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Concentrated Media Inoculant for Food Production Using Metagenomics

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Abstract

A great wealth of information is currently available regarding the role of microbial flora in the various functions that occur in an aquaponics system. It is necessary to begin identifying the microbes present in aquaponics systems and compare those to populations of microbes that are important in nutrient cycling, plant health, and fish health to optimize production within the system. We examine microbes that interact with plant health. Studies have been conducted to evaluate the food safety aspects of aquaponics produce, specifically assessing the risk of foodborne pathogens in an aquaponics system. This study is a continuation of that evaluation. We investigated whether transfer of microbial flora occurs when microbes are or are not present in the media where Romaine lettuce is raised. We found that the diversity of microbial flora is similar regardless of the type of media in which the plants are grown, and if there is a difference of significance, it is a rare event. Therefore, aquaponics systems are safe for growing plants.

Keywords

Aquaponics, Microbial Flora, Microbiome, Metagenomics

1. Introduction

Aquaponics is based on the principles of a biologically closed-loop recirculating system; thus, it is an ecosystem [1] [2]. A basic aquaponic system supports fish, microorganisms, and plants in connected tanks (**Figure 1**). The fish provide waste as a nutrient source to support biological activity in rearing tanks [1] [2]. The fish waste undergoes clarification and then conversion with the use of beneficial microorganisms for usable nutrient uptake by plants (**Figure 1**). A multitrophic relationship is active in an aquaponics system where balance is achieved through a self-regulated, stable ecosystem [1]. We propose using meta-genomic next-gener-

ation DNA sequencing (NGS) techniques to characterize populations of microbes in aquaponics systems that may affect the composition of plants grown in aquaponic systems. Metagenomic analyses use DNA sequences to identify the microbes, unlike traditional culture-based techniques. Less than 1% of microbes are estimated to be culturable in a laboratory setting; therefore, determining the microbial community composition based on DNA is a more robust method of studying microbial populations.

Metagenomic analysis offers a cutting-edge, robust method of analyzing and characterizing the "aquaponics microbiome." We investigate the transfer of microbial organisms present in Romaine lettuce grown in completely individual aquaponics systems in a randomized block design. Here, we identify the upregulation or downregulation of microbial flora based on different treatments composed of media containing different initial concentrations of microbial loads in media beds where plants are raised over three months.

A great wealth of information is currently available regarding the role of microbes in the various functions that occur in an aquaponics system. It is necessary to begin identifying the microbes present in aquaponics systems and compare those to populations of microbes that are important in nutrient cycling, plant health, and fish health to optimize production within the system. We examine microbes that interact with plant health. Studies have evaluated food safety aspects of aquaponics produce, specifically evaluating the risk of foodborne pathogens in an aquaponics system [3] [4]. This study is a continuation of that evaluation.

Microorganisms play a key role in an aquaponics system. They are mainly involved in converting potentially toxic ammonia excreted from fish through nitrification into nitrate from nitrite. As observed in soil, microorganisms in an aquaponic system benefit plants [5] [6]. Studies such as this focus on understanding the complexity of microbial communities present in different compartments of an aquaponic system. The separate units, composed of various concentrations of microorganisms, are also studied, and they are present and available for plants grown in the media.

Sanchez *et al.* (2019) identified bacterial strains from aquaponic systems that could produce siderophores and ammonia and solubilize phosphorus. In our study, we followed the evolution of microbial communities over three months, collecting lettuce at the end of each month after initial planting at the beginning. Microbial communities were characterized with NGS 16S rRNA sequencing.

2. Experimental Procedure

This was a randomized complete block designed study where treatments were defined as those with 0%, 25%, 50%, and 75% application of stones that compose the media beds that had been inoculated with microbial flora over an extended period (up to 3 years) in an enlarged fish tank containing recirculating populations of tilapia. The location of the study was in Green-house 2 on the Main farm of Olive Branch Aquaponics LLP. The temperature was maintained at 82 - 86˚ F. These

fertilized media were divided into four aquaponic systems and four areas to complete a randomized block design study [7] [8].

