



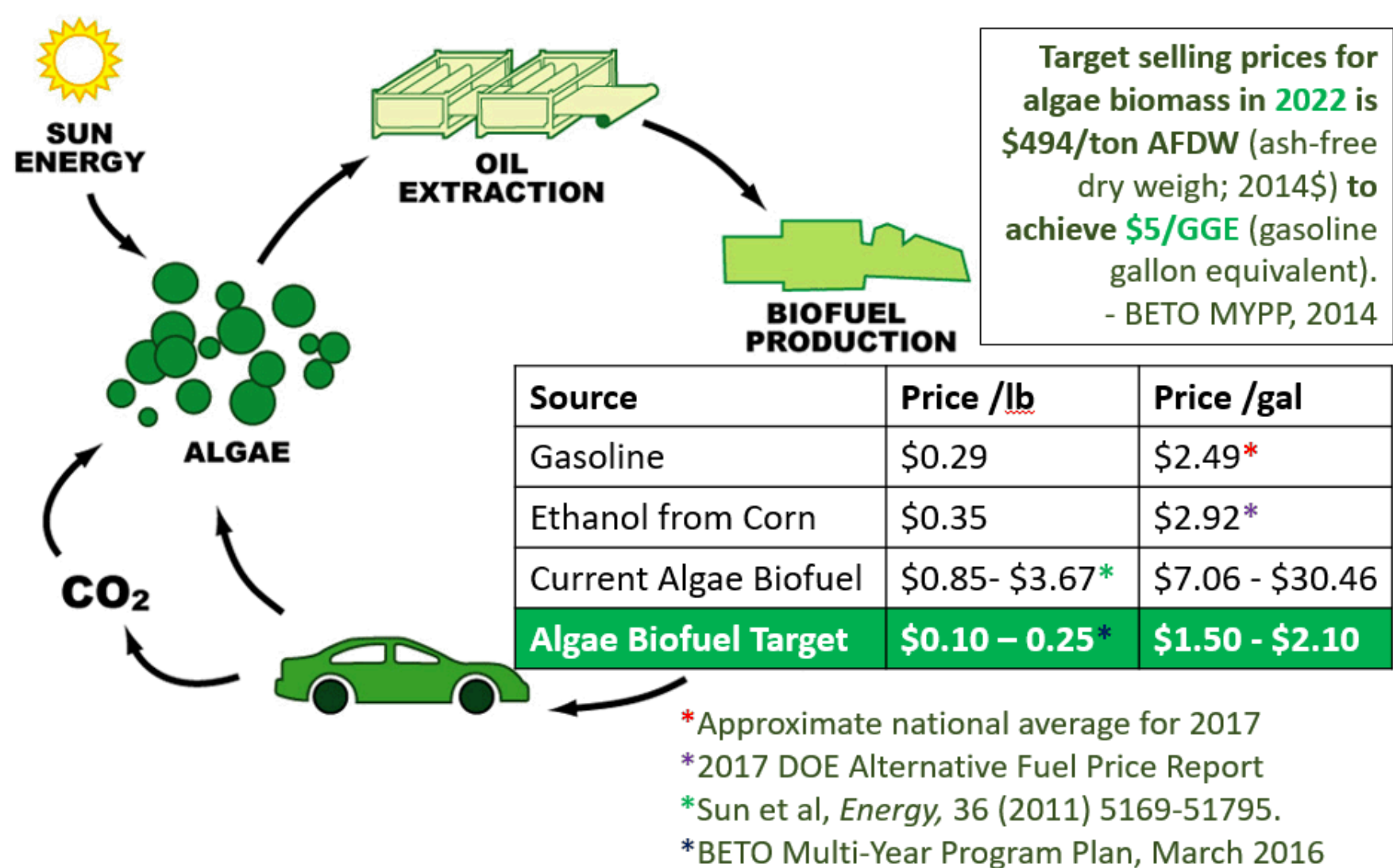
# The Good, the Bad and the Algae:

## Identifying Bacteria that Protect Microalgae from Predators

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Biofuels are the direct energy alternative for the transportation industry.

