



Chicken manure-based bioponics: Effects of acetic acid supplementation on nitrogen and phosphorus recoveries and microbial communities

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ABSTRACT

Bioponics has the potential to recover nutrients from organic waste streams, such as chicken manure and digestate with high volatile fatty acid (VFA) contents through crop production. Acetic acid, a dominant VFA, was supplemented weekly (0, 500, 1000, and 1500 mg/L) in a chicken manure-based bioptic system, and its effect on the performance of bioponics (e.g., plant yield and nitrogen and phosphorus availabilities) was examined. Microbial communities were analyzed using 16S rRNA gene sequencing, and the functional gene abundances were predicted using PICRUSt. Although acetic acid negatively affected plant yield, no significant difference ($p > 0.05$) was noted in the average nitrogen or phosphorus concentration. In terms of nutrient recovery, the bioptic systems still functioned well, although higher concentrations of acetic acid decreased plant yield and altered the bacterial communities in plant roots and chicken manure sediments. These data suggest that an acetic acid concentration of < 500 mg/L or a longer loading interval is recommended for the effective operation of chicken manure and digestate-based bioponics.

1. Introduction

Bioponics is the next revolution of soilless agriculture in which crops can be produced from nutrient-rich waste streams, without the use of chemical fertilizers. Bioponics both recovers nutrients for vegetables and other crops from nutrient-rich waste products (e.g., aquaculture effluent, untreated wastewater, effluent from secondary wastewater treatment, diluted urine, sludge, food waste composts, and manure) and remediates waste through the symbiotic actions of microorganisms and plants (Wongkiew et al., 2021a,b). However, only aquaponics—a type of bioponics that uses aquaculture effluent as a nutrient source—has been commercially applied because of a well-established techno-economic analysis of the system (Love et al., 2015; Wongkiew et al., 2017). Chicken manure-based bioponics has the potential to become the next generation of soilless agriculture technology for producing crops with

yields comparable with those obtained using aquaponic and soil-based agriculture systems (Wongkiew et al., 2021b). The major challenge with chicken manure-based bioponics is the integration of different forms of chicken manures (e.g., fresh, dry, anaerobically digested, and composted chicken manure) with bioponics. To date, only dry chicken manure has been successfully used in bioptic vegetable production (Wongkiew et al., 2021b). Thus, further studies are needed to incorporate the other forms of chicken manure with bioponics.

One of the primary nutrient-rich wastes from agricultural industries (e.g., poultry and swine) is the digestate from the anaerobic digestion process, which is particularly rich in ammonium and phosphate (Möller and Müller, 2012; Sharma et al., 2017). Owing to its high nutrient contents, anaerobic digestate must be treated before disposal into the environment. In this context, anaerobic digestate has a high potential to be a nutrient source when integrated with bioponics. However,

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anaerobic digestate also contains residual volatile fatty acids (VFAs), primarily acetic acid, which may inhibit the growth of both microbes and plants in the bioponic systems (Allen and Allen, 2020; Li et al., 2020a; Tenuta et al., 2002). Moreover, studies on nitrogen and phosphorus recovery from a bioponic system integrated with a VFA-rich nutrient source (e.g., digestate) are limited (Page et al., 2014; Sharma et al., 2017; Yin et al., 2021). Therefore, for the effective incorporation of nutrient-rich waste streams such as chicken manure digestate, the performance of bioponics under various acetic acid concentrations should be evaluated.

Microorganisms in bioponics play significant roles in nutrient recovery such as organic waste degradation to maintain good water quality as well as nutrient mineralization and transformation for plant uptake (Cáceres et al., 2018; Sharma et al., 2017). Because bioponics is a microbially mediated process, understanding microbial functions, key biomarkers, and microbial interactions are critical for the evaluation and improvement of the performance of bioponic systems. Studies on microbiome, such as those on microbial community compositions, functional gene predictions, microbial co-occurrence networks, discriminant analyses, and correspondence analyses, reveal microbial insights into bioponic systems (Hu et al., 2017; Langille et al., 2013; Segata et al., 2011). For example, co-occurrence microbial networks can identify keystone taxa and symbiotic interlinkages, and discriminant

analysis can reveal microbial biomarkers that are significantly predominant at specific conditions (Segata et al., 2011; Tan et al., 2020). Thus, a microbiome study, when incorporated with performance evaluation, may provide strategies to improve bioponic systems integrated with acetic acid-rich nutrient sources (Bao et al., 2021).

The overarching goal of this study was to evaluate the performance of chicken manure-based bioponic systems containing varying concentrations of acetic acid in combination with a microbiome study. This study aimed to (1) investigate nitrogen and phosphorus transformations and bioponic performances based on acetic acid concentrations and (2) evaluate the microbial communities and predict the microbial genes associated with bioponic performance. To evaluate the effects of acetic acid concentrations on the performance of bioponic systems, different acetic acid concentrations were supplemented in dry chicken manure-based bioponic systems each week, to maintain the initial target concentrations. The novelty of this study lies in the preassessment of the bioponic system to integrate with waste containing high acetic acid concentrations, identify ecology, and predict metabolic functions of the microbiota present in the bioponic systems. This study provides information and strategies for integrating anaerobic digestate and raw manure influent in bioponic systems.