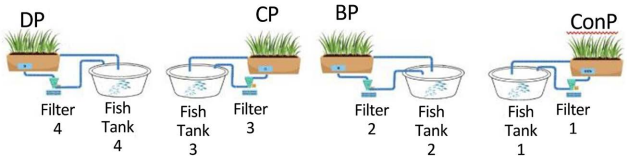


Figure 1. A randomized complete block design of aquaponics media beds was set up for a 0% microbial load Control (ConP), 25% microbial load (BP), 50% microbial load (CP), and 75% microbial load (DP). Each bed was completely independent, functioning in its system.

Specific Objectives

The study examined the meta-genomic conditions of plants (*Lactuca sativa,* Romaine lettuce) in an aquaponic system under controlled environmental conditions. A system was used to record the following parameters in each aquaponic system, where in aquaponic Fish Tank #1-4, aquaponic Filter #1-4, aquaponic Plant beds Con, B, C, and D, monthly for three months for each set of representative samples of individual plant beds**.** Immediate preservation of the collected plant tissue was completed in 1.5 ml centrifuge tubes, labeled, transported in liquid nitrogen, and stored in a −80˚C freezer until processing.

There were 160 tilapias divided equally into four individual aquaponic units. Each aquaponic unit had 40 fish placed in the system's tank. The tilapia used began at the fingerling stage of their developmental age. The tilapias were left to maintain the health of the aquaponic system at an equal level per replication.

Romain lettuce, including roots and a portion of the stem and leaves, was collected monthly at a prescribed time (30 days post planting (dpp), 28 dpp, and 30 dpp).

Laboratory work was completed where nucleic acids were extracted from portions of samples for metagenomic sequencing. Modified Norgen Biotek Microbiome kits (Thorold, Ontario, Canada) were used for nucleic acid extractions.

Sequencing Library Preparation

All cDNA and DNA libraries proceeded through sequencing library preparation procedures using slightly modified kit protocols. Integrated DNA Technology (IDT) XGen library kit preparation (Oklahoma Medical Research Foundation (Oklahoma City, OK)) for DNA samples used to prepare samples.

Bioinformatic Analysis

Differences in procedures will require different types of bioinformatics techniques. For example, the proprietary software suite Partek Flow now a part of Illumina (San Diego, CA) will be used where Kraken software identified OTU community structure, Principal Component Analysis charts and Differential analysis tools such as Kruskal Wallis were used to more descriptively show the activity of microbial community patterns in the aquaponic units. Downstream analysis was completed for operational taxonomic units (OTUs) classification and alpha-beta diversities.

3. Results

Despite the visual indication of high production growth in the zero% media bed as compared to the other media beds, when conducting differential analysis on the samples and comparing them we obtained no significant difference between the media bed with zero% inoculant versus 25% inoculant, zero% inoculant versus 50% inoculant and zero% inoculant versus 75% inoculant.

All the media beds appear to share a similar relative abundance of microbial flora (Figure 2).