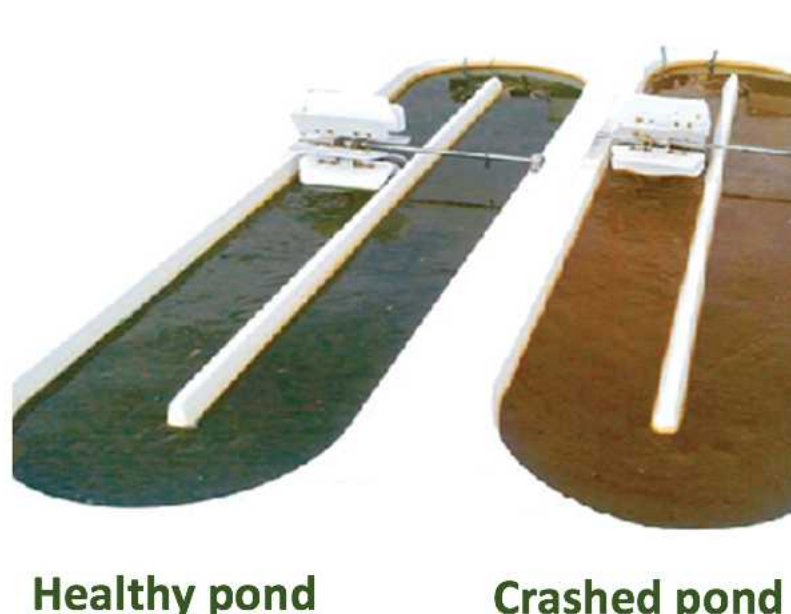
In order to achieve usable algal biofuels, we must overcome the market's barrier to entry.



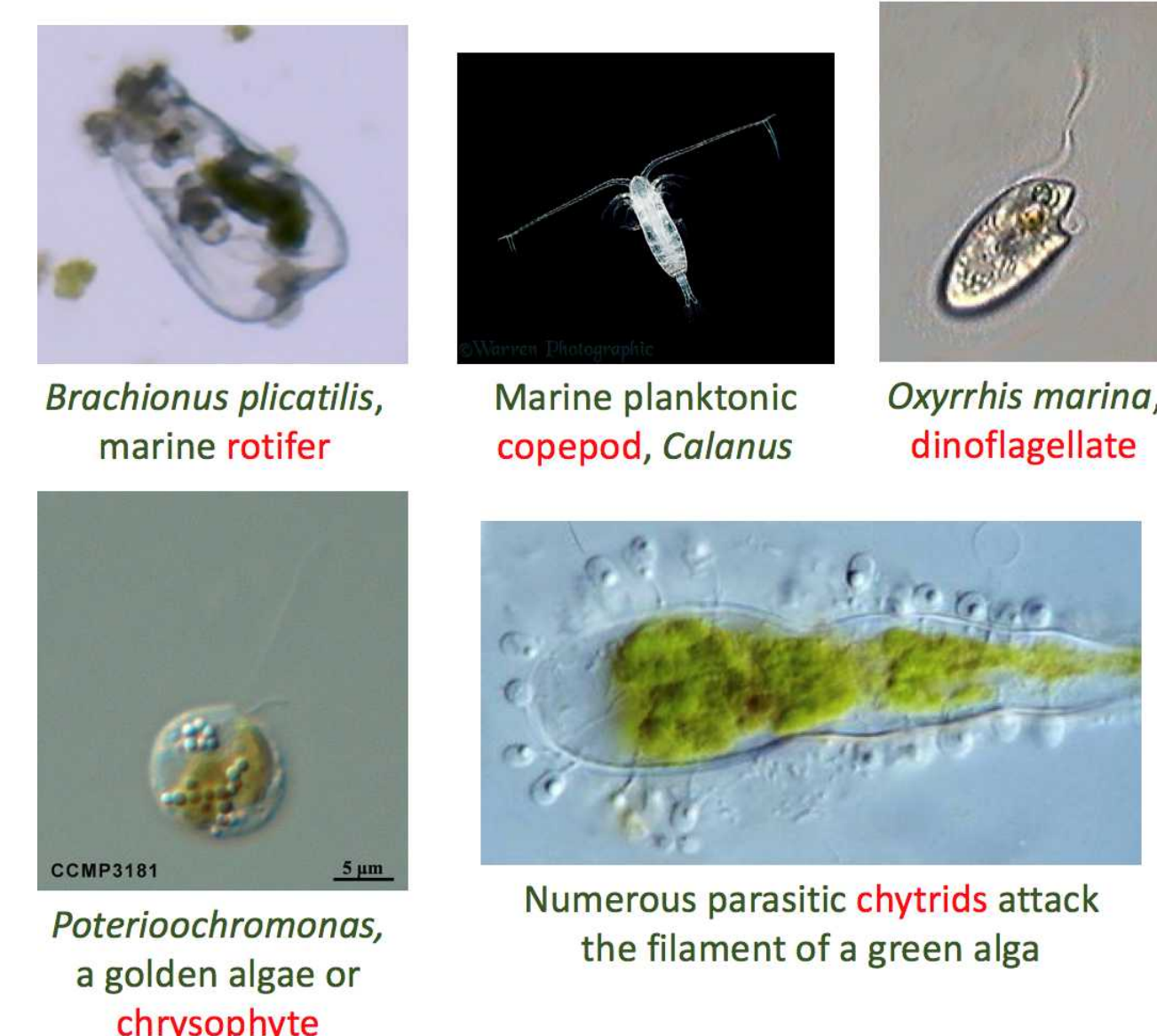
The first step towards biofuel reality is maintaining a substantial crop production.