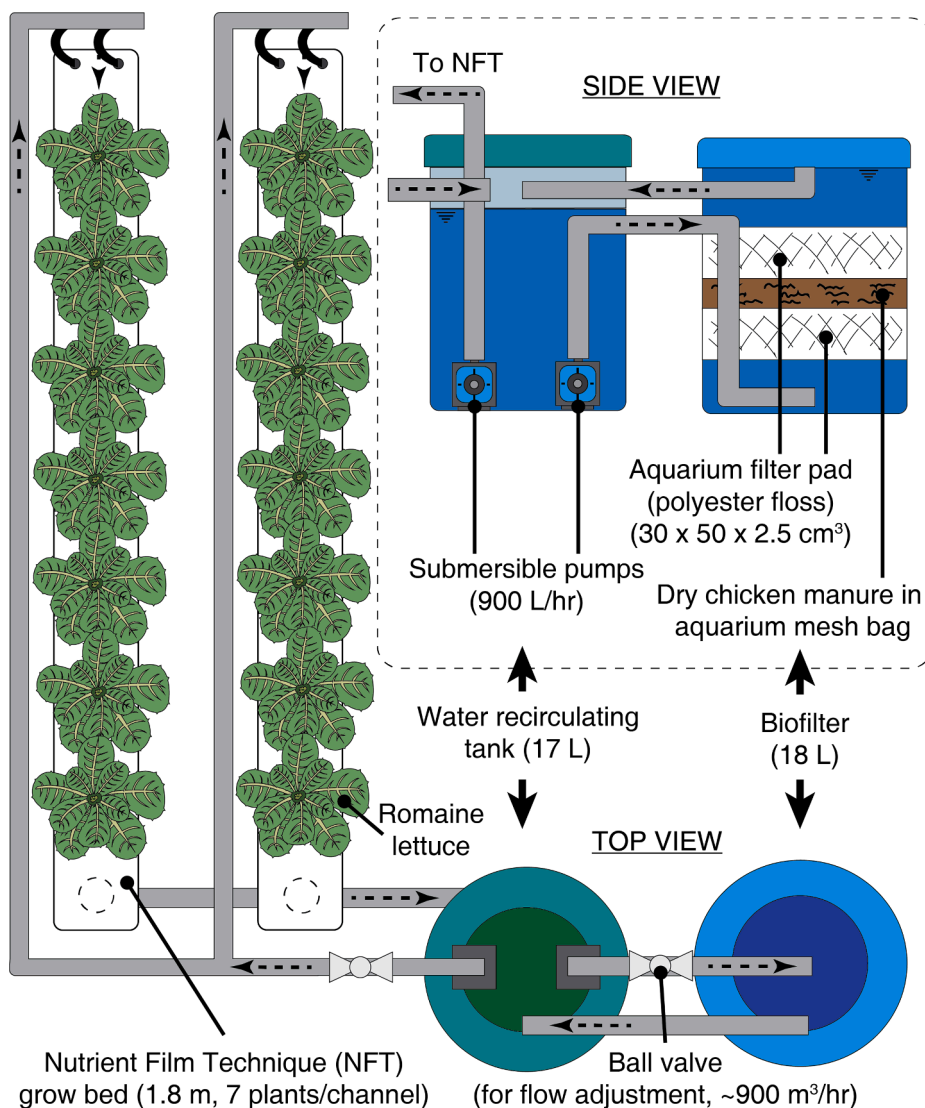


Fig. 1. Schematic representation of a nutrient-film technique bioponic system used in this study.

2. Materials and methods

2.1. Bioponic setup

Chicken manure-based bioponic systems were operated on a roof terrace (daylight hour: approximately 11.5 h; average temperature: approximately 27.5 °C–28.7 °C) in the Department of Environmental Science, Chulalongkorn University, Bangkok, Thailand. The bioponic system (total working volume: 35 L) was a nutrient-film technique (NFT) type (14 plants per system, 1.8-m-long double channel) with an upflow biofilter (working volume: 18 L) for capturing total suspended solids and providing a surface area for microbial growth (Fig. 1). Each biofilter was packed with two layers of polyethylene filter pad (typically used in aquarium filters) with a specific surface area of approximately 2000 m²/m³ and a dimension of 30 × 50 × 2.5 cm³. A recirculating tank (17 L) was used as an equalization sump tank and was connected with the NFT grow bed and biofilter. Each recirculating tank comprised two submersible pumps (JUN aquarium equipment, HX-5000; approximately 900 L/h), one connected to the NFT grow bed and another to the biofilter.

To supply equal nitrogen and phosphorus loadings in all bioponic setups, a mesh bag containing 500 g (dry wt.) dry chicken manure was placed between the filter pads (Fig. 1) as a nutrient source (total nitrogen = 2.03 ± 0.21 % dry wt., P₂O₅ = 8.0 ± 0.72 % dry wt.). In this study, a higher chicken manure loading (500 g dry wt. per system, 35.7 g dry wt./plant) than that used in previous studies (11.1–22.2 g dry wt./plant) (Wongkiew et al., 2021b) was used to assess the performance of the bioponic systems in excessive nutrient and high acetic acid conditions. Before initiating the experiments, all bioponic systems were operated without plants and acetic acid for 4 weeks to ensure the development and acclimation of microbial communities in the biofilters. Romaine lettuce (*Lactuca sativa longifolia* cv. *Jericho*) was used in this study because of its high productivity and nitrogen and phosphorus recoveries in the NFT system.

2.2. Experimental setup

The experiment was designed to evaluate the effects of acetic acid concentrations on nitrogen and phosphorus recoveries and availabilities, microbial community, metabolic functions, and plant growth in the chicken manure-based bioponic system. The experiment was divided into two phases, namely, phase I—control (without acetic acid, 0 mg/L acetic acid) versus 1000 mg/L acetic acid—and phase II—500 mg/L acetic acid versus 1500 mg/L acetic acid. Phase I was designed to evaluate the effects of acetic acid supplementation in bioponics, whereas phase II was designed to compare the two concentrations of acetic acid. Acetate concentrations were below the detection limit at the beginning of each experiment. Acetic acid was added to each system every 7 days (0, 7, 14, 21, and 28) using diluted glacial acetic acid (QR&C, A1020) concentrations of 17.5, 35.0, and 52.5 g/L to adjust the acetate concentrations to 500, 1000, and 1500 mg/L, respectively. These acetic acid concentrations are below the range of those reported in chicken manure digestate and raw manure influent (Page et al., 2014; Yin et al., 2021).

Each phase was operated for 35 days in duplicate. Lettuce seedlings were germinated for 14 days before being transplanted to the bioponic system. Nutrients for the seedlings were supplied using bioponic water. Important water quality parameters and nutrient levels in both phases were measured, and the nitrogen and phosphorus concentrations in all bioponic systems were adjusted to concentrations equivalent to those before the initiation of each phase. The 4-week operating gap was maintained between phases I and II to allow the system to equalize and ensure equivalent conditions for the bioponic microbiota. During the equalization period, the systems were operated using water recirculation without chicken manure, plants, or acetic acid. At the beginning of each phase, 500 g (dry wt.) of dry chicken manure was added along with 2.0 mg Fe/L of iron ethylenediamine-N,N'-bis(2-hydroxyphenylacetic

acid). Water loss due to evapotranspiration was replenished with nutrient-free tap water after the water volume in the bioponic system dropped by 10%.

2.3. Sampling

Water samples were collected from the recirculating tanks before the weekly addition of acetic acid (on days 0, 7, 14, 21, 28, and 35) to measure the total ammonia nitrogen (TAN), nitrite, nitrate, total Kjeldahl nitrogen (TKN), total phosphorus (TP), phosphate, chemical oxygen demand (COD), and VFAs such as acetic acid. At the end of phases I and II, all 14 plants of the system were harvested, total fresh weight was recorded, and total dry weight was determined by drying at 70 °C for 48 h. The dried plants were mixed, ground, and analyzed for TKN and TP. For microbial community analysis, plant roots (n = 2) and biofilter chicken manure sediment (n = 2) were sampled at the end of each experiment for 16S rRNA gene sequencing. The root and sediment samples were taken directly from the bioponic systems and preserved in sterile tubes at – 80 °C before DNA extraction.

2.4. Analytical methods

2.4.1. Physical and chemical analyses

Dissolved oxygen (DO) concentrations, pH levels, and water temperature were monitored weekly in the recirculating tanks using portable DO and pH/temperature meters, respectively (HACH, Loveland, CO, USA). The ambient temperature was recorded weekly using a simple bulb thermometer. Water samples were analyzed for TKN, TAN, nitrite, and nitrate using the macro-Kjeldahl (4500-N_{Org}.B) (APHA, 2005), modified Nessler (Jeong et al., 2013), colorimetric (4500-NO₂.B) (APHA, 2005), and sodium salicylate (Monteiro et al., 2003) methods, respectively. TP and phosphate contents as well as COD were analyzed using sulfuric acid–nitric acid digestion (4500-P.B), the vanadomolybdophosphoric acid method (4500-P.C), and the closed reflux colorimetric method (5220 D), respectively (APHA, 2005). TKN and TP contents in plants and chicken manure were analyzed using digestion methods based on AOAC-991.20 and AOAC-975.03–984.27, respectively (AOAC, 2019). VFAs, including acetic acid concentration, were determined using high-performance liquid chromatography equipped with a reflective index detector (Shimadzu LC10-AD).

