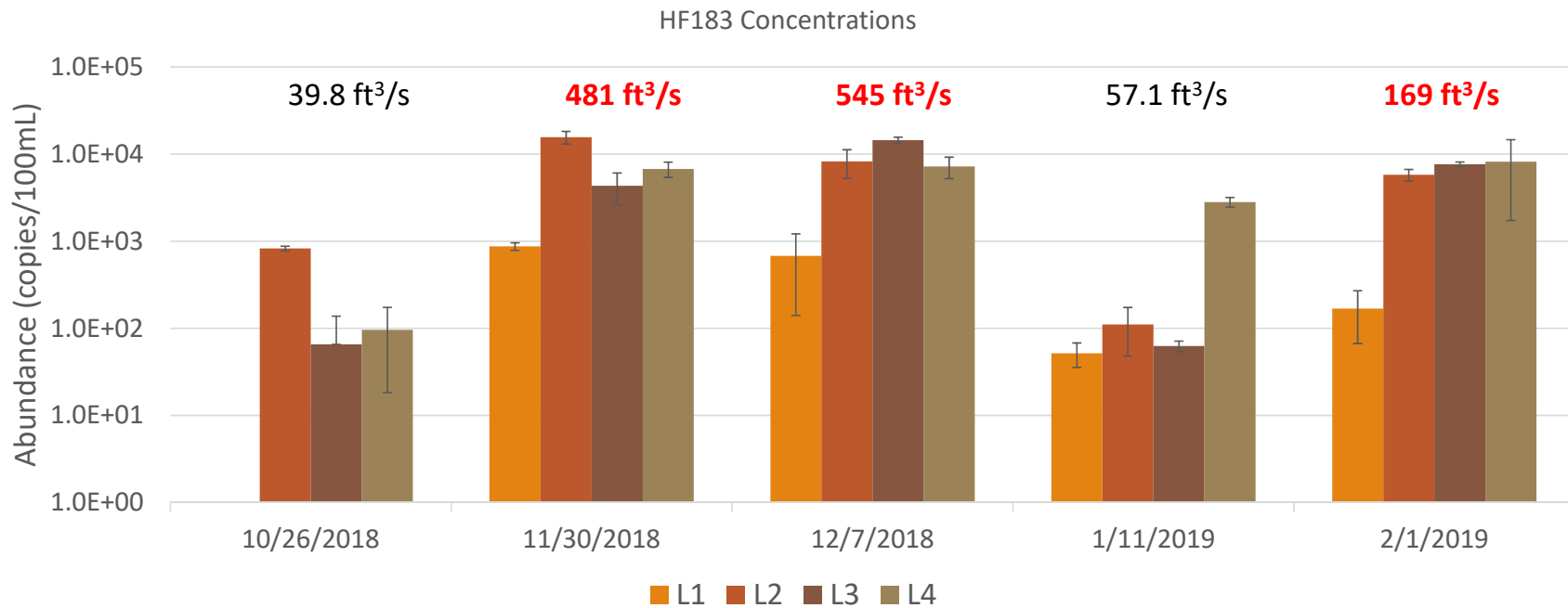
1. Agua Mansa
2. Martha McLean-Anza Narrows Park
3. Van Buren Bridge
4. Paradise Knolls



Results: Human marker HF183



Wet- and dry- weather influence?



Future Work

Quantify gene-specific markers

- Canine, equine, bovine, swine

Microbial community profiles

- Determine all bacterial members from a sampling location
- Associate microbial community profiles with different activities