Algae is grown in large, open-air ponds which are unprotected against microscopic predators, leading to frequent "crashes".

Algal population crashes cause losses of up to 30% of annual crop production from the typical open raceway system.



### Predators of microalga

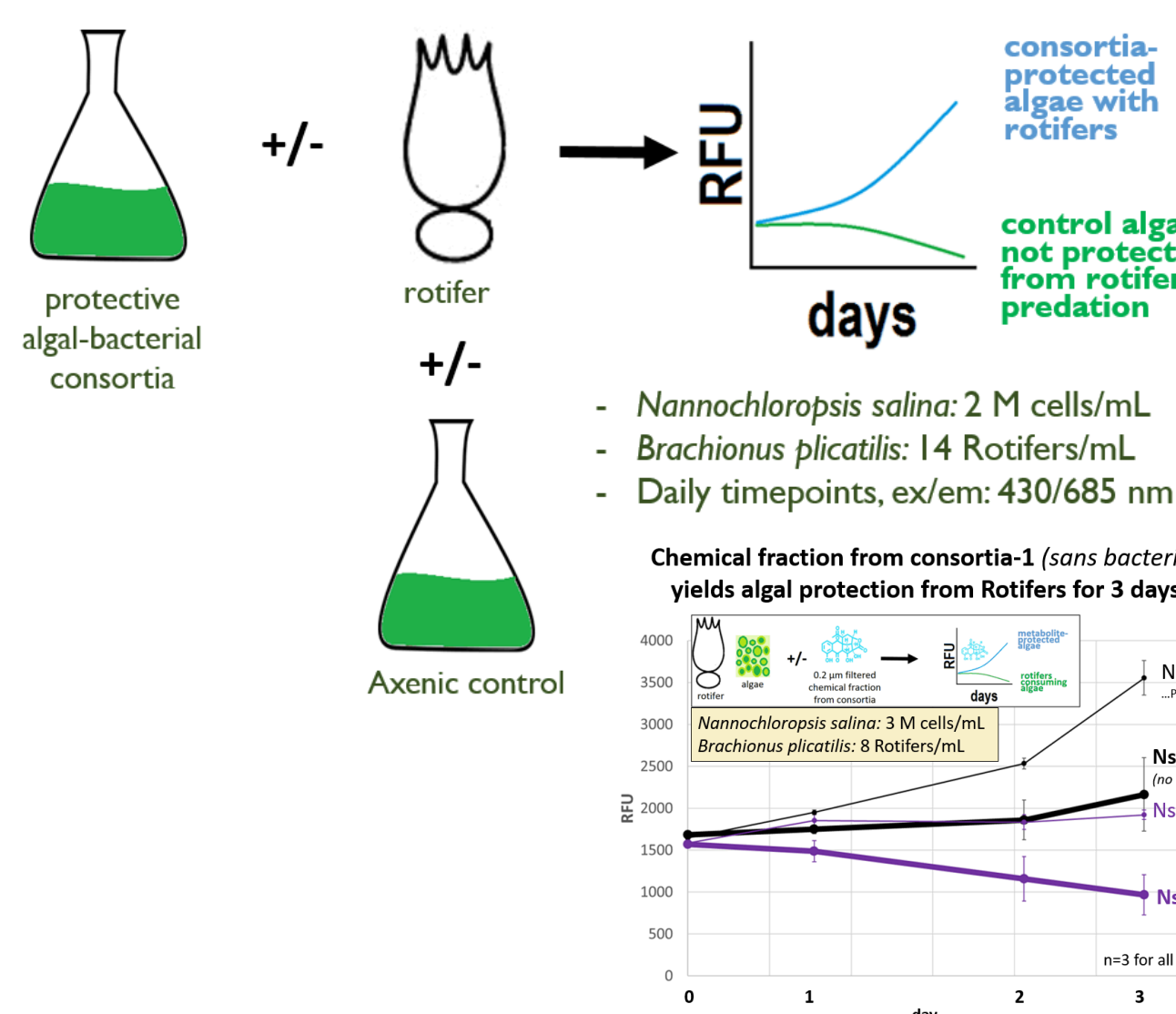


At Sandia, we are studying the chemical ecology of algal culture systems in order to develop biological and chemical tools that will stabilize algal production systems, deter predation, and mitigate pond crashes.