2.4.2. 16S rRNA gene sequencing and bioinformatic analyses

Bacterial 16S rRNA genes were extracted from plant roots and chicken manure sediment samples using the TIANamp Soil DNA Kit (Tiangen Biotech, China). The genes were amplified from the V3-V4 regions using the 341F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 805R (GTCTCTGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) primers and sparQ HiFi polymerase chain reaction (PCR) master mix (Quantabio, USA). Bacterial gene amplifications were performed with one PCR cycle for initial denaturation (94 °C, 3 min) followed by 25 denaturation (98 °C, 20 min), annealing (60 °C, 30 min), and extension (72 °C, 30 min) cycles and a final extension cycle (72 °C, 5 min). Marker amplicons were purified using AMPure XP beads and indexed using 5 µl of Nextera XT index primer in a 50 µl PCR reaction with 8–10 cycles of the abovementioned PCR conditions. Finally, the PCR products were cleaned, pooled, and diluted before sequencing on an Illumina MiSeq to produce clusters of approximately 250-bp paired-end sequence reads. These 16S rRNA gene sequencing steps followed the protocol reported by Wongkiew et al. (2021b). All 16S rRNA sequences have been deposited to the Sequence Read Archive at the National Center for Biotechnology Information under the accession no. PRJNA749753.

Raw sequences were processed following the standard operating procedure for MiSeq data using Mothur (v.1.39.5) tools in Galaxy version 21.01 (Batut et al., 2018; Schloss et al., 2009). In brief, bioinformatics included (a) quality control (ambiguous bases = 0), (b)

sequence alignment (reference = SILVA version 132 as 16S rRNA gene databases, max homopolymers = 8, chimera removal = VSEARCH), (c) taxonomic classification using naive Bayesian classifier (reference taxonomy = Greengenes version 13.5, removed taxon = Chloroplast-Mitochondria-unknown-Archaea-Eukaryota), (d) operational taxonomy unit (OTU) clustering (identity cutoff = 0.03, subsample = yes), and (e) diversity (alpha and beta) analyses. A BIOM file was generated in Mothur and was used to predict gene family abundance (metagenome prediction categorized by Kyoto Encyclopedia of Genes and Genomes [KEGG] Orthology at level 3) using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) in the online Galaxy/Hutlab version 1.0.0 (Langille et al., 2013).

2.4.3. Statistical analyses and data visualizations

The plant weight, nitrogen use efficiency (NUE), phosphorus use efficiency (PUE), pH level, DO concentration, and water temperature between the two groups of acetic acid supplementation were compared using a *t*-test to identify significant differences ($p < 0.05$). Because the number of days of operation was found as a covariance in the experiments for TKN, TAN, nitrite, nitrate, TP, and phosphate concentrations and COD ($p < 0.05$), a comparison between the two groups was performed using the analysis of covariance test followed by the Tukey post hoc test for identifying significant differences ($p < 0.05$). Statistical analyses were performed using Minitab 16 Statistical Software (Minitab 16.1.1, Minitab Inc.). Alpha diversity indices of the microbial communities (e.g., Shannon diversity index) were calculated using Mothur (v.1.39.5). Microbial communities and the metabolic gene abundances were visualized using a heatmap, and dendrograms were prepared using the unweighted pair group method with arithmetic mean in Statistical Analysis of Metagenomic Profiles version 2.1.3 (Parks et al., 2014). Comparisons of the microbial groups and functional genes between the control and treatments (i.e., acetic acid dosages) were performed using the linear discriminant analysis (LDA) effect size (LEfSe) method for finding metagenomic biomarkers ($p < 0.05$) for discriminative features in the online Galaxy/Hutlab version 1.0.0 (Segata et al., 2011). Co-occurrence networks of the microbial community were analyzed using the RStudio software version 1.3.1093 (R version 4.0.3) and Jupyter Notebook 6.0.3 (for Python script) and were visualized using the Gephi software version 0.9.2 (Hu et al., 2017).

3. Results and discussion

3.1. Effects of acetic acid dosages on the water quality and nitrogen and phosphorus availabilities in bioionics

The additions of 500, 1000, and 1500 mg/L acetic acid did not significantly affect the water quality in the bioionics systems ($p > 0.05$; Table 1 and Figs. S1 and S2 in Supplementary Data). The concentrations of TKN, TAN, nitrite, nitrate, TP, and phosphate and COD (Table 1) were within the ranges of nitrogen and phosphorus concentrations for effective plant growth in other aquaponic/bioionics systems (Wongkiew et al., 2021b; Yang and Kim, 2020). Owing to rapid water recirculation through the biofilter, the addition of acetic acid did not reduce DO concentrations, which remained high (i.e., 6.4–6.8 mg/L) during the entire study period. COD did not show any significant difference among the bioionics systems investigated including the control, thereby suggesting the effectiveness of bioionics systems in removing acetic acid. The additions of 500, 1000, and 1500 mg/L acetic acid immediately resulted in a rapid decrease in the pH levels from 7.9 ± 0.2 to 6.5 ± 0.4 , 6.1 ± 0.3 , and 5.1 ± 0.2 , respectively (Table 1). However, within a day, the bioionics system's pH level recovered and was not significantly different from that of the control (7.7–7.9, Table 1). Thus, no significant effects of acetic acid supplementation were found on nitrogen or phosphorus concentrations or the water quality in the bioionics systems. This might be because of the readily biodegradable nature of acetic acid and the efficient buffering capacity of chicken manure in the biofilter

Table 1

Nitrogen and phosphorus concentrations, COD, nitrogen and phosphorus use efficiencies, and lettuce yields in the bioionics systems based on the acetic acid dosage.

Parameters	Acetic acid dosage conditions in the bioionics systems			
	Phase I		Phase II	
	Control (0 mg/L) *	Medium (1000 mg/L)	Low (500 mg/L)	High (1500 mg/L)
TKN (mg N/L)	12.6 ± 3.5 ^a	12.0 ± 2.6 ^a	10.6 ± 2.1 ^c	13.2 ± 4.2 ^c
TAN (mg N/L)	2.8 ± 0.5 ^a	3.0 ± 0.8 ^a	2.5 ± 0.8 ^c	2.7 ± 1.0 ^c
Nitrite (mg/L)	2.0 ± 0.4 ^a	1.8 ± 0.5 ^a	1.7 ± 0.5 ^c	1.6 ± 0.6 ^c
Nitrate (mg/L)	5.1 ± 2.2 ^a	5.3 ± 1.8 ^a	6.5 ± 2.2 ^c	6.6 ± 2.5 ^c
TP (mg P/L)	33.8 ± 8.0 ^a	37.9 ± 10.1 ^a	41.4 ± 15.6 ^c	41.3 ± 15.8 ^c
Phosphate (mg P/L)	24.9 ± 7.3 ^a	29.6 ± 9.3 ^a	32.0 ± 8.4 ^c	31.6 ± 8.8 ^c
COD (mg/L)	321.5 ± 92.2 ^a	306.6 ± 88.0 ^a	302.3 ± 101.8 ^c	315.5 ± 101.8 ^c
Initial pH (after adding acetic acid)	7.9 ± 0.2 ^a	6.1 ± 0.3 ^b	6.5 ± 0.4 ^c	5.1 ± 0.2 ^d
pH (before adding acetic acid)	7.9 ± 0.2 ^a	7.7 ± 0.2 ^b	7.8 ± 0.2 ^c	7.7 ± 0.2 ^d
DO (mg/L)	6.5 ± 0.3 ^a	6.4 ± 0.4 ^a	6.6 ± 0.2 ^c	6.8 ± 0.2 ^c
Water temperature (°C)	30.0 ± 1.9 ^a	30.1 ± 1.9 ^a	29.9 ± 1.8 ^c	29.8 ± 1.8 ^c
Lettuce yields (fresh wt. g/system)	1185 ± 13.4 ^a	593 ± 60.1 ^b	741 ± 62.9 ^c	536 ± 19.8 ^d
NUE (%)	16.3 ± 0.2 ^a	8.1 ± 0.8 ^b	10.2 ± 0.9 ^c	7.3 ± 0.3 ^d
PUE (%)	3.3 ± 0.1 ^a	1.7 ± 0.2 ^b	2.1 ± 0.2 ^c	1.5 ± 0.1 ^d

