Science of the Total Environment 852 (2022) 158426

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Microbial diversity across compartments in an aquaponic system and its connection to the nitrogen cycle

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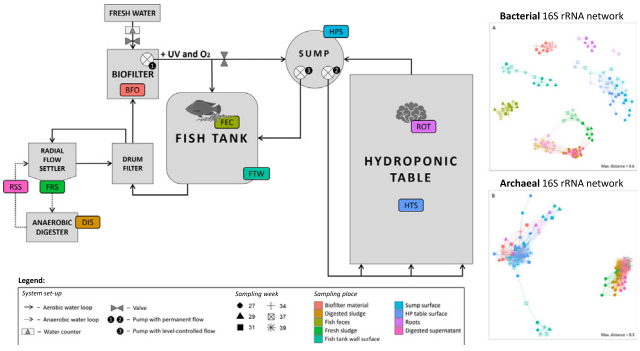
HIGHLIGHTS

- First insight into the presence of archaeal communities in an aquaponic system
- Insight into unique microbial communities of different system compartments
- Higher microbiome diversity observed in the aerobic loop of an aquaponic system
- Temporal changes in microbial composition observed within the anaerobic compartment
- Nitrifiers in hydroponic compartments play larger role in system nitrogen cycle

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Warish Ahmed

Keywords:
Aquaponics
Community analysis
bacteria
archaea
tilapia

ABSTRACT

Aquaponics combines hydroponic crop production with recirculating aquaculture. These systems comprise various compartments (fish tank, biofilter, sump, hydroponic table, radial flow settler and anaerobic digester), each with their own specific environmental pressures, which trigger the formation of unique microbial communities. Triplicated aquaponic systems were used to investigate the microbial community composition during three lettuce growing cycles. The sampling of individual compartments allowed community patterns to be generated using amplicon sequencing of bacterial and archaeal 16S rRNA genes. Nitrifying bacteria were identified in the hydroponic compartments, indicating that these compartments may play a larger role than previously thought in the system's nitrogen cycle. In addition to the observed temporal changes in community compositions within the anaerobic compartment, more archaeal reads were obtained from sludge samples than from the aerobic part of the system. Lower bacterial diversity was observed in fresh fish feces, where a highly discrete gut flora composition was seen. Finally, the most pronounced differences in microbial community compositions were observed between the aerobic and anaerobic loops of the system, with unique bacterial compositions in each individual compartment.

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http://dx.doi.org/10.1016/j.scitotenv.2022.158426
Received 6 May 2022; Received in revised form 25 August 2022; Accepted 27 August 2022
Available online 31 August 2022

1. Introduction

An aquaponic system combines aquaculture with soilless plant cultivation (Graber and Junge, 2009). The system's function relies on a diverse consortia of microorganisms with metabolic pathways (Bartelme et al., 2019; Schmautz et al., 2017) that convert nutrients provided by fish excreta and non-consumed fish feed into nutrients for plants (Goddek et al., 2016). Nutrient removal through plant absorption and growth reduces nutrient concentrations in the system, improving overall water quality and benefiting fish production (Bartelme et al., 2018), where the nitrogen cycle plays an important role. While practically all processes involved in the nitrogen cycle are based on the metabolic activities of selected microorganisms (Karl and Michaels, 2019; Robertson and Groffman, 2007), fundamental knowledge of the microorganisms responsible for these nitrogen transformations remains incomplete (Robertson and Groffman, 2007; Stein and Klotz, 2016). Especially in aquaponic systems, where system health and productivity are heavily dependent on the stability of the nitrogen cycle, there is a significant lack of knowledge of the nitrogen transforming microorganisms involved (Robertson and Groffman, 2007).

