

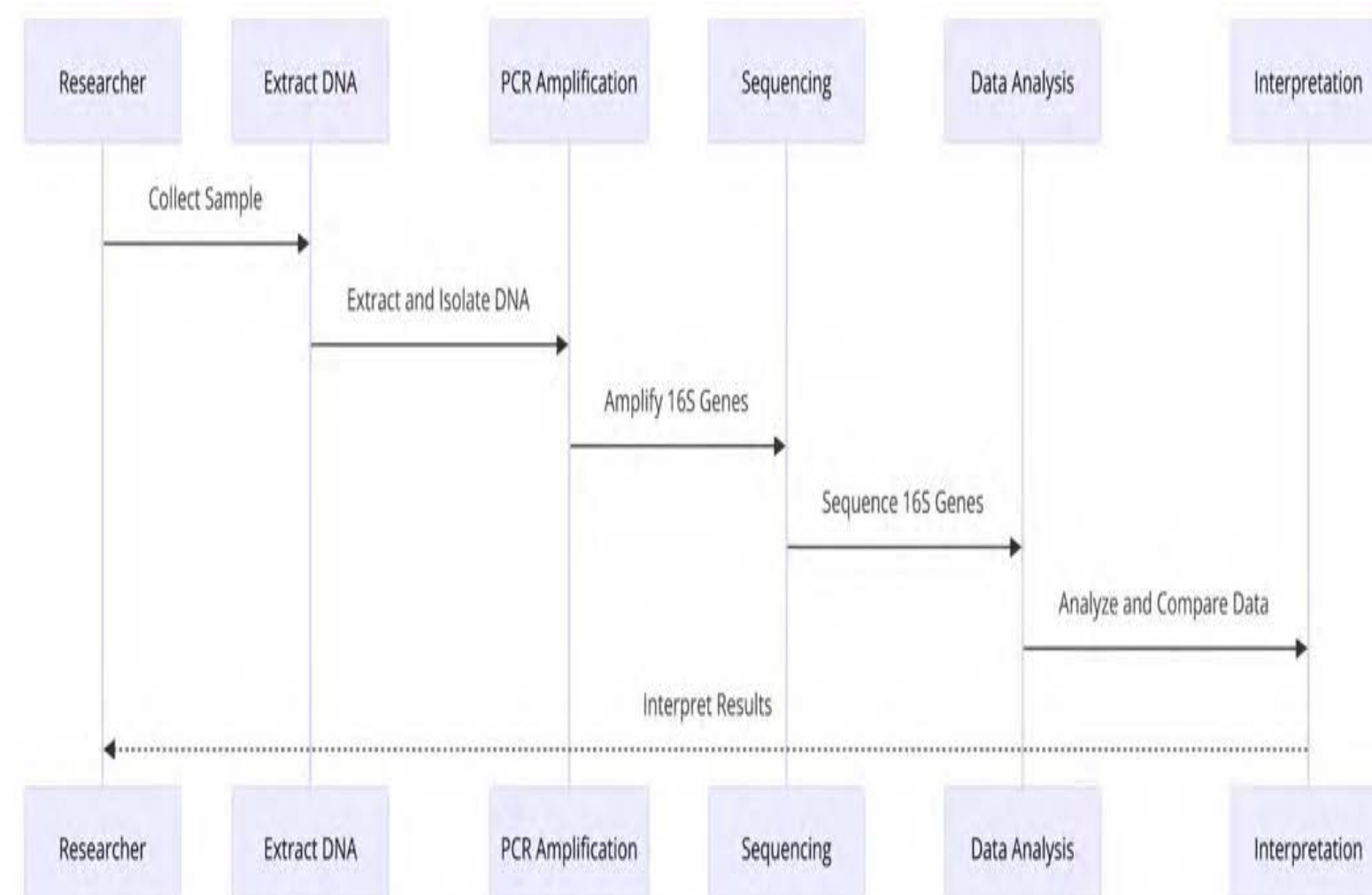
# Microalgal Application on the Composition of the Soil Microbiome

## Background

We utilized qPCR of a nitrogen fixing gene, deep sequencing of the microbial community in order to identify changes in the composition and function of the soil microbial community with and without the addition of microalgae. This provides an insight into the changes of nutrient cycling and the presence or absence of an official bacteria to plant growth. Previously, it has been found that microalgae addition results in enhanced plant productivity and a reduction in fertilizer use.