Values reported as mean ± standard deviation (n = 12 for TKN to water temperature, n = 2 for lettuce yield, NUE, and PUE). The superscripts a, b, c, and d represent statistical differences ($p < 0.05$). Comparisons were made within each phase. NUE and PUE (%) were calculated based on the percentage of nitrogen and phosphorus assimilated to plant biomass per nitrogen and phosphorus mass applied. *The initial acetic acid concentration.

(Cáceres et al., 2018; Drózdź et al., 2020). Moreover, acetic acid was not detectable in all the bioionics systems a week after acetic acid supplementation. Therefore, the weekly acetic acid dosage did not significantly affect the amount of nitrogen or phosphorus available ($p > 0.05$) for plant uptake in the bioionics systems.

3.2. Effects of acetic acid dosages on plant yield, NUE, and PUE

The weekly acetic acid dosages negatively affected plant yield, NUE, and PUE (Table 1). The lettuce yield negatively correlated with the weekly acetic acid dose concentration ($p < 0.05$). The yield reduction might be due to a pH shock resulting from the entry of the undissociated acetic acid (with $pK_a = 4.76$) into the cell membrane (Allen and Allen, 2020), thereby reducing transpiration (as observed by temporary wilt, but recovered within a day) and inhibiting root and shoot growths (Fig. S3). Biofilm and solids due to microbial growth around the roots of lettuce were observed in the bioionics systems with acetic acid supplementation (Fig. S4). The supplementation of acetic acid in the system enhances microbial biofilm formation because acetic acid is a readily available carbon source for microbial growth (Iannacone et al., 2021). A thick biofilm layer may decrease oxygen diffusion into plant roots (Wongkiew et al., 2021a), thereby reducing the plant yield. Thus, avoiding acetic acid concentrations of > 500 mg/L, supplementing acetic acid at longer intervals, and pretreating chicken manure digestate (e.g., multistage biofilter) to reduce VFA concentrations, particularly that of acetic acid, may alleviate the issues associated with the formation of undissociated acetic acid, which inhibits plant growth. However, studies using real digestates with VFA concentrations of < 500 mg/L must be conducted in bioionics systems to ensure the effective removal of VFAs and evaluate the adverse effects of VFAs on plant growth and

nutrient recovery.

The plant yield observed in the control (chicken manure loading = 35.7 g dry wt./plant without acetic acid) in this study was within the range of plant yields reported previously (chicken manure loading = 11.1–22.2 g dry wt./plant) (Wongkiew et al., 2021b). However, the overloading of chicken manure in this study resulted in a lower NUE (16.3%) and PUE (3.3%) than those reported previously (NUE = 35.1%–41.8%, PUE = 6.8%–8.0%) (Wongkiew et al., 2021b). This is because the amount of nitrogen and phosphorus supplied by chicken manure (nitrogen loading = 10.2 ± 1.1 gN/harvesting cycle; phosphorus loading = 17.5 ± 1.6 gP/harvesting cycle) were much higher than that required by the plants (Wongkiew et al., 2021a; Wongkiew et al., 2017). The NUE and PUE also negatively correlated with acetic acid concentrations ($p < 0.05$), which is also supported by the plant yield data (Table 1 and Fig. S5). On the basis of the findings of this study, the NUE and PUE may be improved by reducing the amount of chicken manure loaded to balance between the nutrient input and output, supplementing with other nutrient sources (e.g., aquaculture effluent and algae co-cultivation) (Wang et al., 2018; Wongkiew et al., 2021a), and/or combining with nanobubble-aeration to enhance VFA degradation (Chuenchart et al., 2021). Other techniques for pretreatment or in situ treatments include micro- and nanobubble aeration; multiple biofilm reactors; and real-time monitoring and control of acetic acid, pH level, and nutrient level. These changes might improve the overall performance of bioptic systems, especially when using nutrient-rich waste streams such as the anaerobic digestates from animal manures (Möller and Müller, 2012).

3.3. Microbial communities and predicted metabolic genes in the bioptic systems

Plant roots and chicken manure sediments harbored diverse microbial communities (Good's coverage = 0.958–0.971). The microbial community compositions at the genus level (top 25 OTUs) in roots (28.5%–70.7% coverage of total microbial OTUs; Fig. 2A) and chicken manure sediments (37.1%–43.0% coverage; Fig. 2B) were visualized as heatmaps. The metabolic gene abundances in the same roots (48.6–49.8% coverage of total metabolic genes; Fig. 2C) and sediments (49.7%–50.6% coverage; Fig. 2D) were also visualized as heatmaps. The microbial composition in plant roots was different from that in sediments, and only four OTUs overlapped in the list of top 25 OTUs (Fig. 2A and B). However, the top metabolic functional gene abundances in plant roots were similar to those in sediments (23 gene abundances overlapped, Fig. 2C and D). Principal component analysis also revealed a difference in microbial OTU and functional metabolic gene compositions between plant roots and sediments (Fig. S6). Therefore, plant roots and sediments may provide different habitats for microorganisms in bioptic systems. Moreover, the abundance and composition of microbial community and functional metabolic genes in plant roots grown under the control condition differed from those in plant roots grown under acetic acid supplementation (Fig. 2, S7, and S8).

As visualized in the heatmaps (Fig. 2A), the dominant microbial OTUs in plant roots under the control condition were *Hydrogenophaga* (4.06%–6.49%), *Zoogloea* (3.89%–5.00%), *Azorhizophilus* (3.59%–5.38%), *Pseudoxanthomonas* (1.21%–1.66%), and *Flavobacterium* (0.89%–1.09%), whereas the dominant OTUs in plant roots supplemented with acetic acid included *Acinetobacter* (0.01%–12.61%), *Azohydromonas* (0.19%–8.49%), *Pseudomonadaceae* (0.53%–11.38%), *Comamonadaceae* (2.62%–24.97%), *Cellvibrio* (0.25%–3.17%), and *Chloronema* (0.12%–1.61%). There is evidence that *Hydrogenophaga* (denitrifier and phosphorus-accumulating organism), *Zoogloea*, *Pseudoxanthomonas*, *Comamonadaceae* (denitrifier), *Azorhizophilus* (potassium-solubilizing bacteria), *Acinetobacter*, *Flavobacterium*, *Cellvibrio* (organic carbon degrader), and *Azohydromonas* (bacteria associated with nitrogen cycle) are responsible for organic degradation and nutrient availability (Chen et al., 2018; Iannacone et al., 2021; Li et al.,

2020a; Marak et al., 2020; Wongkiew et al., 2017; Zhang et al., 2021a, b).