Explore Live/Dead assays for gene markers

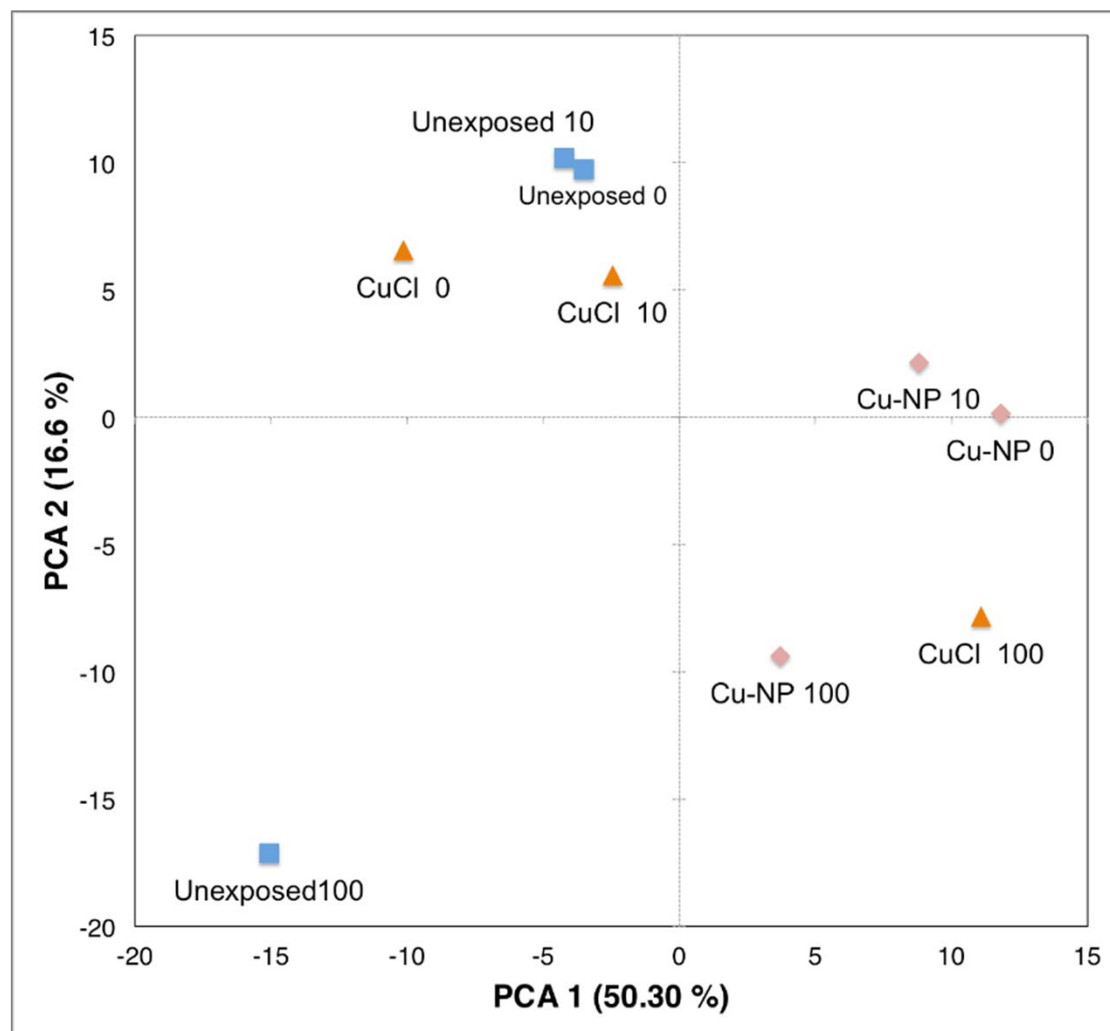
Distribution of Microbial Communities is Associated with Metal Exposure

- Square = Unexposed control
- Triangle = CuCl_2 exposed
- Diamond = CuNP exposed

Clustering of 0 and 10 day microbial communities

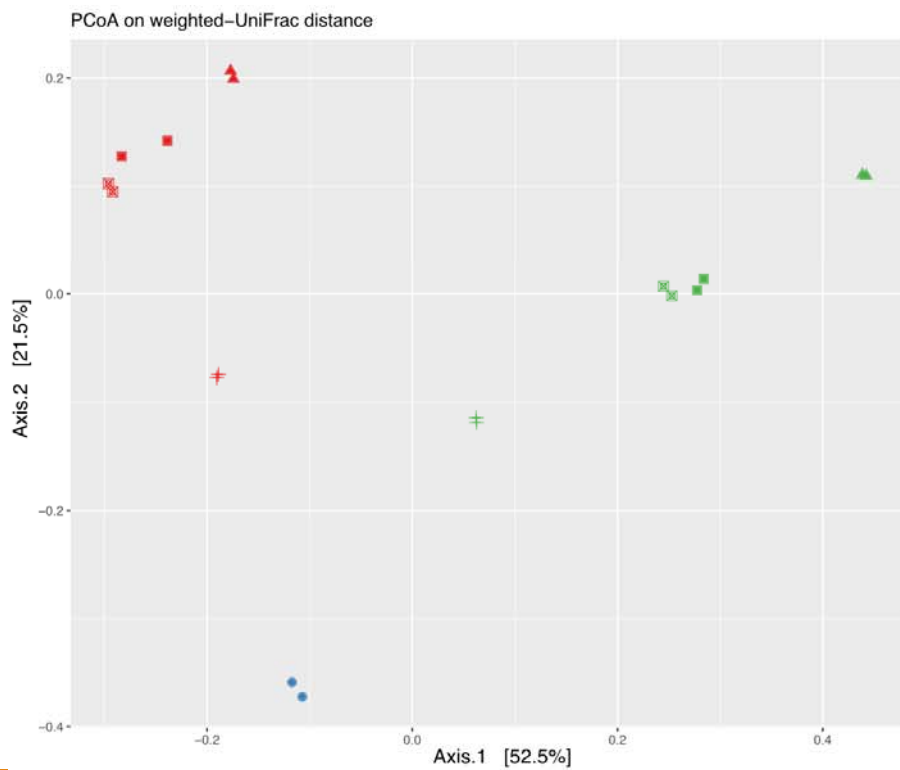
Vastly different microbial communities after 100 days

