# Using Chemical Tracers to Supplement Microbial Source Tracking in the Estero River

Michael A. Kratz[1]; Taylor L. Hancock[1,3]; Haruka E. Urakawa[2]; L. Donald Duke[1]; Serge Thomas[1]; Hidetoshi Urakawa[1,3]

[1]Department of Ecology and Environmental Studies, Florida Gulf Coast University, Fort Myers, FL
[2]Department of Marine and Earth Sciences, Florida Gulf Coast University, Fort Myers, FL
[3]School of Geosciences, University of South Florida, Tampa, FL

## Abstract

Detecting fecal pollution is a crucial component in water quality monitoring due to the human health risk involved. Fecal indicator bacteria (FIB), like *Escherichia coli* and enterococci, have been the primary analyte for human wastewater contamination in U.S water quality monitoring since the 1960s. The methods used to enumerate these bacteria are relatively cheap and quick to use, but researchers have recommended using other tracers in tandem with *E. coli* and enterococci. This is due to their presence in a variety of warm-blooded animal species as well as their extra-enteric environmental persistence. In this study, we used the chemical tracers, sucralose (a man-made sugar), and 15N/18O stable isotopes to help identify the source of FIB contamination in the Estero River via surface and groundwater sampling. The Estero River has had chronic exceedances of the Florida Department of Environmental Protection (FDEP) FIB standard for recreational waterways but monitoring efforts have not identified the main driver of these elevated FIB levels. There are multiple wastewater treatment plants (WWTPs) and septic tanks along the river, potentially leaching FIB and pathogens into the groundwater and eventually the river if not treated properly. Using tandem-tracing methods, we found that there was no significant correlation between any of the chemical tracer and FIB combinations. Groundwater FIB levels were below detection levels in the majority of samples but also had sucralose levels near the level of wastewater (>1000 ng/L). This result indicates that the residential WWTPs are eliminating FIB and potential human pathogens before entering the waterway and are not the primary cause of elevated FIB levels in the Estero River. *E. coli* correlated strongly with electric conductivity, which may be an indication that tidal resuspension is leading to elevated *E. coli* levels in the downstream portion of the Estero River. We also observed that poor correspondence between sucralose and 15N levels that is likely due to microbial transformation of nitrogen via denitrification and the fact that the majority of groundwater nitrogen came from fertilizer. Overall, we demonstrated the effectiveness of the use of sucralose and 15N/18O stable isotope signatures as chemical tracers to assist in identifying the source of FIB contamination.