On the basis of the heatmap (Fig. 2B), some microbial groups such as *Dok59* (an unclassified genus of the family *Rhodocyclaceae*, 0.38%–2.89%), *Coprococcus* (0.73%–3.59%), *Treponema* (1.38%–5.31%), and *Porphyromonadaceae* (0.09–6.58%) were highly abundant in the chicken manure sediments during acetic acid supplementation. Other dominant microbial groups in the sediments during all growth conditions included *Bacillus* (0.51%–6.32%), *Dysgonomonas* (0.02%–4.06%), *Pseudoxanthomonas* (0.02%–1.54%), *Hydrogenophaga* (0.10%–1.19%), and *Ruminococcus* (0.15%–2.50%). Although some of the dominant OTUs in sediments were not identified in the top genera present in plant roots, they were likely responsible for the organic degradation and nutrient mineralization of chicken manure necessary for plant growth. These OTUs included *Dok59* (denitrifier), *Treponema* (a marker for human and chicken gut microbe), *Bacillus* (phosphate-solubilizing bacteria and denitrifier), and *Dysgonomonas* (organic carbon degrader) (Cerozi and Fitzsimmons, 2016; Huang et al., 2016; Tao et al., 2020; Wongkiew et al., 2017; Wongsaroj et al., 2021). Bacteria associated with ammonia and nitrite oxidations—for example, *Nitrospira* (0.12%–1.04% in roots and 0.05%–0.24% in sediments) and *Nitrosomonadaceae* (0.01%–0.09% in roots and 0.1%–0.7% in sediments)—were also present and are important in nitrification (Wongkiew et al., 2018). Although the OTUs of autotrophic nitrifiers were found in low abundance in these bioptic systems (<2%), results suggested that these relative abundances promote sufficient nitrification in bioptics, based on the low concentrations of TAN and nitrate in bioptic systems (Table 1, Fig. S1). Despite the effect of acetic acid supplementation on microbial communities, the water quality and nitrogen and phosphorus availabilities between bioptic systems with and without acetic acid did not differ significantly ($p > 0.05$, Table 1). The results suggest some interconnection between key microbial groups that may, together, facilitate stable metabolic functions to maintain nitrogen and phosphorus availabilities even with acetic acid supplementation.

The Shannon (H_s) index of bacterial diversity in these experiments (H_s of 4.23–5.88 in roots and 4.97–6.13 in chicken manure sediments; Tables S1 and S2) were within the range of those noted in aquaponic systems (H_s of 4.74–6.30) (Heise et al., 2021) and aquaculture effluent treatment systems (H_s of 5.1–5.8) but were higher than those of biological wastewater treatment systems (H_s of 2.25–2.92) and lower than those of soil (H_s of 7.57–9.39) and aquaculture sediment (H_s of 8.3–8.4) (Wongkiew et al., 2018). In this study, the diversities in roots and sediments grown in the control condition did not differ significantly ($p > 0.05$) from those grown in the presence of acetic acid. The results suggest that the groups of microbes present in the bioptic systems worked symbiotically for nitrogen and phosphorus transformations and organic compound degradation.

Plant roots and chicken manure sediments had similar metabolic profiles of over 155 metabolic gene types of KEGG orthologs predicted through OTU abundances (Fig. 2C and 2D, 25 top abundant genes), which provided an overview of the metabolic functions in bioptics. The top metabolic genes in plant roots included those involved in purine metabolism (3.49%–3.73%), oxidative phosphorylation (2.69%–3.05%), peptidase (2.62%–2.88%), pyrimidine metabolism (2.30%–2.54%), and arginine and proline metabolism (2.18%–2.30%). Some dominant metabolic genes in chicken manure sediments included those involved in purine metabolism (3.83%–3.97%), biosynthesis of peptidases (3.03%–3.43%), pyrimidine metabolism (2.73%–3.11%), methane metabolism (1.98%–2.41%), biosynthesis of amino acid-related enzymes (2.35%–2.69%), and oxidative phosphorylation (2.46%–2.98%), which suggests differing microbial functions between the microbiota in plant roots and those in chicken manure sediments. Nitrogen metabolism (1.31%–1.72%), lipid biosynthesis (1.47%–1.63%), and energy metabolism (1.55%–1.96%) were also important processes of nitrogen recovery in bioptics. The results suggest that the dominant genes may be those responsible for organic degradation and