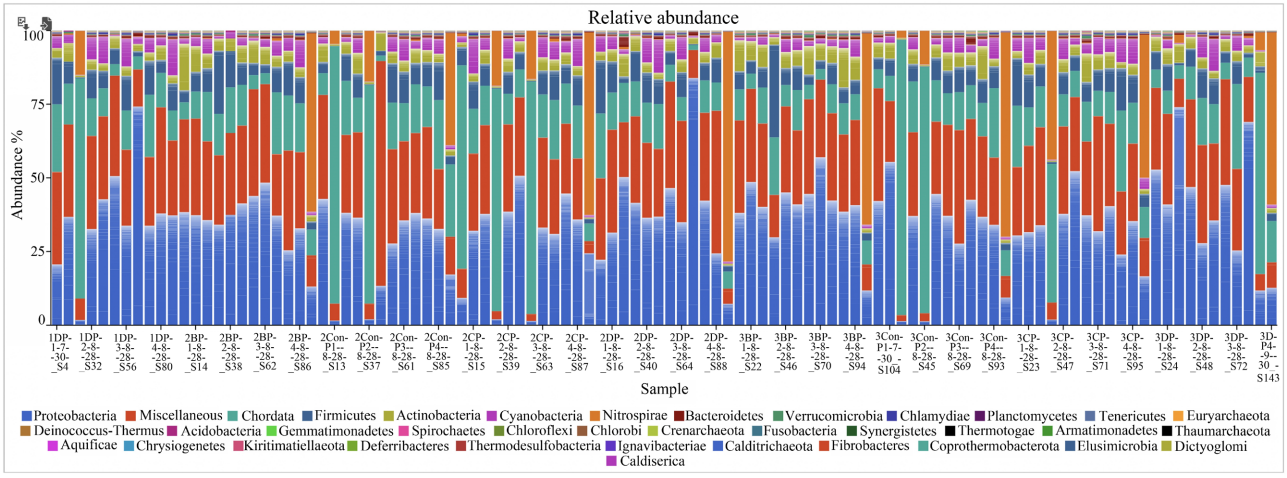
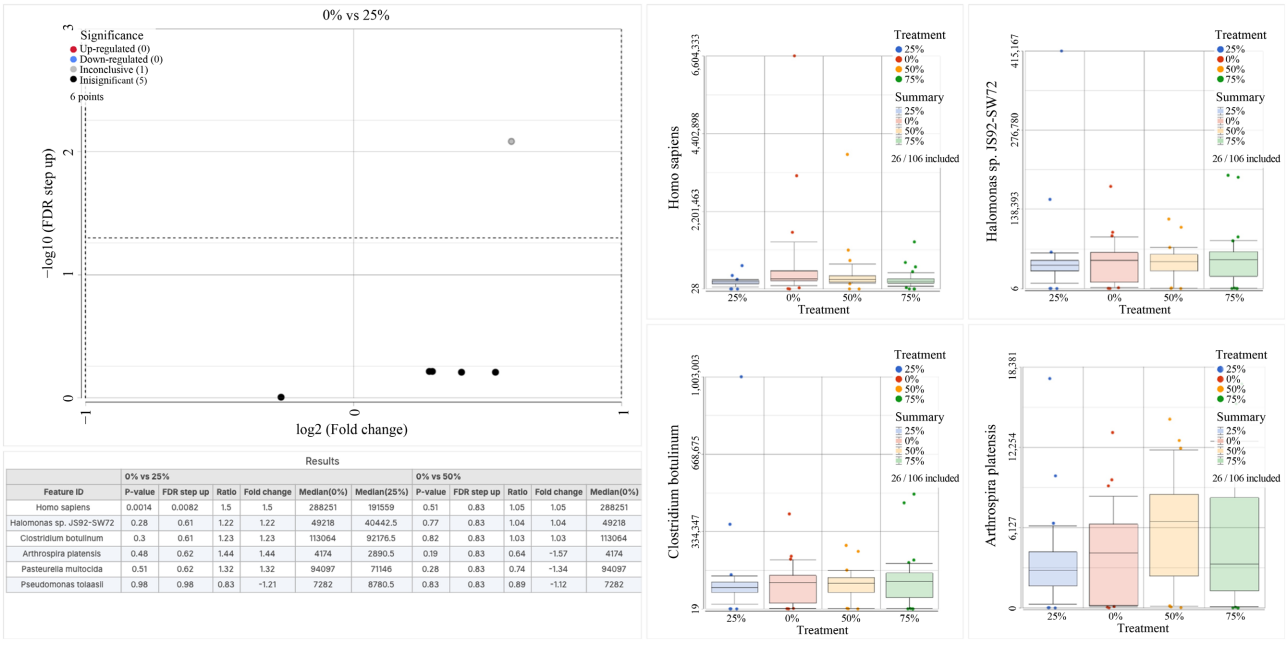


Figure 2. Individual samples from the control (CONP) media bed, the media bed with 25% microbial load (BP), the 50% microbial load (CP), and the 75% microbial load or exposure (DP) share a similar diversity of microbial flora.



Results

	0% vs 25%						0% vs 50%				
Feature ID	P-value	FDR step up	Ratio	Fold change	Median(0%)	Median(25%)	P-value	FDR step up	Ratio	Fold change	Median(0%)
Homo sapiens	0.0014	0.0082	1.5	1.5	288251	191559	0.51	0.83	1.05	1.05	288251
Halomonas sp. JS92-SW72	0.28	0.61	1.22	1.22	49218	40442.5	0.77	0.83	1.04	1.04	49218
Clostridium botulinum	0.3	0.61	1.23	1.23	113064	92176.5	0.82	0.83	1.03	1.03	113064
Arthrospira platensis	0.48	0.62	1.44	1.44	4174	2890.5	0.19	0.83	0.64	-1.57	4174
Pasteurella multocida	0.51	0.62	1.32	1.32	94097	71146	0.28	0.83	0.74	-1.34	94097
Pseudomonas tolaasii	0.98	0.98	0.83	-1.21	7282	8780.5	0.83	0.83	0.89	-1.12	7282

Figure 3. Comparison of zero% inoculant with 25% inoculant in media beds where Romaine lettuce was grown.