Removal of feed remains and fish metabolites, such as ammonium and fecal matter from the fish tanks and their reuse as plant nutrients within an aquaponic system requires that individual compartments such as the fish tanks, biofilters, sumps, hydroponic growing systems and any additional elements, such as anaerobic reactors, are connected by a circular water flow (Palm et al., 2019). In most of these compartments, microbial activity mainly occurs on surfaces, and therefore, the majority of microbial communities are organized in biofilms (Blancheton et al., 2013; Davey and O'Toole, 2000; Donlan, 2002). Hitherto attempts to characterize the microbial communities in aquaponic systems have focused on the sampling of the water column and biofilter material (Blancheton et al., 2013; Eck et al., 2019; Hu et al., 2015; Schmautz et al., 2017). However, little consideration has been given to the microbial populations developing in each individual compartment.

As aquaponic system design is not standardized, each compartment has distinct process conditions caused by different configurations, dimensions and flow rates. Therefore, environmental conditions will differ (Bartelme et al., 2017; Eck et al., 2019; Menezes-Blackburn et al., 2021), resulting in the development of unique, localized microbial communities. Due to the interconnection between compartments, these localized communities may influence each other (Bartelme et al., 2019, Bartelme et al., 2018), changing process conditions over time and supporting or hindering productivity either directly or indirectly through their metabolites. Thus, understanding the compartment-specific microbial communities in aquaponic systems becomes essential, especially in terms of water quality, fish welfare, plant health and food safety (Bartelme et al., 2018; Blancheton et al., 2013; Munguia-Fragozo et al., 2015). At the same time, more in-depth insights into the microbiomes also open up the possibility of further controlling microbial communities by modifying environmental conditions (Blancheton et al., 2013). Microbial research supporting aquaponic crop production, however, still lags behind its traditional agricultural system counterparts (Bartelme et al., 2018), even though the microbes in both soil and aquaponic systems seem to play similar roles (Bender et al., 2016; Hartman et al., 2018).

In this study, we used three identical aquaponic systems running in parallel to investigate the microbial community composition of their different compartments over 12 weeks, corresponding to three cycles of lettuce production. Community patterns were generated using amplicon sequencing of bacterial and archaeal 16S rRNA genes. The results provide a better understanding of microbial profiles, their resilience to changes, and their connection to the nitrogen cycle.

2. Materials and methods

Between June and September 2017, experiments were carried out in the greenhouse facilities of the Zurich University of Applied Sciences (Wädenswil, Switzerland). Three replicates of a 4.3 m^3 aquaponic system (Fig. 1) were