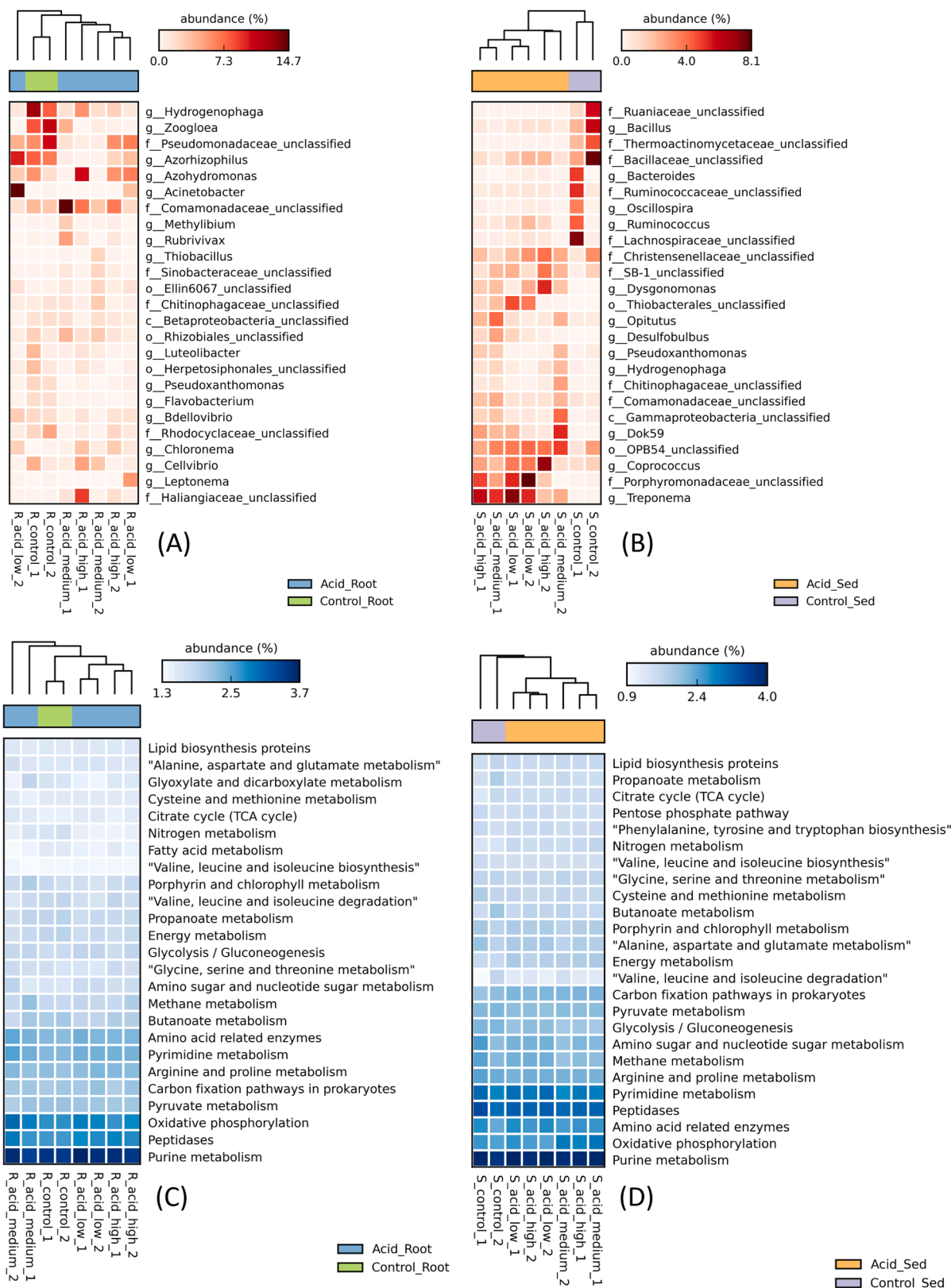


Fig. 2. Heatmaps of microbial communities (A and B) and metabolic functions (C and D) in plant roots (A and C) and chicken manure sediments (B and D) with and without acetic acid supplementation.

removal, nutrient mineralization, microbial cell synthesis from nitrogen and phosphorus, and energy utilization from the microbial breakdown of organic molecules.

3.4. Effects of acetic acid dosages on microbial OTUs and metabolic genes in the bioponic systems

The weekly acetic acid dosages (500–1500 mg/L) significantly ($p < 0.05$) affected some microbial OTUs (biomarkers) and metabolic genes in the bioponic systems (Fig. 3). This might have resulted from acetic acid supplementation, which enhanced the growth of acid-tolerant microbes that dominated over pre-existing microbes in the bioponics in the absence of acetic acid (Ho et al., 2017). In plant roots, *Clostridiales* (0.08%–0.34%) exhibited a significantly higher abundance in the absence of acetic acid (0.97%–3.95%) than in the control, whereas significantly higher abundances of *Hydrogenophaga* (4.06%–6.49%), *Zoogloea* (3.89%–5.0%), *Rhodocyclaceae* (2.88%–4.11%), *Pseudomonadaceae* (7.54%–11.38%), and *Xanthomonadaceae* (1.05%–1.16%) were found in the control condition (Fig. 3A). *Clostridiales*, the acid-forming bacteria, are reported to utilize protein and glucose and produce VFAs under anaerobic conditions, which could partially occur in plant roots under high acetic acid concentrations (Zhai et al., 2020). However, this family was found at low relative abundances in the bioponic system, suggesting that some microbes with a low relative abundance are dependent on acetic acid to maintain the stability of microbial degradation and nitrogen and phosphorus availabilities in bioponic systems.

Several microbes found in plant roots positively correlated with those found in the control condition, which suggests that chicken manure-based bioponics (without acetic acid) conserved microbial biomarkers (Fig. 3). For example, *Hydrogenophaga* was reported as a phosphorus-accumulating microbe and hydrogenotrophic denitrifier, which could contribute to the nitrate removal and nitrogen loss of bioponic plant roots (Iannacone et al., 2021; Liu et al., 2020). Other dominant key OTUs, such as *Zoogloea* (denitrifier), *Rhodocyclaceae* (denitrifier and found in enhanced biological phosphorus removal processes), *Pseudomonadaceae* (the family of *Pseudomonas* that contributes to phosphorus recovery), and *Xanthomonadaceae* (heterotrophic

nitrifier/denitrifier at low DO conditions) were found in significant abundance under the control condition (Li et al., 2020b; Li et al., 2020a; Tian et al., 2016; Wongkiew et al., 2017).

In sediments, *Treponema* (1.38%–5.31%) and *Rhodocyclaceae* (1.03%–4.73%) were significantly more abundant with acetic acid supplementation, whereas *Bacillus* (2.68%–6.32%), *Thermoactinomycetaceae* (1.61%–3.22%), and *Ruaniaceae* (0.79%–3.93%) were significantly more abundant in the control (Fig. 3B). *Treponema* is a plant pathogen present in fecal compost and is a probable human pathogen (Wongsaroj et al., 2021). The genus *Bacillus* was reported to enhance the availability of phosphorus and act as a plant growth promoter (Cerozi and Fitzsimmons, 2016). Other studies have reported the effects of several organic acids (e.g., acetic, citric, and succinic acids) and pH levels on microbial communities including an increase in the abundance of phosphate-solubilizing bacteria, thus facilitating phosphate solubilization (Wei et al., 2018). *Thermoactinomycetaceae* was associated with hyperthermophilic conditions, which could be inherited from the chicken gut, and *Ruaniaceae* was associated with the presence of antibiotic-resistance genes in the compost (Bao et al., 2021; Qiu et al., 2021). The abundance of these two OTUs may be due to microbial succession in chicken manure, although acetic acid supplementation could shift their abundances. Thus, a disinfection system (e.g., ultraviolet ray and ozone) is necessary to eliminate possible pathogens from the chicken manure-based bioponic system and ensure the safety of food products.

During acetic acid supplementation, the microbial metabolisms in roots were associated with the metabolisms of amino acid (alanine, aspartate, and glutamate) and carbohydrates (fructose and mannose metabolisms) as well as the biosynthesis of pantothenic acid (vitamin B₅) and CoA (Fig. 3C), which are essential for fatty acid metabolism, phospholipid synthesis, and cellular energy production (Leonardi et al., 2007; Zhang et al., 2021a,b). These findings suggest that organic carbon utilization, nitrogen assimilation, and exopolysaccharide synthesis are dominant pathways at this range of acetic acid supplementation. In this study, biofilm formation was also found around plant roots during growth with acetic acid (Fig. S4), thereby supporting the association of these metabolic functions with the utilization of sugar and acetic acid.