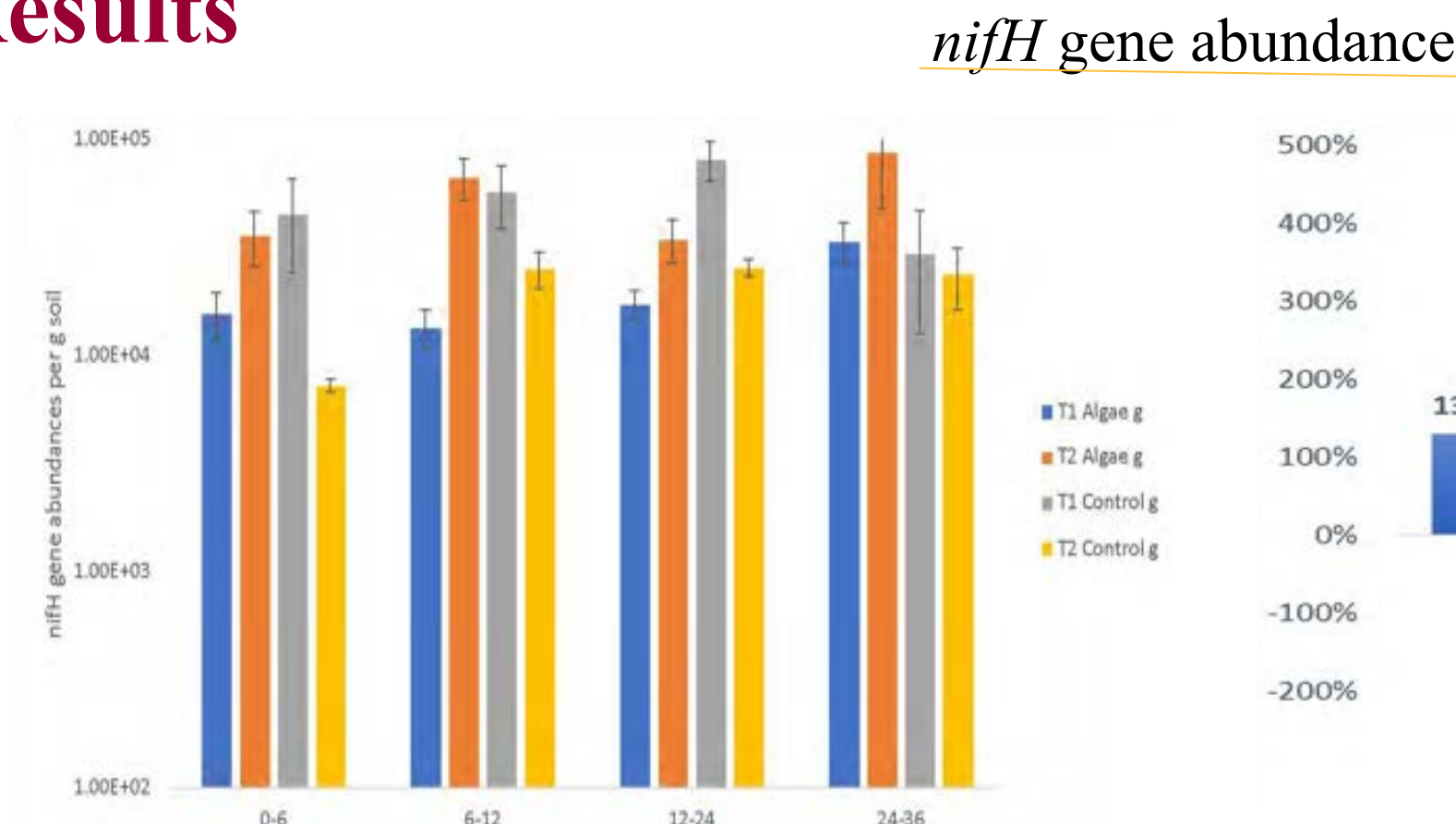
## Methods

Targeted amplicon sequencing was performed on the 16s rRNA gene which is the biomarker used to identify the taxonomic composition of the bacterial community. This was performed on the Illumina MiSeq platform utilizing 2x250 chemistry. A total of 4.1 million reads were initially obtained with 3.69 million filtered and de-noised 16S rRNA gene sequences from 78 submitted samples with an average of 47,300 sequences per sample. A total of 27,104 unique sequences were obtained, representing unique features (amplicon sequence variants, ASVs). Classification was based on the Greengenes database. Statistical analyses were performed based on Bray-Curtis dissimilarity matrices including a dummy variable after Hellinger transformation of raw sequencing data using PRIMER.



