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Diversity of Nitrogen Fixing Bacteria in a Sample Aquaponics Ecosystem

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Objective

In this study, we characterize the community composition of nitrogen-fixing bacteria present in a nitrogen-rich aquaponics ecosystem.

Introduction

- In **aquaponics**, fish waste from ponds or aquariums is used to fertilize cultivated plants.
- **Nitrifying** bacteria in the system convert ammonia found in the fish waste to nitrates and nitrites (figure 1).
- **Nitrates** and **nitrites** are two forms of nitrogen that are biologically available to plants (figure 1).
- **Nitrogen fixing bacteria** source their nitrogen from the atmosphere through respiration. These species use **nitrogenase** to cleave atmospheric nitrogen. Nitrogen fixing bacteria are commonly found in the rhizosphere.
- The **rhizosphere** is made up of roots and surrounding dirt.
- **USM's aquaponics gardens** uses a two-step microbial cultivation system before waste is introduced to the gardens.
 - 1. An **anaerobic** habitat where fish waste is first introduced.
 - 2. An **aerobic** habitat that is fed water from the anaerobic habitat.
- Anaerobic systems have been found to eliminate the production of nitrates but have a minimal production of compounds containing soluble reactive phosphorus relative to those found in aerobic conditions (Monsees et. al. 2017).

Methods

- **Sampling**
 - Water from aquaponics beds and aquariums
 - Aquaponics rhizosphere
 - Legume and clover rhizosphere from conventional garden (for comparison)
- **Microscopy**
 - Nitrogen-free substrates
 - Lowenstein-Jensen media
 - Lowenstein-Jensen broth
 - *Azotobacter* media
 - Nitrogen-containing control substrates
 - Lowenstein-Jensen control media
 - TSA media
 - Isolation streaks
- **Polymerase chain reaction (PCR)** (figure 3)
 - A form of genomic analysis
 - Nitrogenase primer
 - 16S DNA primer
- **Metagenomics** (figure 4)