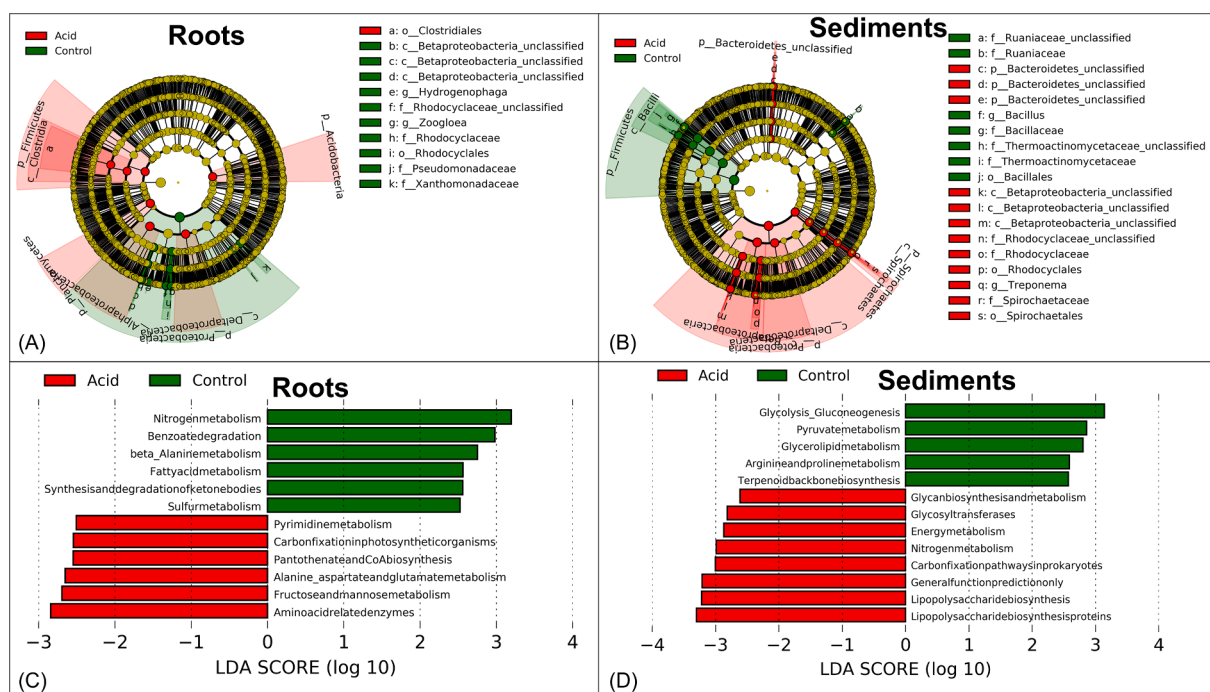


Fig. 3. Differential abundance of microbial communities (A and B, LDA score > 4) and their predicted metabolic functions (C and D, LDA score > 2.5) based on LefSe in plant roots and sediments from bioponics under acetic acid supplementation and control condition ($p < 0.05$).

Such biofilm formation may also result in a rapid depletion in DO around the root zone, which is likely to affect the plant growth (Wongkiew et al., 2021a). In contrast, metabolic functions in the control condition were highly associated with nitrogen, beta-alanine, fatty acid, and sulfur metabolisms and benzoate degradation. These metabolic pathways are associated with several organic degradation and nutrient metabolism outcomes, which suggests that the pathways involved in organic degradation and nitrogen and sulfur availabilities are dominant around the plant roots during control conditions (Singh and Schwan, 2011; Yin et al., 2019). Nitrogen and sulfur metabolisms are important for the formation of amino acids, particularly cysteine, which is then utilized in protein synthesis and mediate nutrient availability (e.g., nitrogen and sulfur) for plant growth (Ren et al., 2017; Singh and Schwan, 2011). On the basis of metabolism-associated results, the control condition appears to be more favorable to plants in terms of nutrient uptake and thinner biofilm formation on plant roots than acetic acid supplementation.

The metabolic functions in sediments were different from those in roots (Fig. 3D). Under acetic acid supplementation, sediments showed distinct metabolic functions such as nitrogen and energy metabolisms, lipopolysaccharide biosynthesis, carbon fixation, and glycan biosynthesis, suggesting that microbial cell synthesis (particularly gram-negative) and inorganic nitrogen transformation (e.g., nitrification and denitrification) in chicken manure sediments are induced by acetic acid (Melamed and Brockhausen, 2021; Ren et al., 2017). Thus, both microbial growth and nitrogen transformation in the sediments may contribute to nitrogen loss, mineralization, and nitrogen assimilations for microbial growth in the biofilter. However, in the control condition, the microbial groups contributed to glycolysis–gluconeogenesis as well as pyruvate, glycerolipid, arginine, and proline metabolisms, which are metabolic functions required for organic degradation and biosynthesis, nutrient mineralization, and energy production (Fuji et al., 2018; Öhlund and Näsholm, 2002). Although there were differences in metabolic functions, the overall functional roles (organic degradation, mineralization, and nutrient transformation) of the sediment microbiota did not differ between the control and acetic acid supplementation.

These results suggest that plant roots and sediments perform

different functions, in which several metabolic pathways are affected by acetic acid. Microbial growth, including cell biosynthesis, may be enhanced by the addition of acetic acid. Previous studies have reported that the high levels of acetate increase microbial growth rates as well as COD and total nitrogen removals (Biros et al., 2014; Li et al., 2020a). Moreover, acetic acid has been reported to contribute toward protein/polysaccharide synthesis in wastewater treatment systems (Li et al., 2020a). In bioponics, plant roots play an important role in nutrient mineralization and uptake, whereas sediments contribute to organic degradation. In this study, the functions were predicted through KEGG pathway analysis using PICRUSt based on 16S rRNA OTUs, which only has accuracies of approximately 80% and 40%–80% compared with that of shotgun metagenome analysis of soil and mammal gut microbiomes, respectively (Langille et al., 2013). Thus, gene expression, shotgun sequencing, and metabolomics should be considered in order to gain insights into system metabolic profiles and other important functions.

3.5. Microbial co-occurrences and their interlinkages

The co-occurrence of microorganisms identifies the interlinkages of microbial symbiosis and keystone taxa in the bioponic systems. A dominant phylum *Proteobacteria* (12.9%–75.7%) showed a low degree of co-occurrence (Fig. 4A), suggesting that this group functioned independently. Some minor phyla co-occurred with other dominant phyla, suggesting that the ecosystem of minor microbial groups in bioponic systems may be significant for the optimal functioning of bioponics in addition to the individual dominant microbial OTUs. At the phylum level, the *TM7* (*Saccharibacteria*, 0.07%–0.53%) lineage was found to have the highest degree of co-occurrence followed by the phyla *Cyanobacteria* (0.11%–2.43%), *Nitrospirae* (0.07%–1.16%), *Chloroflexi* (1.20%–7.12%), *Acidobacteria* (0.56%–5.87%), *Planctomycetes* (1.53%–8.26%), *Firmicutes* (0.32%–54.07%), and *Actinobacteria* (1.38%–16.57%; Fig. 4A and 4B), which correlated with nitrogen, phosphorus, and DO concentrations as well as COD in bioponic systems (Fig. S5). In bioponics, a desirable microbial ecosystem may be enhanced by maintaining good water quality and using sufficient unit components (multiple biofilters and pretreatment systems) for high nutrient release (e.g.,