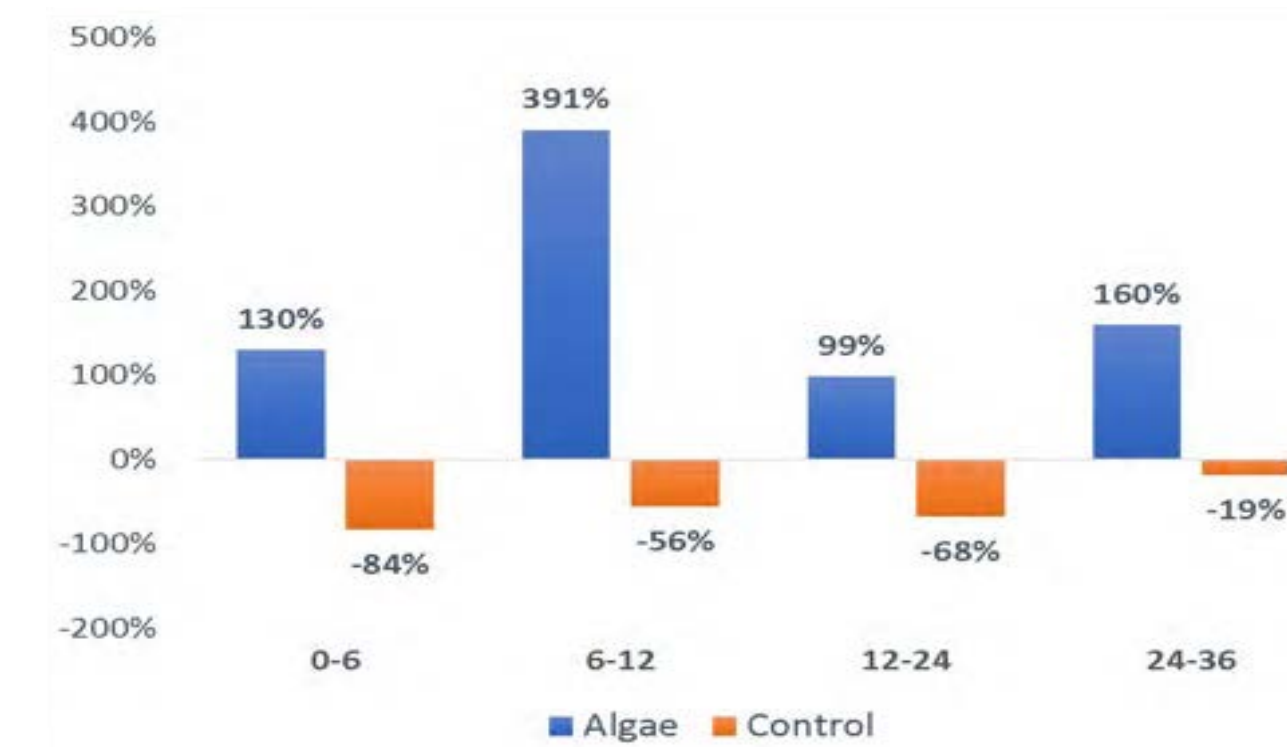
**Figure 1:** 16S rRNA sequencing is a technique utilizes PCR and sequencing technologies, like Next-Generation Sequencing, to analyze the microbial diversity. This method is crucial for understanding microbial communities in various environments and has significantly advanced our knowledge in microbial ecology.