There was no significant presence of microbial contaminants in the Romaine

lettuce when comparing zero% inoculant with 25% inoculant in the media where the Romaine lettuce was growing (Figure 3).

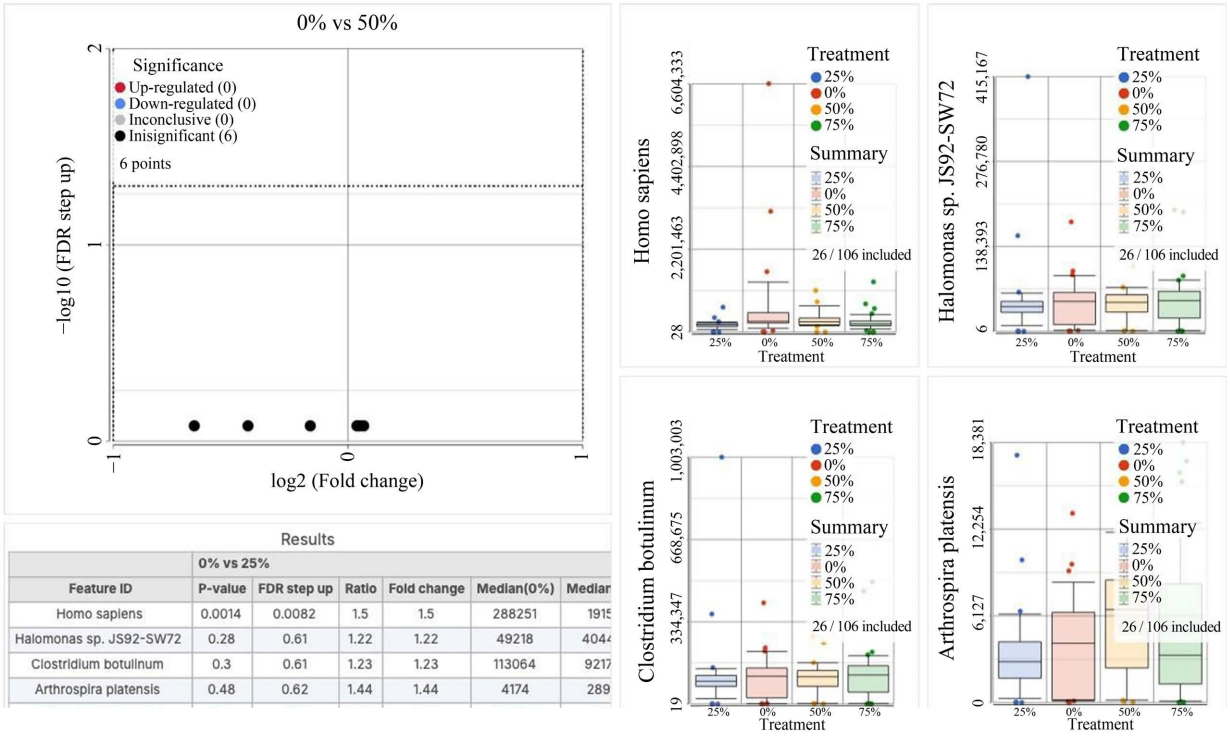


Figure 4. Comparison of zero% inoculant with 50% inoculant in media beds where Romaine lettuce was grown.