### Experimental Methodology

#### Consortia screen

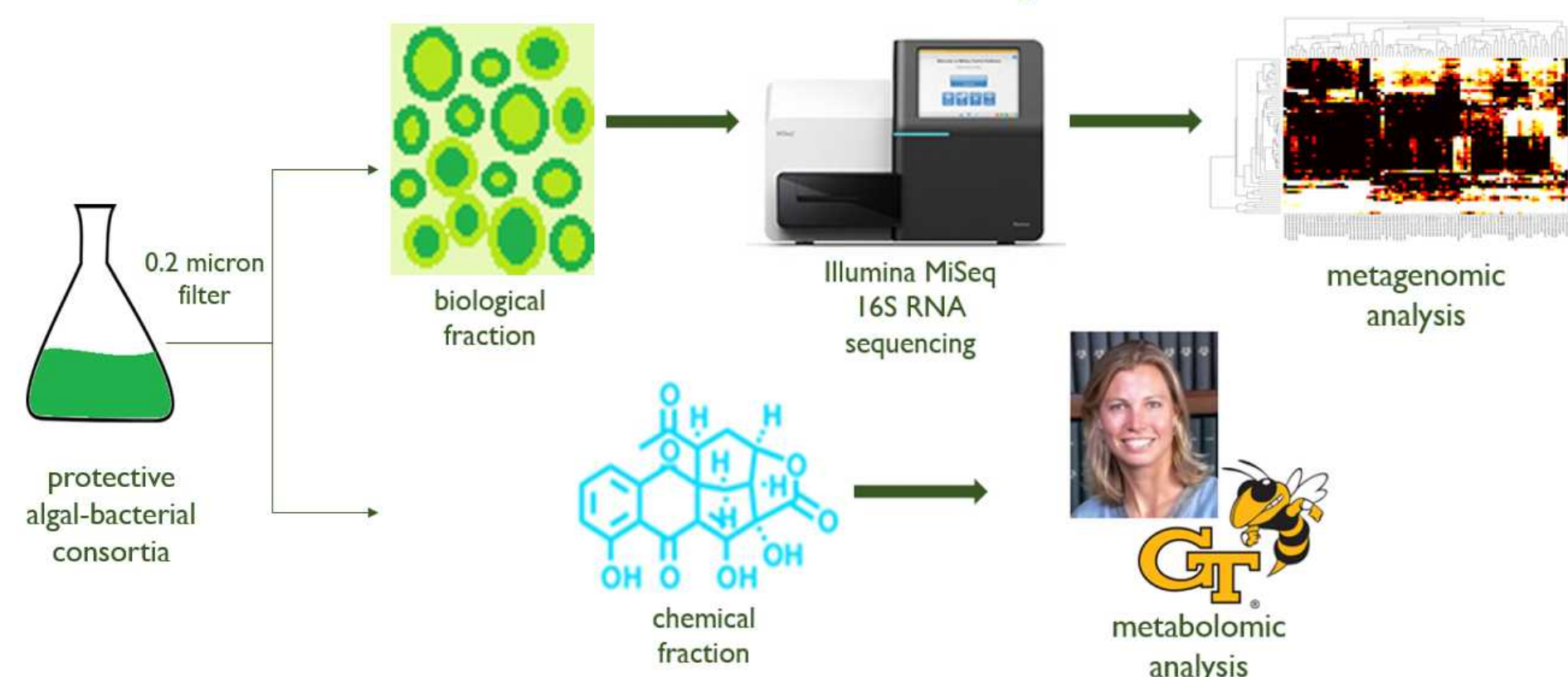
Algae survival assay (Rotifer live/dead assay)



Consortia protection is determined by the fluorescence readings taken daily during the screen. The subsequent analysis of the bacterial and chemical fractions will determine the protective organisms and compounds.

Past consortia data from Fisher, Lane, et al, demonstrates protection might be incurred through a chemical produced by the bacteria.

#### Consortia Analysis



### Results

The Consortia Screen experiment tested twenty unique consortia against an axenic control. Six consortia demonstrated protective properties when challenged, with two growing beyond the unchallenged axenic control. DNA was extracted from each biological sample and the V3/V4 region of the 16S ribosome was amplified and tagged for sequencing. All samples were sequenced on an Illumina MiSeq and initial analysis was performed in QIIME2.