## Results



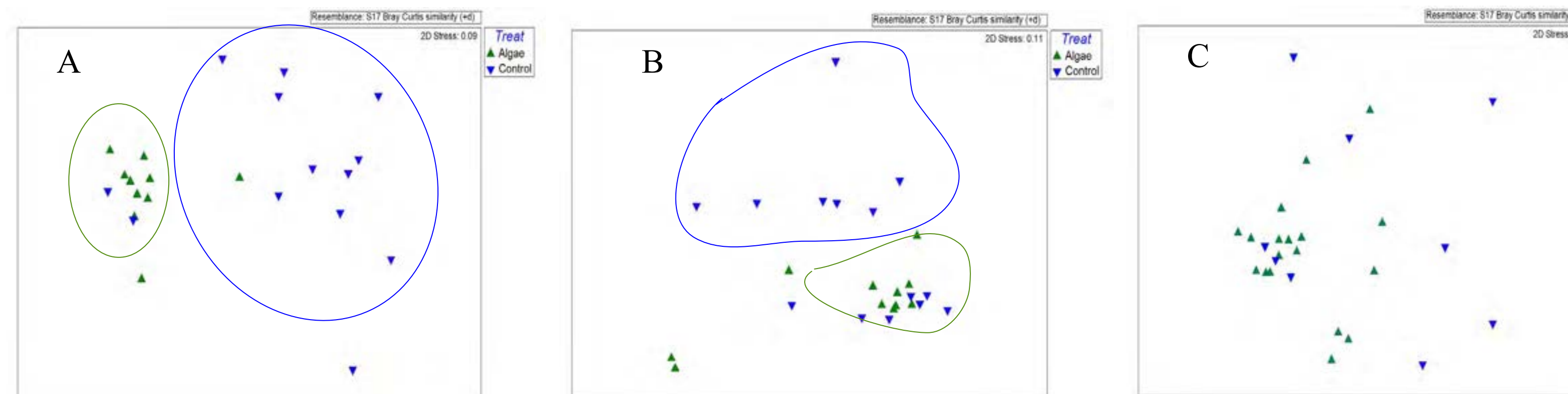
**Figure 2.** NifH gene abundance of the soil bacterial community increased with time for both the controls in the algae fields.  
- Likely due to the impact of root Rhizosphere (during crop growth).  
- Seen in surface layer 0-6 and at 24-36 the algal fields had a significant higher increase over time.

## nifH gene abundance



**Figure 3.** Percent increases or decreases in the abundances of N fixing populations over the cropping period in the algae and control treatments by depth.