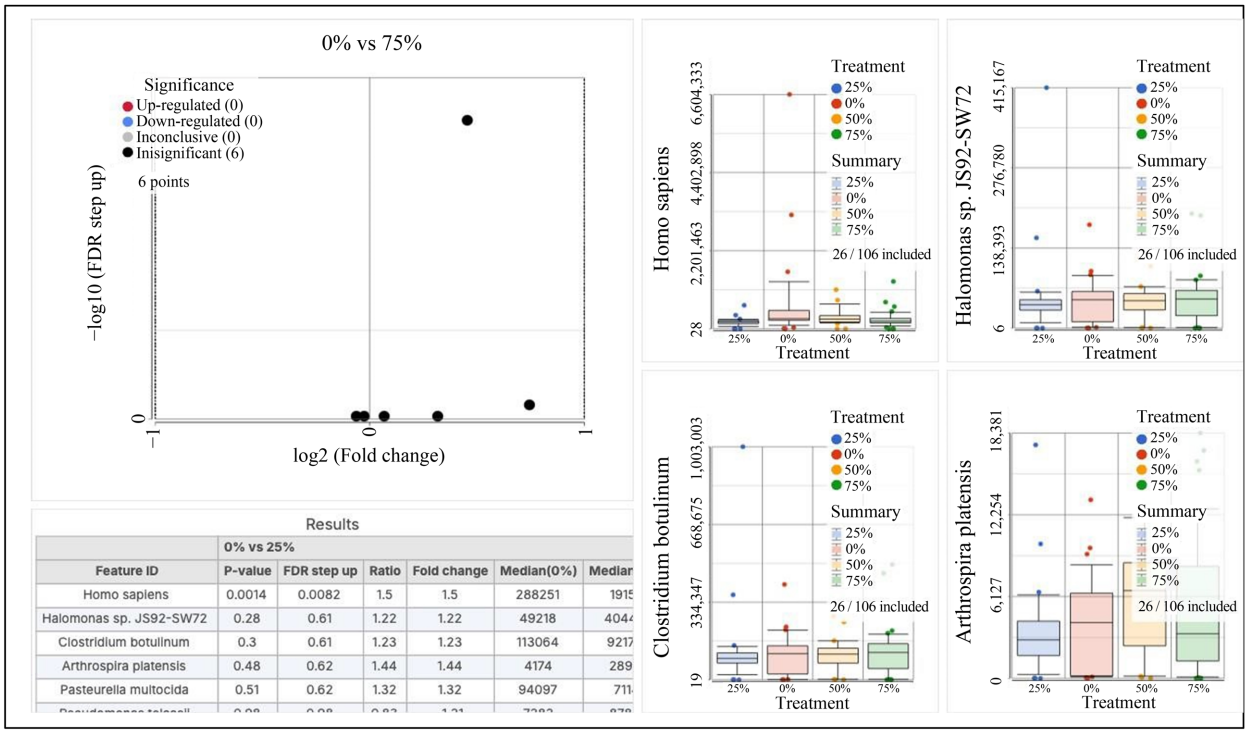
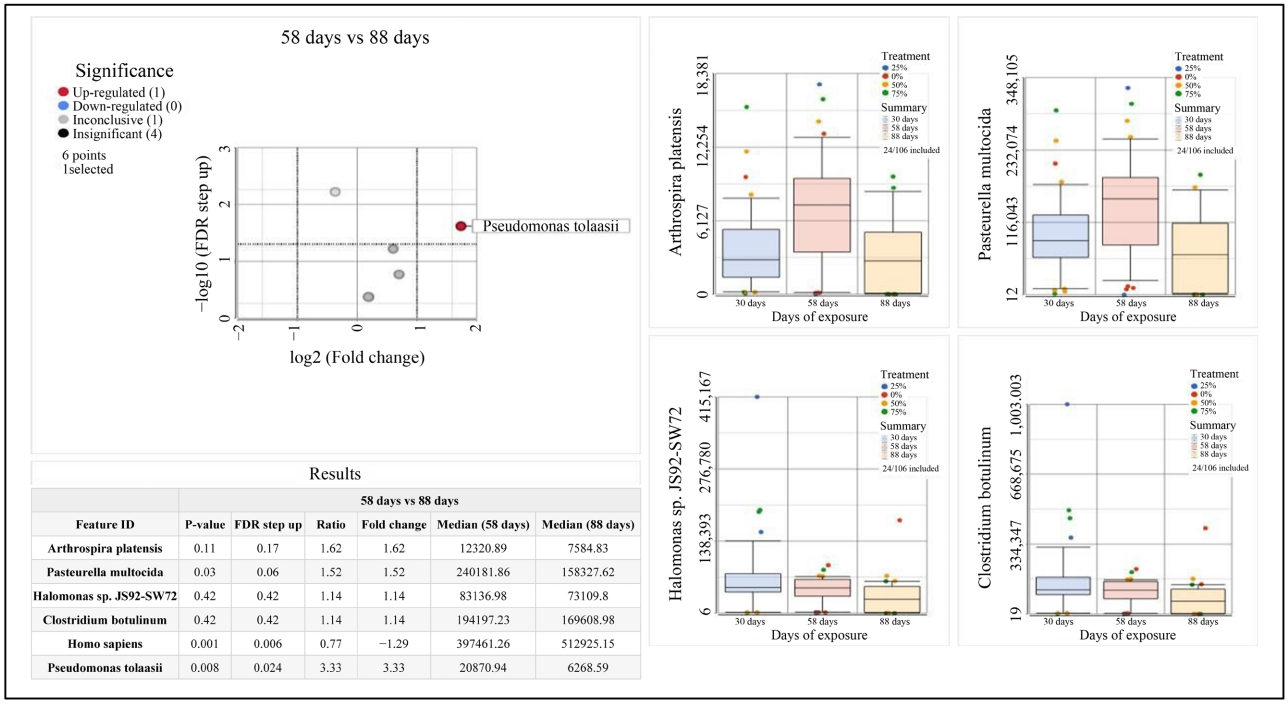