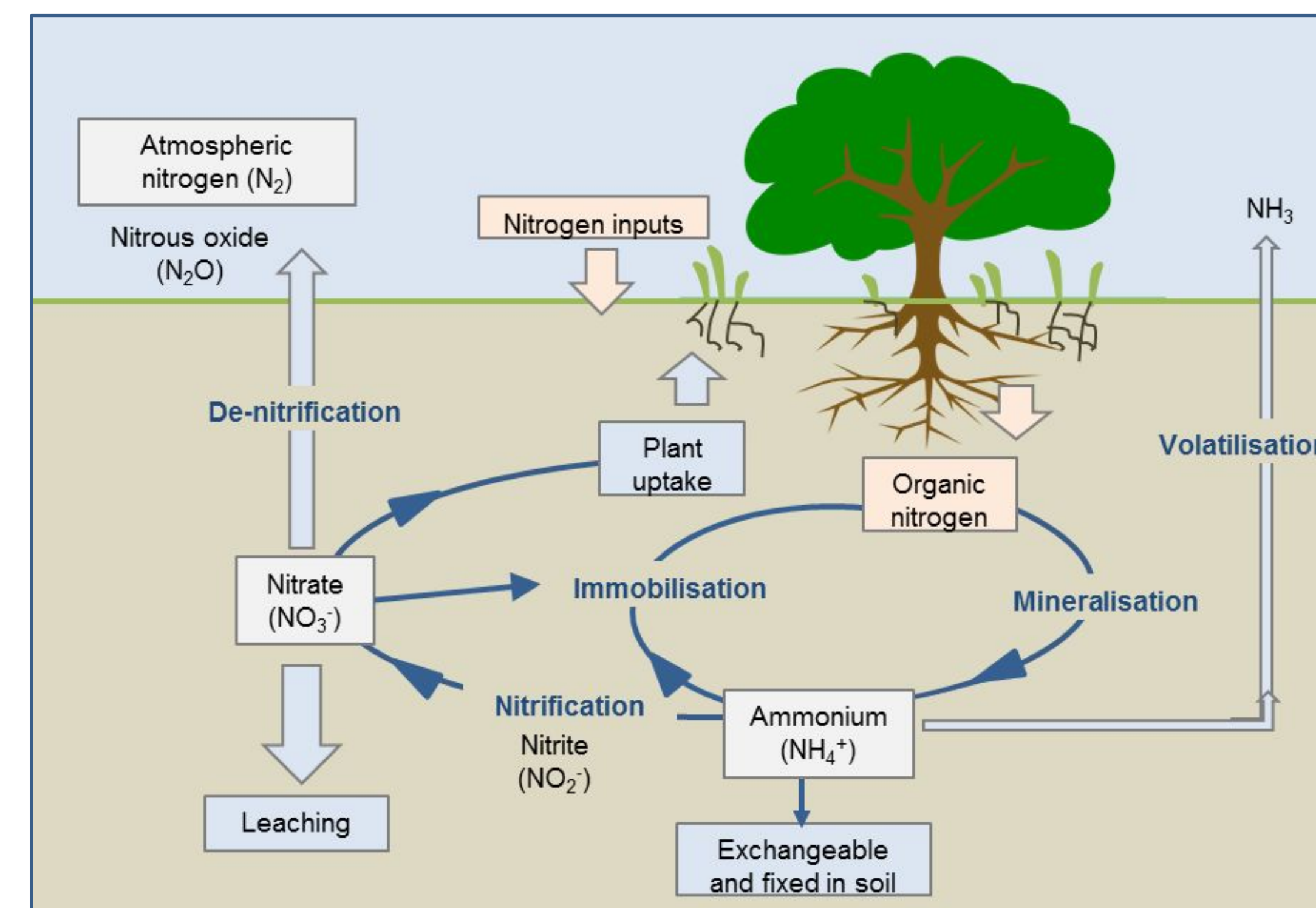


Figure 1. Overview of the nitrogen cycle
Source: Hoyle et. al. 2017



Figure 2.
Bacterial colonies that strongly resemble *Azotobacter* from USM's aquaponics system