## Introduction

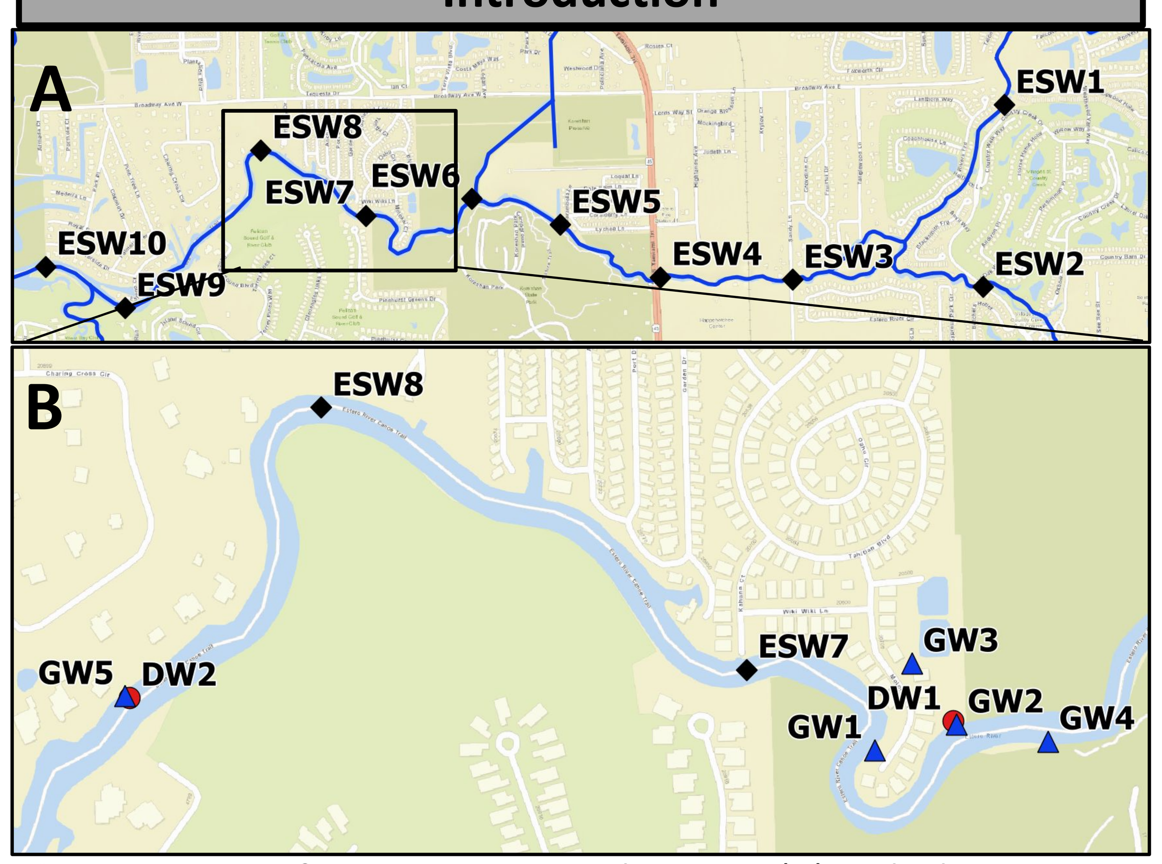


**Figure 1.** Map of Estero River sampling sites (A) with close-up on ditch and groundwater sites (B).

- Fecal Indicator Bacteria (FIB) such as *E. coli* and enterococci are commonly used tracers for human fecal contamination into waterways[1,2]
- FIB can also be present in wild animal feces and can persist in high levels in sediments[1,3]
- Chemical source tracking can be used in tandem with FIB monitoring to confirm origins of FIB[3,4]
- Sucralose is found in man-made sugars (Splenda) and does not break down through wastewater treatment[5]
- Wastewater 15N levels are enriched compared to atmospheric N levels[6]
- High sucralose and 15N levels indicate human inputs of FIB[4]

## Acknowledgments

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## Methods

- Estero River surface water samples and YSI readings were taken via boat from August 2019 to August 2020.
- Groundwater samples were collected from five wells along the Estero River, with three wells near a wastewater treatment plant (WWTP).
- Water samples were analyzed for FIB (*E. coli* & enterococci), nutrients, and chemical tracers (sucralose and 15N).