Figure 5. Comparison of zero% inoculant with 75% inoculant in media beds where Romaine lettuce was grown.

There was no significant presence of microbial contaminants in the Romaine lettuce when comparing zero% inoculant with 50% inoculant in the media where the Romaine lettuce was growing (Figure 4).

There were no significant presence of microbial contaminants in the Romaine lettuce when comparing zero% inoculant with 75% inoculant in the media where the Romaine lettuce was growing (Figure 5).

The only significant difference in terms of operational taxonomic units (OTUs) is when comparing 58 days versus 88 days of growth (Figure 6).



Results

	58 days vs 88 days					
Feature ID	**P-value**	**FDR step up**	**Ratio**	**Fold change**	**Median (58 days)**	**Median (88 days)**
Arthrospira platensis	0.11	0.17	1.62	1.62	12320.89	7584.83
Pasteurella multocida	0.03	0.06	1.52	1.52	240181.86	158327.62
Halomonas sp. JS92-SW72	0.42	0.42	1.14	1.14	83136.98	73109.8
Clostridium botulinum	0.42	0.42	1.14	1.14	194197.23	169608.98
Homo sapiens	0.001	0.006	0.77	−1.29	397461.26	512925.15
Pseudomonas tolaasii	0.008	0.024	3.33	3.33	20870.94	6268.59

Figure 6. Indication of significant presence of microbial contaminants in the Romaine lettuce when comparing zero% inoculant with 75% inoculant in the media where the Romaine lettuce was growing was only found in the comparison of 58 days and 88 days. *Pseudomonas tolaasii* was identified as the single OTU upregulated in Romaine lettuce between the two days of exposure.

4. Discussion

There have been many speculations as to whether harmful components of fish waste are transferred to plants in an aquaponic system. In the period between 1998 and 2015, a review of the data of the Centers for Disease Control and Prevention of the United States found 857 foodborne disease outbreaks associated with fish, resulting in 4814 illnesses, 359 hospitalizations, and four deaths. Most hospitalizations were caused by *Salmonella* spp. (31%) and ciguatoxin (31%). Here, we find no significant presence of microbial contaminants transferred to Romaine lettuce except for *Pseudomonas tolaasii,* which is manageable if proper hygiene and production management are followed.

We examined if there was any significant presence of microbial flora by analyzing the microbiome of Romaine lettuce following different concentrations of exposure of microbial inoculants to plants in individual aquaponic units. We exam-

ined whether zero percent inoculum would indicate less presence of microbial flora compared to 25%, 50%, and 75% exposure of microbial inoculum to the plants and fish waste in each individual system. We identified that although there were increased inoculum concentrations and visually increased biomass of Romaine lettuce in the zero% inoculum aquaponic bed, there was only one indication that microbial flora was significantly upregulated.

5. Conclusion

We surmise that there is no significant transfer of microbial flora to plants despite the presence of microbial flora in media beds and waste from fish in aquaponics systems [9]. We gather this conclusion from the conclusion of differential analyses, principal component analyses, alpha and beta diversity analyses, and additional use of statistical analyses as shown above. Further studies may be warranted to strengthen the conclusion of this manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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