## 16s rRNA gene sequencing

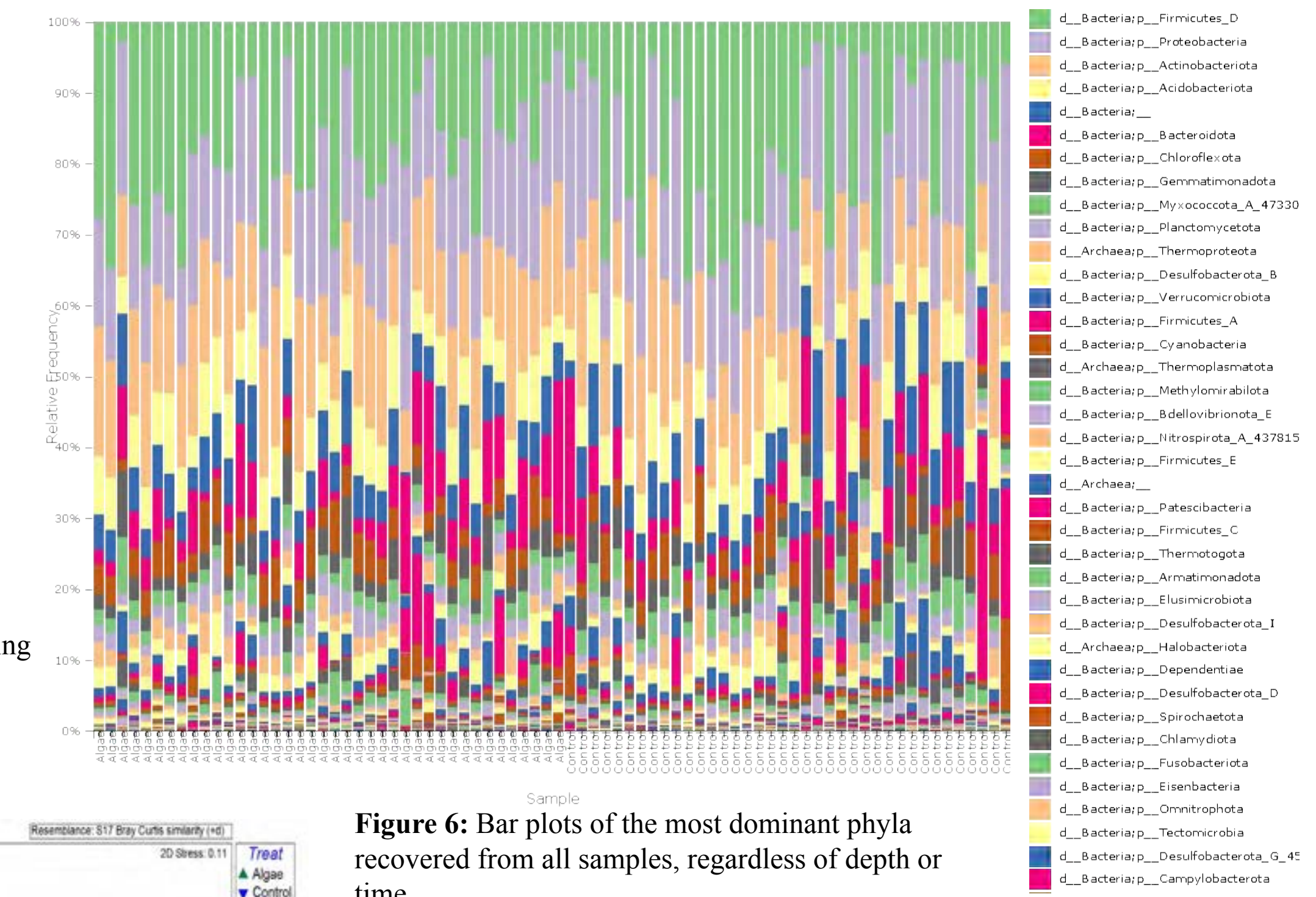


**Figure 4.** NMDS ordination of genus-level assignments at depths 0-6 (a), 6-12 (b), and 12-24 (c) inches, regardless of sampling time.

Class	Genus	Algae 0-6 in	Control 0-6 in
Methanomicrobia	Methanocorpusculum	0%	1525%
Clostridia	Uncultured Clostridia	0%	764%
Bacteroidia	Uncultured Bacteroidetes	0%	712%
Gammaproteobacteria	Uncultured Xanthomonadales	0%	702%
Bacteroidia	Uncultured Salinimicrobium	0%	628%
Acidobacteria	Uncultured Acidobacteria	0%	507%
Cyanobacteria	Tychonema	0%	385%
Abditibacteria	Abditibacterium	0%	348%
Bacteroidia	Uncultured Segetibacter	0%	330%
Gammaproteobacteria	Uncultured Burkholderia	0%	318%
Alphaproteobacteria	Uncultured Beijerinckia	0%	301%
Negativicutes	Anaeromusa	0%	232%
Clostridia	Clostridium	0%	219%
Desulfovibrionia	Desulfovibrio desulfuricans	0%	199%

Class	Genus	Algae 0-6 in	Control 0-6 in
Gammaproteobacteria	Neisseria	0%	72%
Negativicutes	Veillonella	0%	24%
Clostridia	Dorea	0%	23%
Clostridia	Unclassified Ruminococcaceae	0%	22%
Verrucomicrobia	Akkermansia	0%	21%
Clostridia	Anaerostipes	0%	20%
Clostridia	Subdoligranulum	0%	20%
Bacilli	Gemella	0%	18%
Clostridia	Blautia	0%	17%
Clostridia	Fusicatenibacter	0%	15%
Bacilli	Erysipelotrichaceae	0%	15%
Clostridia	Agathobacter	0%	15%

**Table 1:** Percent changes in the top 30 most responding genera for decreases in the algae treatment from initial to final sampling for depth 0-6 inch showing the difference in plant growth promoting bacteria.



**Figure 6:** Bar plots of the most dominant phyla recovered from all samples, regardless of depth or time.

## Discussion and Conclusion

- A significant impact of algae treatment on the composition of the microbial community, especially in surface layers (0-6 inches and 12-24 inches depth).
- Algae treatment led to a decrease in Firmicutes and Actinobacteria and an increase in Verrucomicrobia and Gemmatimonadetes, indicating a shift in the microbial gene pool.
- The impact was significant from the phylum to the genus level.
- This suggests a strong selection for PGPR due to the algae treatments.
  - Particularly for Bacilli who are known to be very potent plant growth promoting bacteria, that can fix Nitrogen, reduce plant stress and inhibit pathogens.

- The increase in genera responsible for nitrification and nitrogen fixation, like Nitrososphaeria and Microvirga, correlates with potentially enhanced nitrogen cycling and fixation in algae-treated soil.

- Evidence that algae treatment alters the soil microbiome, enriching certain beneficial microbial groups and potentially enhancing soil fertility and plant growth.

## Acknowledgements

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