## Results

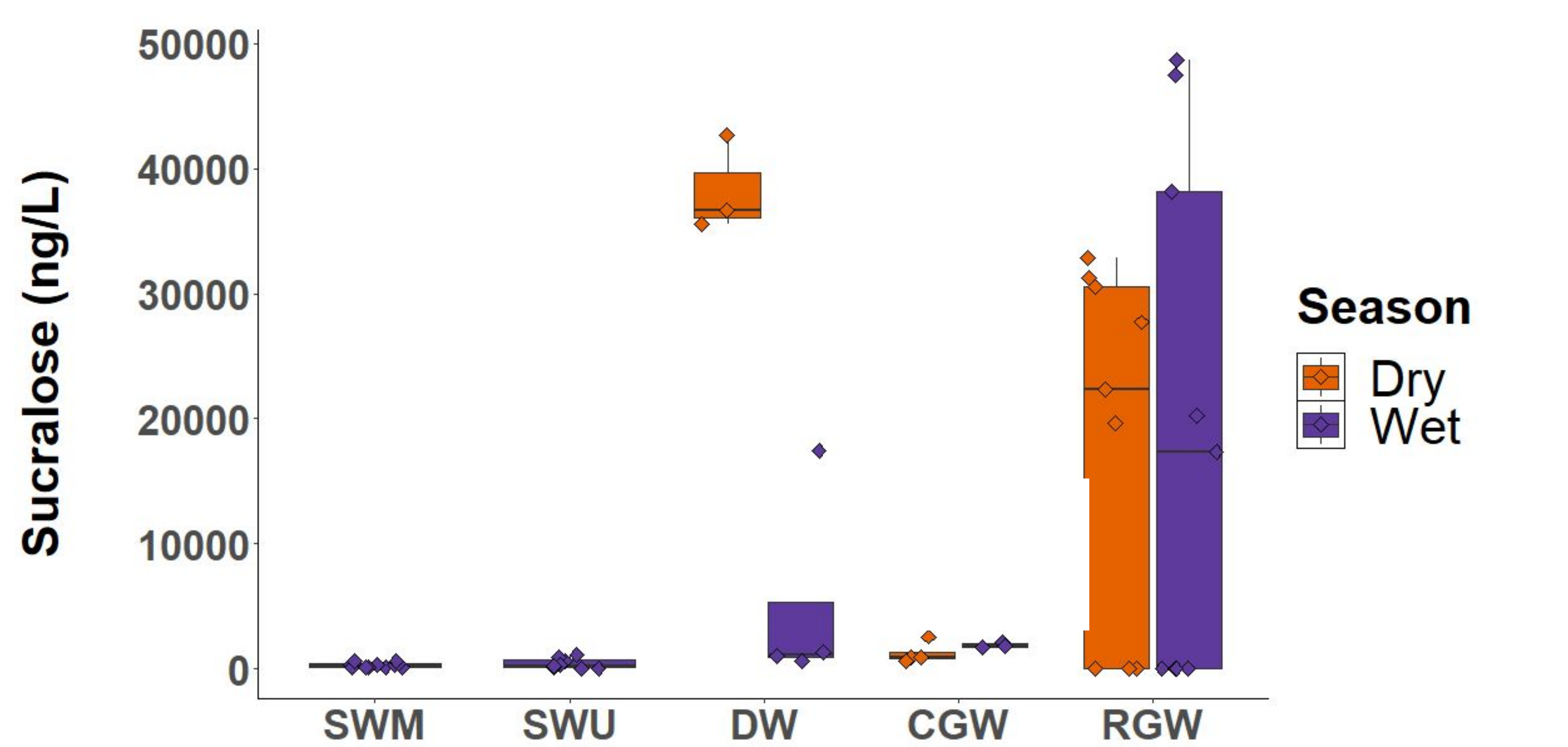


**Figure 2.** Boxplot of sucralose levels of sampling locations grouped by season. SWM = Surface water midstream, SWU = Surface water upstream, DW = Ditch water, CGW = Control groundwater, and RGW = Residential groundwater. Wet season = May – September, Dry season = October – March.