Denitrification performance recovered from initial acute differences

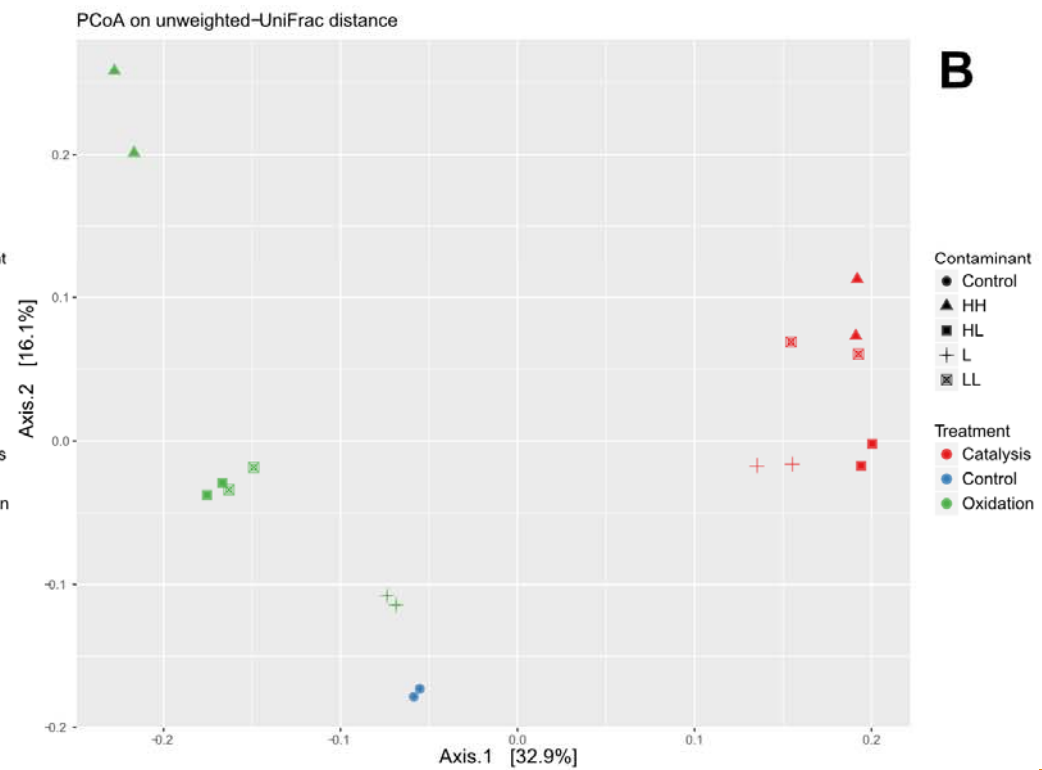


Reyes et al., 2018 *ACS Sustain. Chem. Eng*

Distribution of microbial communities is associated with contaminant and treatments

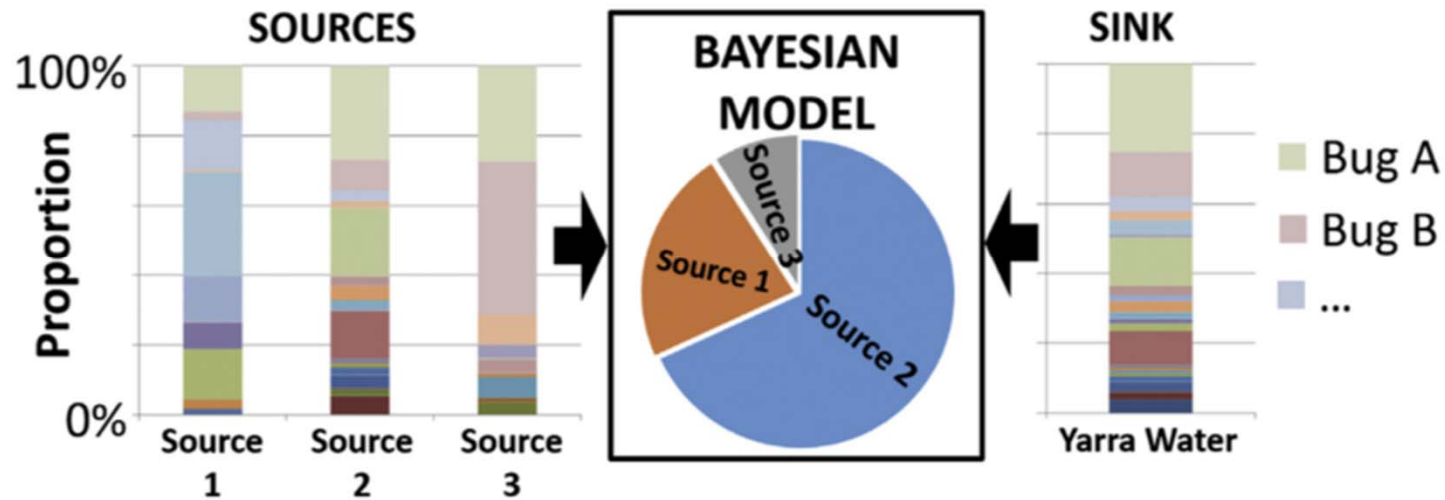


A



B


Microbial Community Strategy for MST



Visual representation of microbial community analysis for pollution source identification from McCarthy et al. 2017

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Questions?

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