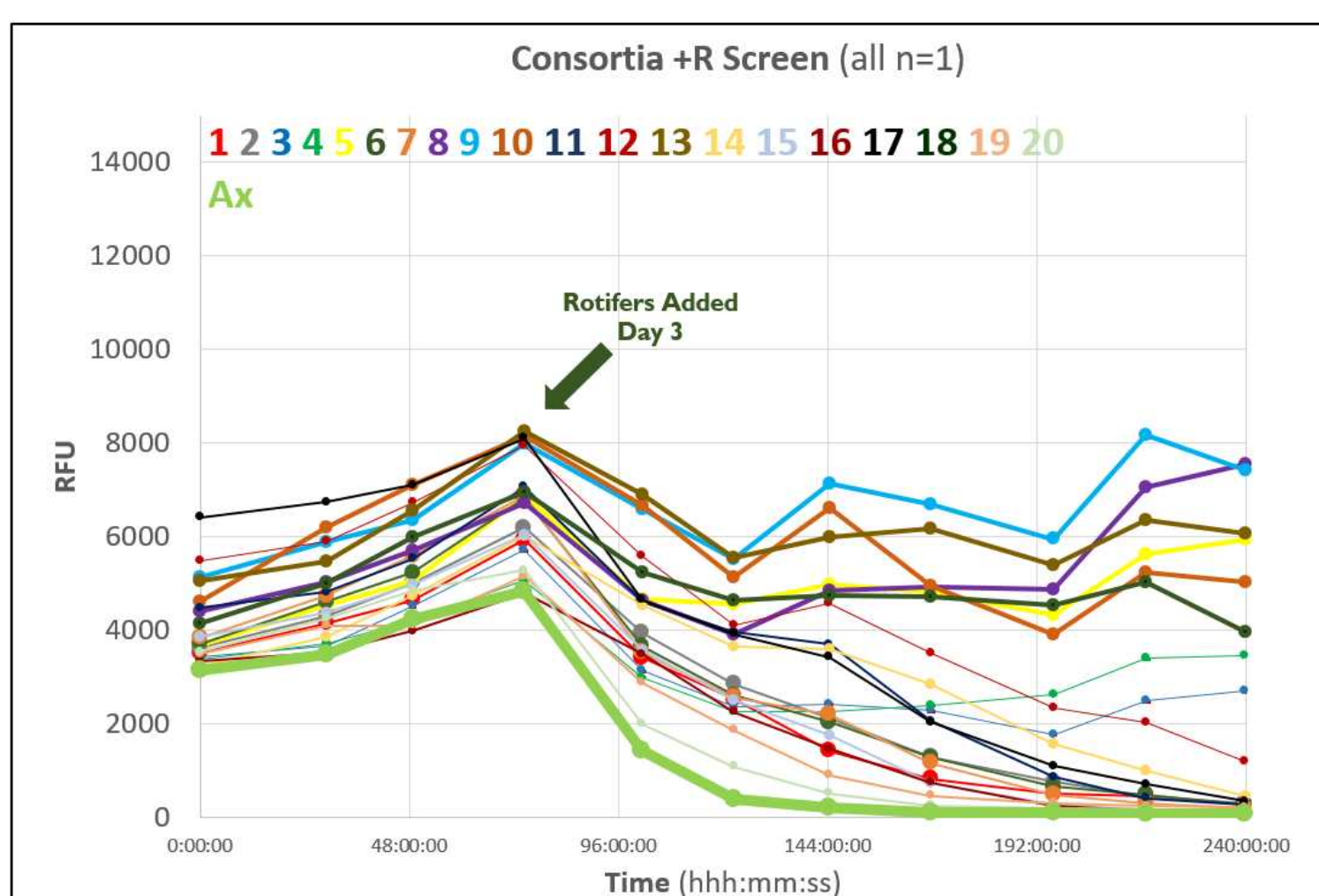


Figure 1. Timepoint data for all consortia challenged with 14 rotifers/mL. Chlorophyll fluorescence is reported in Relative Fluorescence Units.