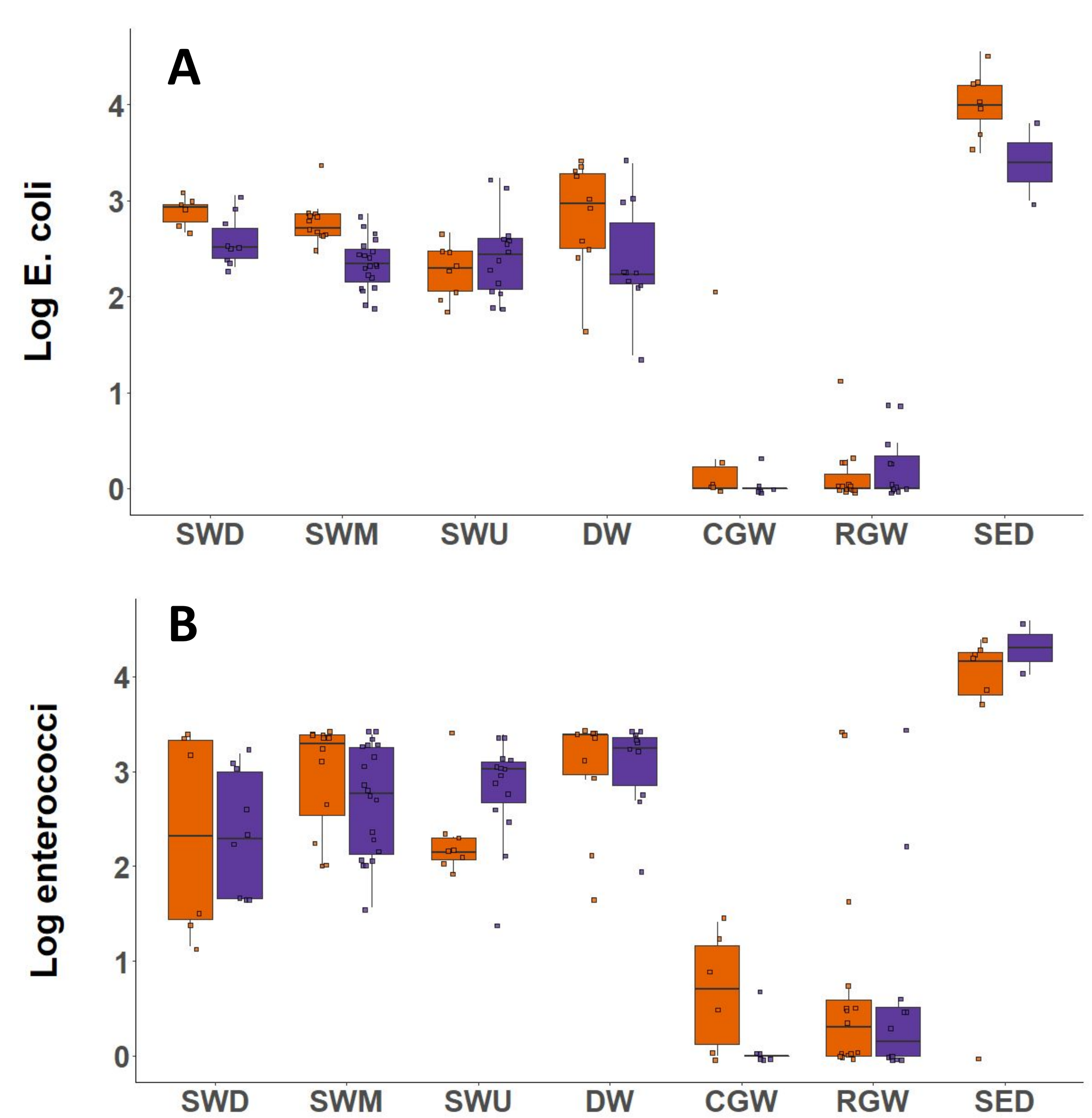


**Figure 3.** Boxplots of *E. coli* (A) and enterococci (B) levels by sampling location/type. SWD = surface water downstream, SWM = Surface water midstream, SWU = Surface water upstream, DW = Ditch water, CGW = Control groundwater, RGW = Residential groundwater, and SED = sediment. Wet season = May – September, Dry season = October – March.

## Discussion

- **No strong correlations between any pairings of FIB and chemical tracer**
  - Sources appear to be different
- **Residential groundwater and ditch water had enriched sucralose levels**
  - Sucralose >1000 ng/L indicates wastewater as the water source
- **FIB undetected in a majority of groundwater samples**
  - Residential wastewater is treated well and likely kills off FIB and human pathogens
- **15N:18O levels for groundwater fell almost exclusively in the range of fertilizers**
  - Fertilizer infiltration into groundwater likely masks elevated 15N levels from wastewater
    - Elevated sucralose levels corroborate this conclusion
- ***E. coli* was strongly correlated with electric conductivity (r = 0.58, p = 0.002)**
  - Surface downstream and midstream levels significantly higher than upstream, may be caused by tidal resuspension of sediments

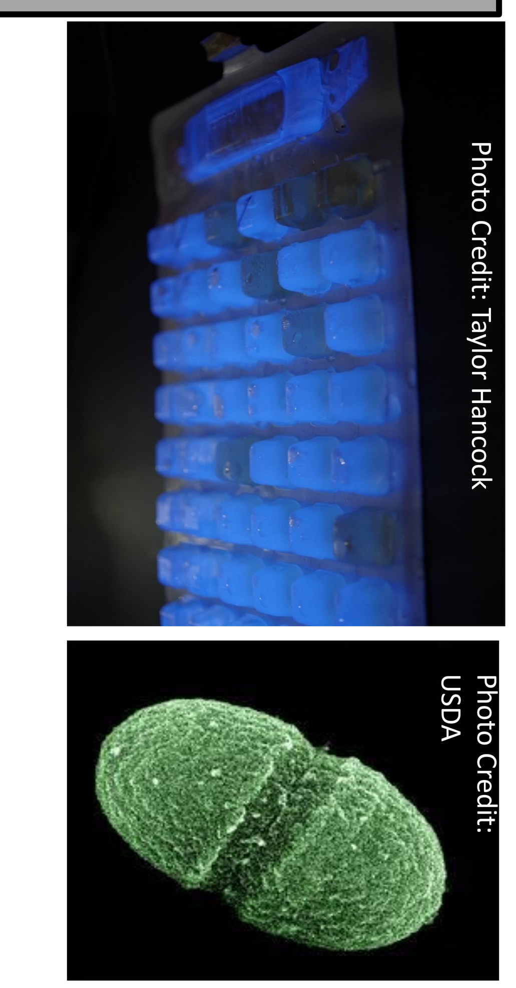


**Figure 5.** (A) IDEXX Quantitray from Enterolert assay under UV light. (B) Electron microscope photo of *Enterococcus faecalis*.