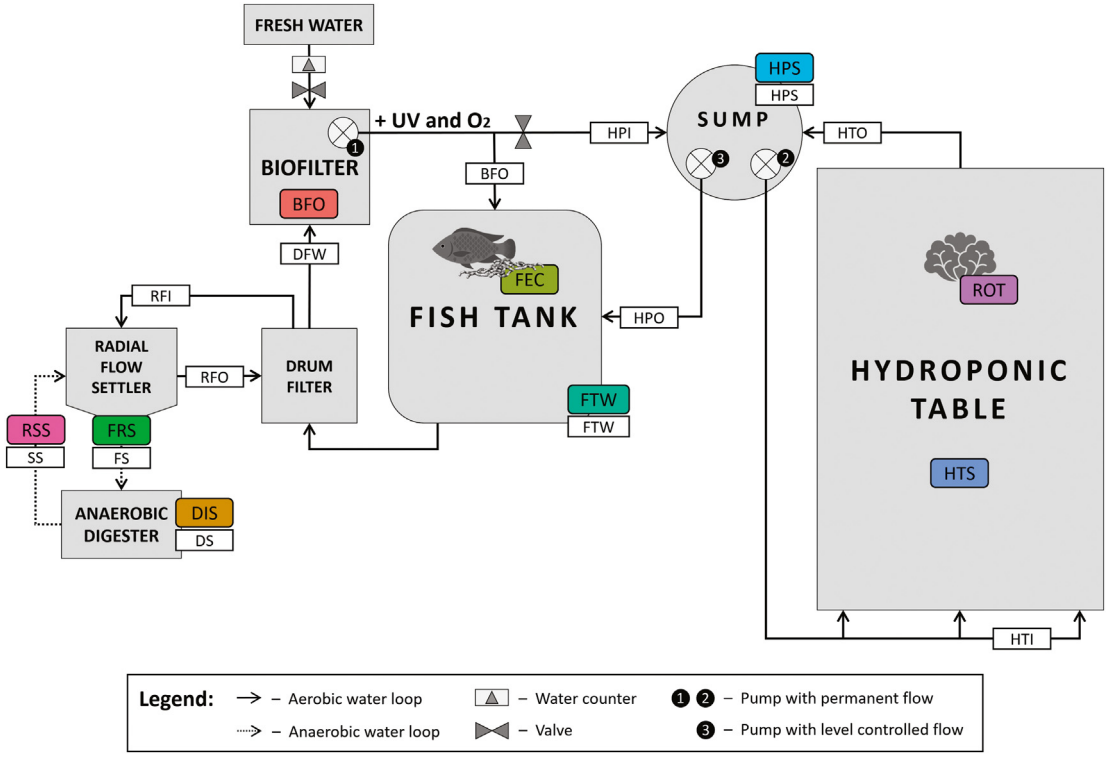

Fig. 1. Water flow and sampling points in the aquaponic system. Microbial sampling points (FTW, fish tank wall surface; HPS, sump surface; HTS, hydroponic table surface; BFO, biofilter material; ROT, roots; FEC, fish feces; FRS, settled fresh sludge; DIS, digested sludge; RSS, supernatant of digested sludge returned back to the system) are marked in color and the chemistry sampling points (DFW, drum filter outflow water; BFO, biofilter outflow water; FTW, fish tank water; HPI, inflow into hydroponic part of the system; HPS, sump water; HTI, hydroponic table inflow; HTO, hydroponic table outflow; RFI, radial flow settler inflow; RFO, radial flow settler outflow; FS, settled fresh sludge; DS, digested sludge; SS, supernatant of digested sludge returned back to the system) are marked in white rectangles.

stocked with Nile tilapia (*Oreochromis niloticus*, stocked at 20 ± 0.2 kg per system) and 63 lettuce plants (*Lactuca sativa* cultivar "YACHT" Salanova) for three lettuce cycles (Supplementary file: Table S1), as previously described (Schmautz et al., 2021b). Experiments were conducted with the authorization of the Veterinary office of Kanton Zürich (Switzerland), no. ZH020/17.

2.1. Sampling procedure

Sampling for chemical and microbial analysis was performed six times (Supplementary file: Table S1) at different locations (Fig. 1) in the systems, at the beginning and at the end of each lettuce growth cycle. Samples were taken from the fish tank (FTW), sump (HPS) and hydroponic table (HTS) surfaces using a cotton swab, by scrubbing an approx. 100 cm^2 surface area. The moving-bed biofilter material (BFO) was sampled by collecting approximately 20 biocarriers (HDPE biocarrier media, 10 × 10 mm, surface of approx. 800 m^2 per m^3). The sampling of the roots (ROT) was performed by cutting a few fine roots from three randomly selected lettuce plants from each replicate system. Fish feces (FEC) were obtained by stripping one anesthetized fish from each system. To obtain samples of microbial community in fresh sludge (FRS), digested sludge (DIS) and the supernatant of digested sludge returned to the system (RSS), 1.5 mL of each was collected (Supplementary file: Table S2). The sampling procedure for chemical analyses is described in the supplementary material (Supplementary file: Supplementary methods and Table S3).