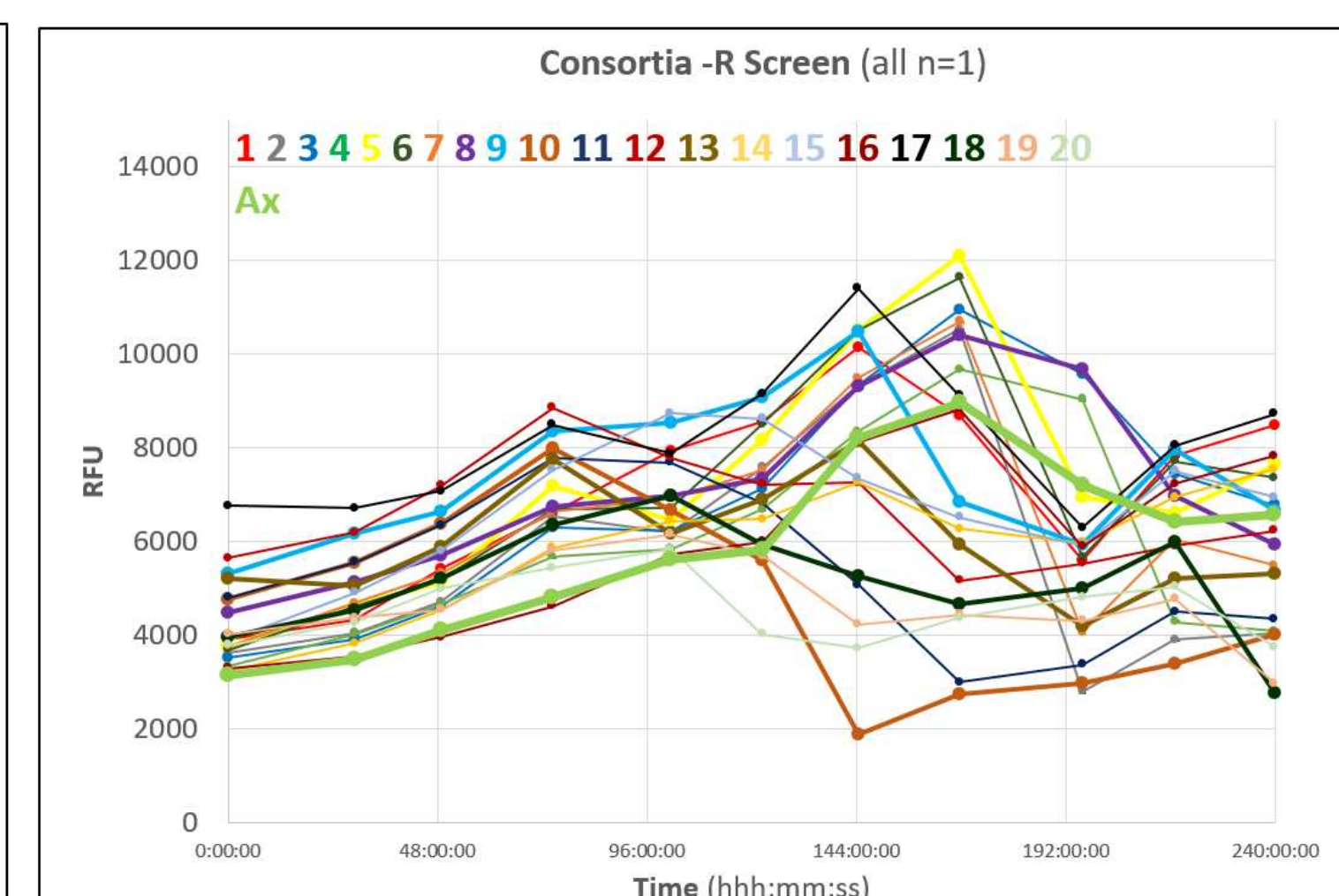
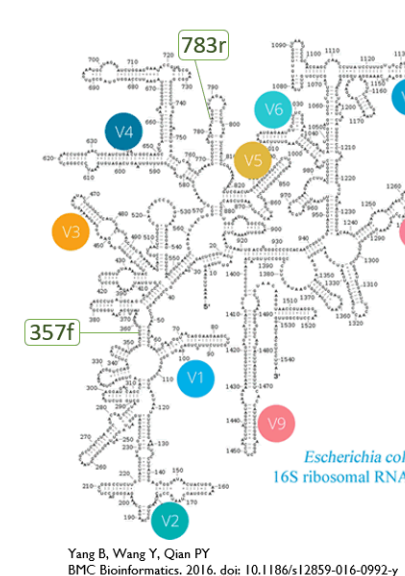


Figure 2. Timepoint data for all consortia without rotifer predation.



The 357f and 783r primers were used to amplify the V3/V4 region of the 16S ribosome. The 16S ribosome is present in all bacteria and can therefore be used to identify bacterial genera.