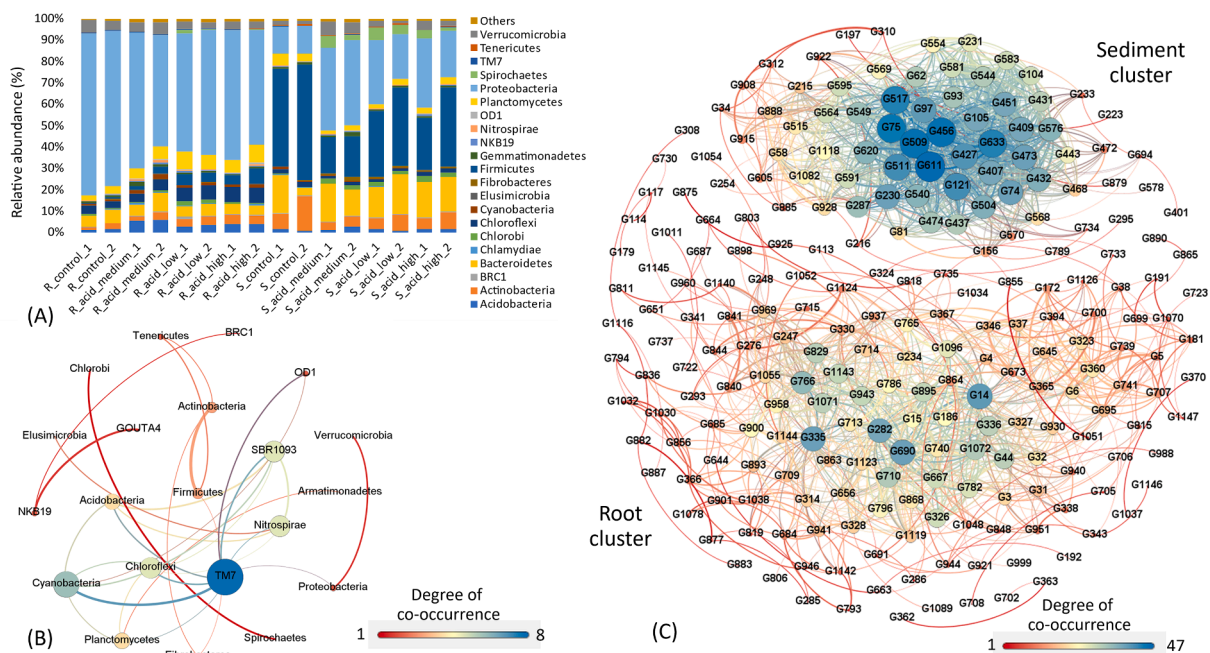


Fig. 4. Relative abundance of microbial phyla (A) and microbial community networks at phylum (B) and genus (C) levels among all samples. Co-occurrence nodes and connections were calculated based on Spearman’s correlation using $\rho \geq 0.6$ and the adjusted false discovery rate (p -value < 0.01). Each node size is proportional to the degree of co-occurrence. See co-occurrence degrees and codes of bacterial genera in Tables S5 and S6.

nanobubble technology and black soldier fly larvae) (Chuenchart et al., 2021; Isibika et al., 2019). Because diverse microbial communities function symbiotically for organic degradation and nutrient cycling (Fig. 4B and S5), the performance of bioponics may be enhanced by controlling and maintaining the environmental factors that promote the symbiosis of these microbial groups. Another option is to incorporate the groups of effective microorganisms to bioponic systems rather than focusing on individual beneficial microbes.

Microbial groups at the genus level functioned symbiotically for acetic acid degradation and nutrient mineralization for nutrient uptake by plants, facilitating the recovery of nitrogen and phosphorus. The microbial networks showed that roots and sediments harbored different groups of microbial genera that were interconnected. Microbial OTUs at the genus level were found to co-occur with two network clusters (Fig. 4C), suggesting that bioponic systems symbiotically function under two main processes and activities—plant roots and chicken manure sediments (see co-occurrence degrees and codes of bacterial genera in Table S6). The first group (sediment cluster) includes *Clostridiales* (G611, organic utilizer (Zhai et al., 2020)), *Sporosarcina* (G456), *Christensenellaceae* (G509), *Gordonia* (G75), *Clostridium* (G517), *Firmicutes* (G633, with some phosphorus solubilizing species (Liang et al., 2020)), and *Nocardioseae* (G121, organic degrader and phosphorus-solubilizing bacteria (Wongkiew et al., 2021b)) as the top six in the co-occurrence list, which were found in sediments with the relative abundance of 1.40%–2.93%, 0.05%–0.31%, 0.75%–2.41%, 0.03%–0.12%, 0.47%–2.47%, and 0.62%–1.99%, respectively. For the second group (root cluster), *PK29* (G14), *envOPS12* (G335), *Phycisphaerales* (G690, ammonia oxidizer (Miao et al., 2019)), *Saprosiraceae* (G282, denitrifier, and nitrous oxide producer (Shen et al., 2021)), and *Hyphomonadaceae* (G766) were the highest co-occurring genera in root samples with the relative abundance of 0.08%–0.26%, 0.07%–0.14%, 0.39%–0.80%, 0.30%–1.66%, and 0.22%–0.57%, respectively. *Nitrospira* (G667) was also found with a high degree of co-occurrence and was dominant in plant roots (0.12%–1.04%). In plant roots, the dominant genera with a high degree of co-occurrence were *Comamonadaceae* (G829, 2.62%–24.97%), *Azohydromonas* (G836, 0.19%–8.49%), and *Hydrogenophaga* (G84, 0.46%–6.49%). The co-occurrence results also confirmed the symbiotic significance of key microbes in bioponic systems, in which the microbes in plant roots and sediments serve different roles in nutrient recovery. Organic degradation, including acetate degradation, and carbon utilization by microbes were beneficial and occurred symbiotically in sediments, which allowed nutrient mineralization and maintained low organic carbon and acetate concentrations in water. Root microbes functioned symbiotically for nutrient transformation (organic degradation, biofilm formation, nitrification, and denitrification), which were beneficial for both nutrient recovery and the maintenance of good water quality in bioponics. Overall, the microbial communities in both plant roots and sediments were capable of degrading acetic acid and maintaining nitrogen and phosphorus availabilities (initial acetic acid concentrations of 500 to 1500 mg/L per week). Further studies are warranted on digestate-based bioponic systems with VFA concentrations of < 500 mg/L to cover a broader range of VFA concentration in the implementation of bioponics in the circular economy of biowaste management.

4. Conclusions

Chicken manure-based bioponic systems were tested with the supplementation of 0, 500, 1000, and 1500 mg/L acetic acid, in which the higher dose decreased plant yields and nitrogen and phosphorus recoveries. The plants were stressed due to rapid acetic acid supplementation. The average nitrogen and phosphorus concentrations as well as COD did not differ significantly based on acetic acid supplementation because of rapid microbial degradation. Microbial communities present in chicken manure sediments may tolerate acetic acid more than those present in plant roots. The metabolic functions present in bioponic

systems suggests that organic degradation and nutrient mineralization are the main functions of the microbial communities present in these systems. Microbial communities and metabolic functions were affected by the acetic acid; however, the overall organic degradation (including acetate removal), phosphorus mineralization, and nitrogen transformation were still maintained at high acetic acid concentration. In conclusion, to use chicken manure digestate in bioponics, a digestate load with mild acetate concentrations <500 mg/L or using loading intervals longer than a week are recommended.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Sumeth Wongkiew: Conceptualization, Methodology, Experiment, Data Analysis, Bioinformatic analysis, Visualization, Writing - original draft preparation, Funding acquisition. **Chongrak Polprasert:** Supervision, Review & Editing. **Thammarat Koottatep:** Supervision, Review & Editing. **Tawan Limpiyakorn:** Supervision, Review & Editing. **K.C. Surendra:** Interpretation, Review & Editing. **Samir Kumar Khanal:** Supervision, Interpretation, Review & Editing.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2021.11.023>.

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