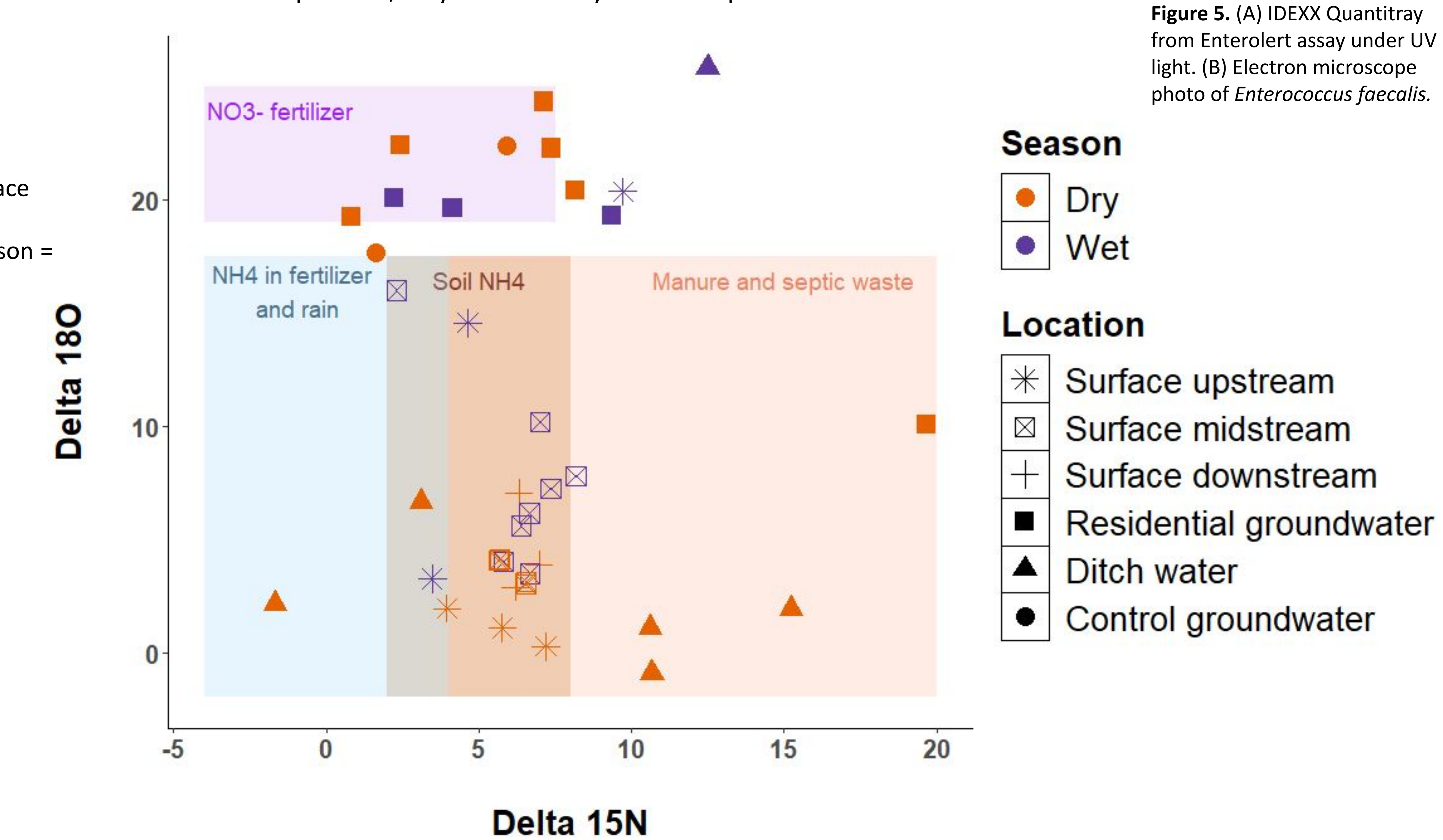


**Figure 4.** Scatterplot of 15N:18O stable isotopes from Estero River samples. Wet season = May – September, Dry season = October – March.

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