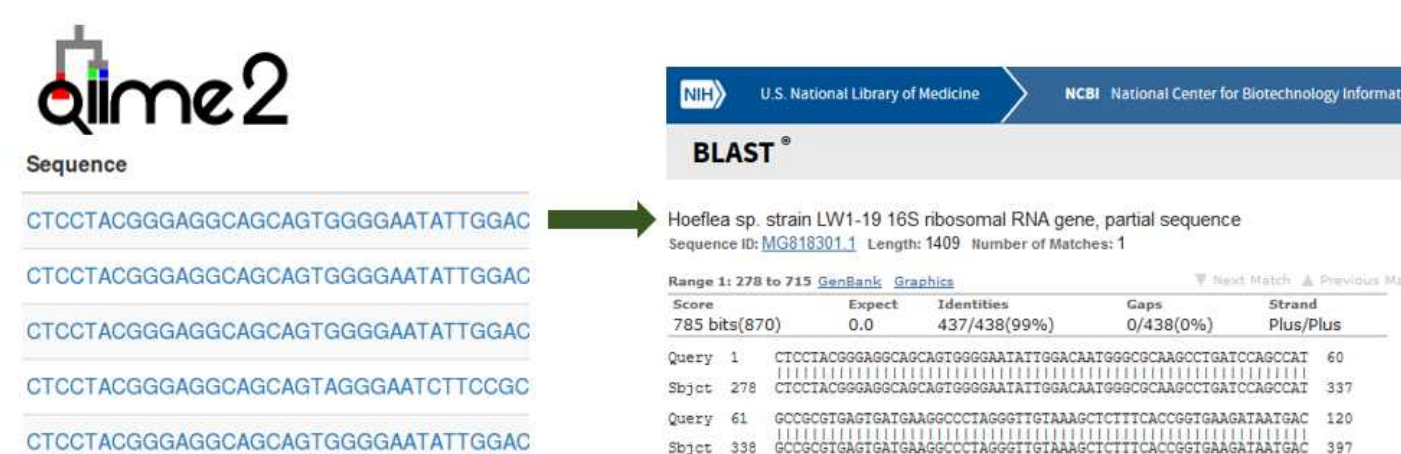


Figure 4. (below) Quality Plots generated in QIIME2 from MiSeq data for both the forward and reverse read of a subset of samples.

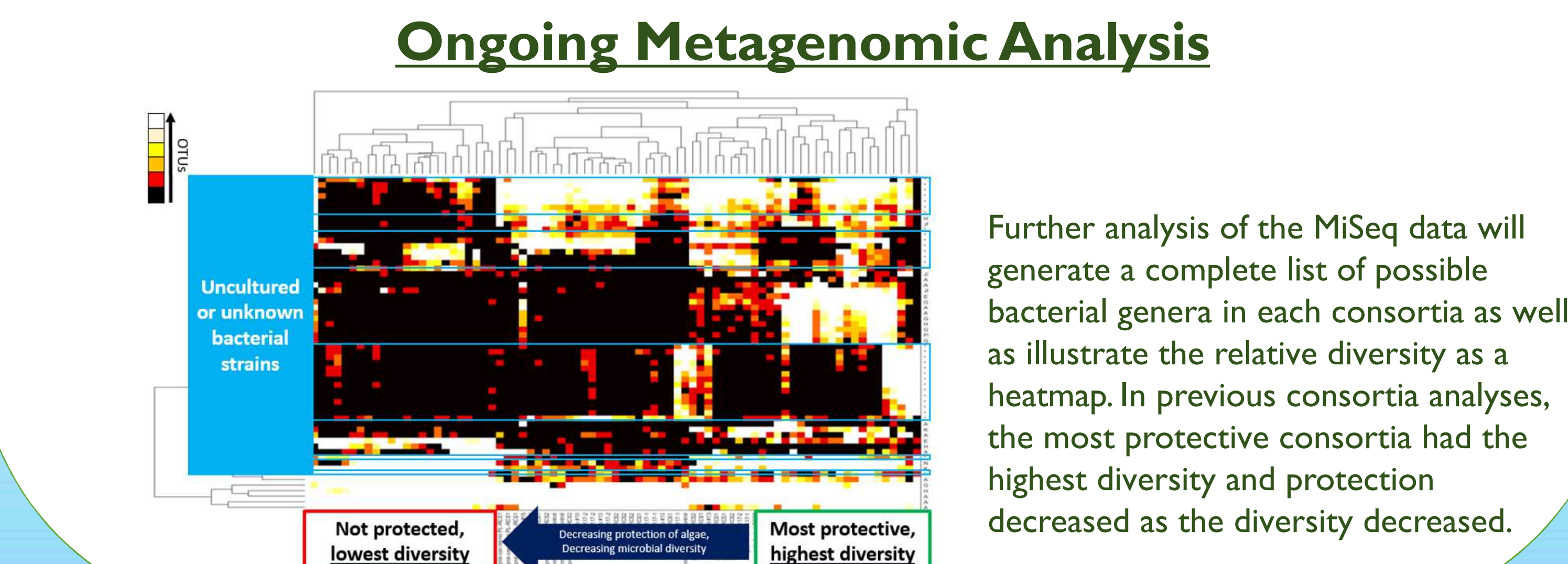


Figure 5. QIIME2 sequence results. These sequences were sent through the NCBI BLAST database and matching sequences were identified.