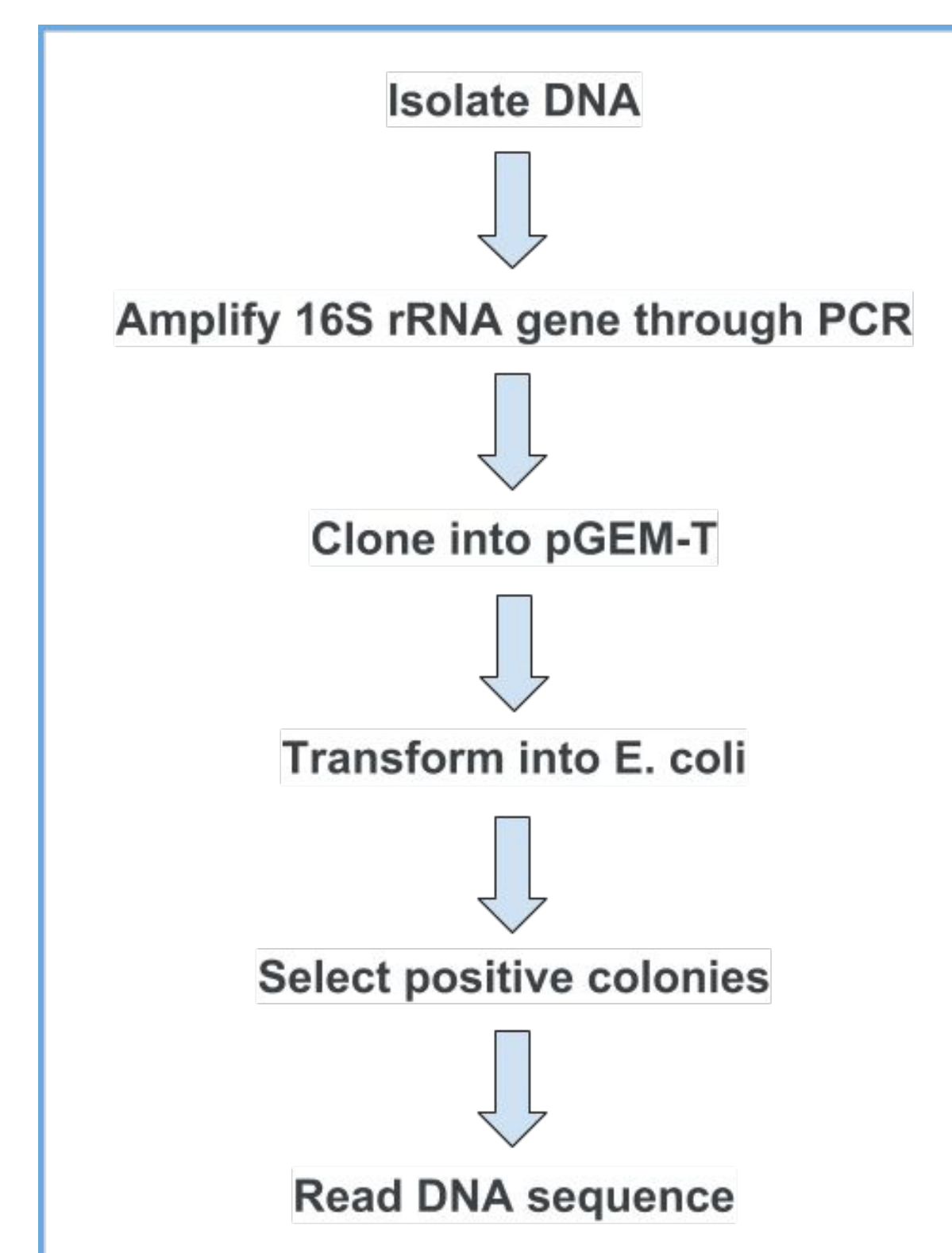


Figure 4.
Overview of the process of metagenomics.