### Ongoing Metagenomic Analysis

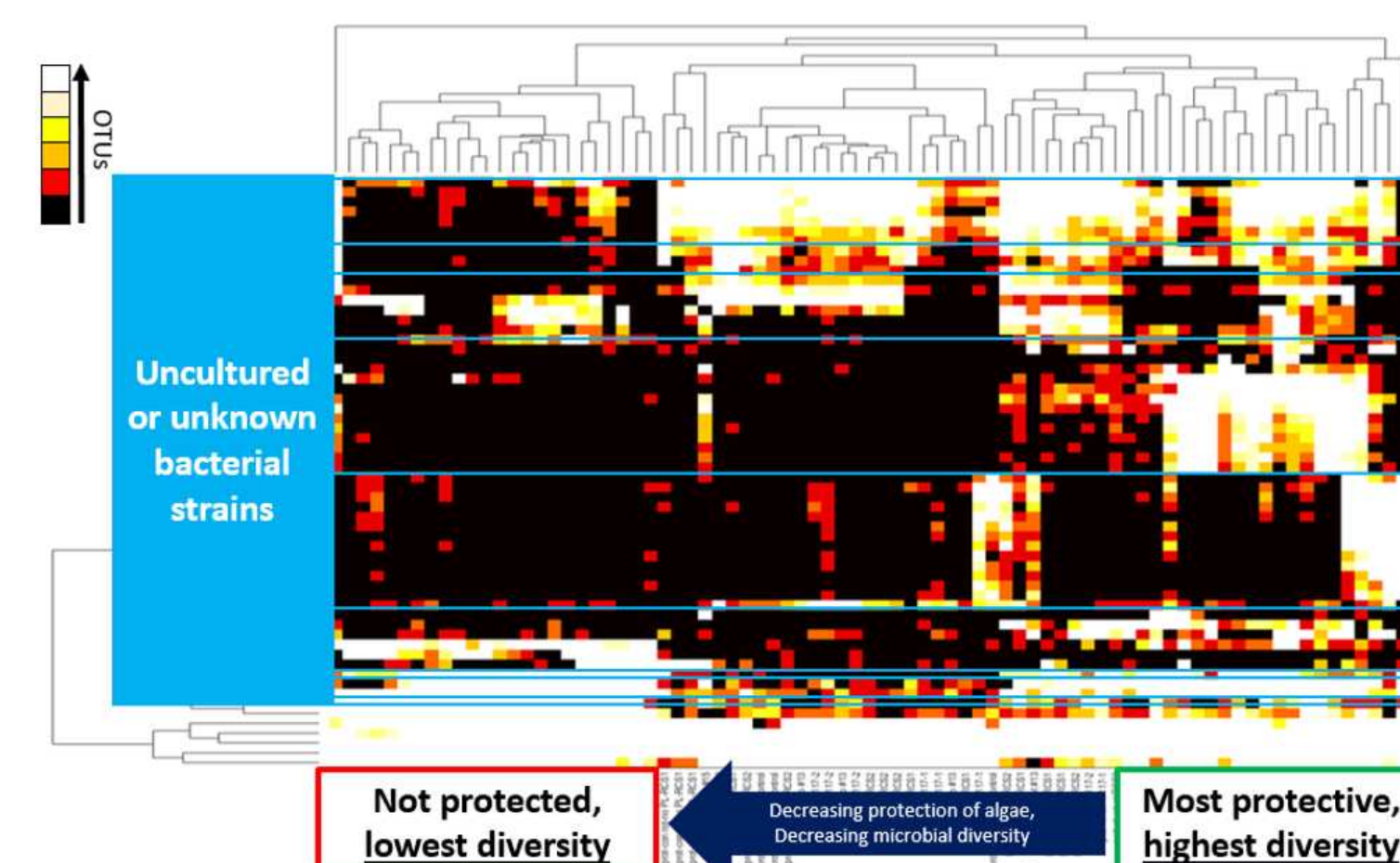


Figure 6. Fisher, Lane, et al, 2018. Heatmap of previously analyzed consortia data.

Further analysis of the MiSeq data will generate a complete list of possible bacterial genera in each consortia as well as illustrate the relative diversity as a heatmap. In previous consortia analyses, the most protective consortia had the highest diversity and protection decreased as the diversity decreased.

### Future Work

- Working with Dr. Julia Kubanek at Georgia Tech to analyze the chemical fractions of each consortia sample.
- Analysis of the metagenomics and metabolomics of each sample to determine the organism(s) and chemical(s) responsible for protection of algae from predation.
- metabolomics: identification and quantification of chemicals produced in biological systems
- metagenomics: identification and classification of organisms in biological systems