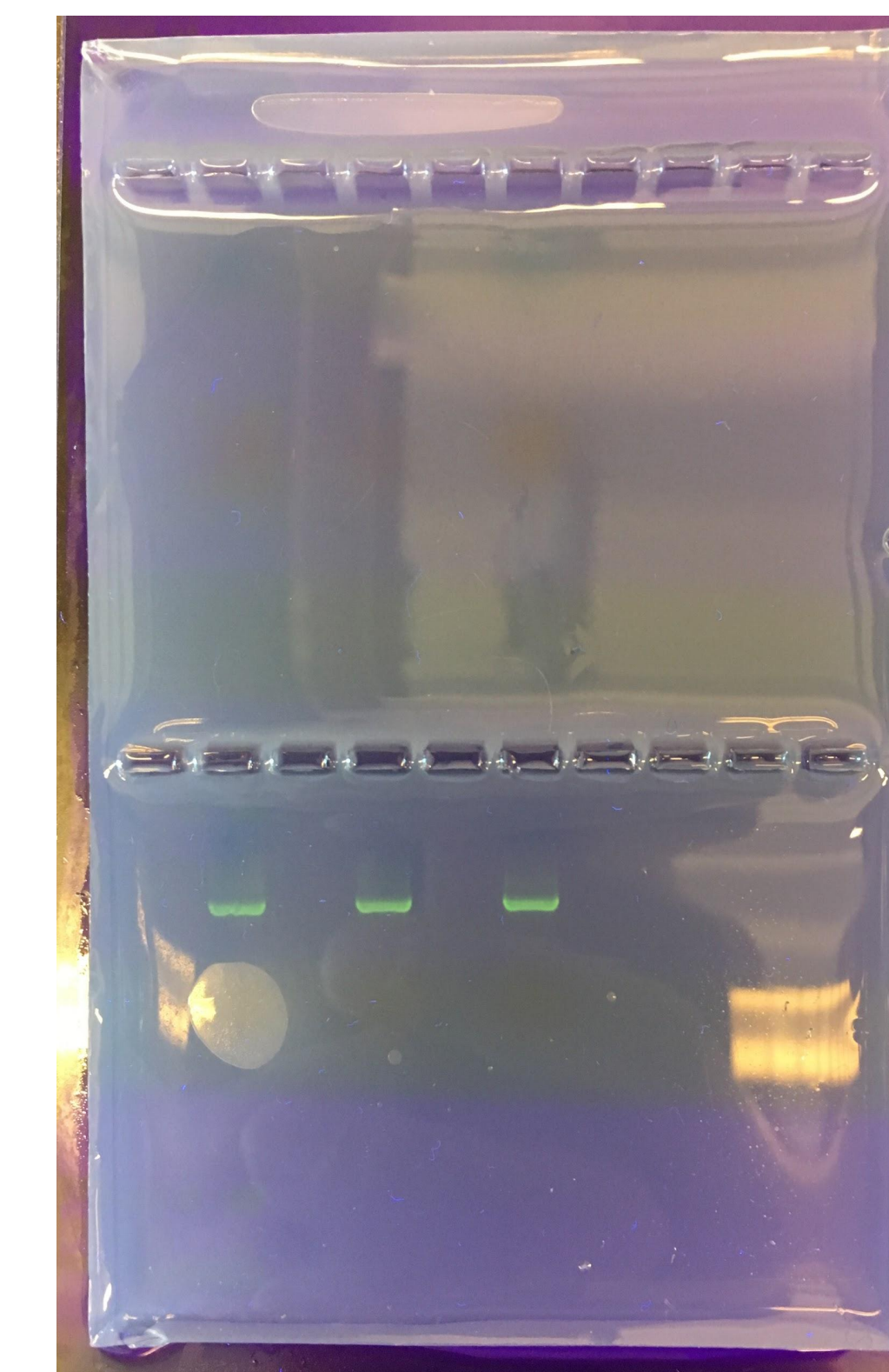


Figure 3.
PCR analysis gel. Samples analyzed via nitrogenase primer (top row) and via 16s primer (bottom row)

Results

- At least two distinct phenotypes were successfully cultured on nitrogen-free media
- Colony morphology suggests that one of these species is *Azotobacter* spp. The other species, "unknown A", remains unidentified.
- *Azotobacter* spp. presented as raised, off-white to yellow colonies with a large volume of mucus/slime production (figure 2), which turned black over time.
- Unknown A presented as tiny, clear, water-droplet shaped colonies.
- *Azotobacter* spp. was grown on plates inoculated with soil samples, while Unknown A was grown on plates inoculated with water samples.
- Metagenomic analysis revealed the presence of at least one species of *Flavobacterium*, likely *F. columnare*, a non-diazotrophic fish-pathogen. Thus far, no nitrogen-fixing bacteria have been identified through metagenomic analysis.

Discussion/Conclusion

Previous research has shown that the introduction of diazotrophic bacteria can have a positive effect on the activity of nitrifying bacteria and can improve overall plant growth in hydroponic systems (Ke, 2019). Research investigating bacterial communities in aquaponics systems is very limited, but it is likely that similar interspecies interactions are occurring in these systems. If this is the case, the presence/diversity of nitrogen-fixing bacteria could have a major impact on the efficiency of nitrification and the overall health of microbial communities in these systems. We recommend that the effects of inoculation with nitrogen-fixing bacteria be studied in these systems.

Our findings indicate that nitrogen-fixing bacteria are indeed present in all areas sampled. Further, the differences between phenotypes grown from water samples vs. soil samples indicates that these areas may be home to distinct communities of nitrogen fixing bacteria. Interestingly, root samples appear to contain both of these distinct phenotypes. There are two possible reasons for this: First, plant roots represent a source of carbohydrates and may be the preferred environment for many diazotrophic organisms; second, plant roots share features of both the soil and aquatic environment - that is, they present a solid surface for attachment and in many cases are fully submerged in water. Finally, preliminary metagenomic analysis revealed the presence of multiple diazotrophic species which were not grown on nitrogen-free media, indicating that many members of this community are non-culturable, at least under the conditions used in our experiments.

In conclusion, we have determined that there are indeed nitrogen-fixing bacteria present in these aquaponic systems and that there may be distinct differences in the communities found in soil, water, and plant roots. While no diazotrophic species have been identified through partial metagenomic analysis, we hope that by analyzing more/fresher samples we will be able to identify diazotrophic bacteria in these systems.

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