2.2. Microbial sample preparation and DNA extraction

Microbial biomass from the FTW, HPS, HTS, BFO, ROT samples was obtained by adding ultrapure water (Supplementary file: Table S2), vortexing the tubes with the sample material for 1 min, followed by 5 min in an ultrasonic bath at room temperature (Sonorex, Bandelin, Berlin, Germany). The tubes were then vortexed for an additional 2 min, followed by 10 min in the ultrasonic bath (37 kHz, 100 % power). Subsequently, any remaining material, such as biocarriers, plant roots or cotton swabs, were removed from the tubes using forceps. Prior to DNA extraction, the samples (excluding FEC) were formed into pellets of microbial biomass by centrifugation (2300 *rcf*, 10 min). All of the samples were extracted using a DNeasy PowerSoil Kit (Qiagen, Venlo, The Netherlands), according to the manufacturer's instructions but with the following changes: instead of 0.25 g soil sample, a pellet mixed with homogenization buffer was transferred to the glass bead tube and the FEC samples were directly transferred to the tube. For homogenization and cell lysis, a FastPrep-24 (MP Biomedicals, Irvine, California, United States) was used for 45 s at a speed of 4 m s^{-1}. Following extraction, the samples were stored at −20 °C until further analysis.

2.3. Microbial sample analysis

Amplicons for the bacterial 16S rRNA genes (V1-V3) were generated using the primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 534R (5′-ATTACCGCGGCTGCTGG-3′), which have already been used in previous 16S rRNA studies (Eck et al., 2019; Schmautz et al., 2017), and the primers Arch516F (5′-TGYCAGCCGCCGCGGTAAHACCVGC-3′) and Univ806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used for the archaeal 16S rRNA genes (V4) (Kuroda et al., 2015; Liu et al., 2018). Both primer sets used their respective adapters (Supplementary file: Table S4). After a first targeted amplification and clean-up, the samples were uniquely barcoded using dual-indexing with a Nextera XT Index kit v2 (Illumina, San Diego, CA, United States). After a second amplification and clean-up, the samples were normalized, amplicon size and quality verified, before being pooled into two separate libraries for the bacteria and archaeal primer sets (Supplementary file: Supplementary methods). Library preparation and sequencing were done at the Genetic Diversity Centre (ETH Zurich, Switzerland) using an Illumina MiSeq platform with v3 reagents and 600 cycles (paired-end 300 nt).

2.4. Bioinformatics

Quality control of the raw data was performed using FastQC (Andrews, 2010) within the Illumina sequencing pipeline. To trim the read-ends and merge read pairs into amplicons "seqtk" (https://github.com/lh3/seqtk) and "FLASH" v1.2.11 (Magoč and Salzberg, 2011) were used with default parameters. The primer sites was trimmed using USEARCH (*in-silico* PCR) v10.0.240_i86linux64 and v11.0.667_i86linux64 for bacterial and archaeal primer sets, respectively (Martin, 2011). Quality filtering was carried out using "prinseq" v0.20.4 (Schmieder and Edwards, 2011) with a size range of 250–550 bp and 100–500 bp for bacterial and archaeal primer set, respectively, a GC range of 30–70 %, a minimum quality mean > 20, and a Ns of 0 (Supplementary files: Bact_181113_ReadReport.xlsx and Arch_181210_ReadReport.xlsx). USEARCH (Edgar, 2010) was used to dereplicate the quality filtered data (usearch:fastx_uniques), followed by correction of any sequencing errors and removal of chimeras (usearch:unoise3). All unique sequences with abundances greater than eight produced with "unoise3" generated zero-radius operational taxonomic units (ZOTUs) (Edgar, 2016a). The data were further clustered (usearch:cluster_smallmem) so that the ZOTUs were defined at 99 % nucleotide sequence identity. A heuristic approach to mapping the amplicons to the ZOTUs for finding the alignment with the highest score was used and a count table with information on read numbers was generated (usearch:otutab). Taxonomic predictions were made with SINTAX (Edgar, 2016b) using the SILVA 16S rRNA reference database v132 (Quast et al., 2013). The raw data is available under the study accession number PRJEB40280 in the database of the European Nucleotide Archive (ENA).

2.5. Data analysis

All further data analysis was carried out using the R statistical software v3.6.1 (R Core Team, 2019). The analysis of the microbial communities was performed separately for the bacterial and archaeal datasets. For the alpha diversity analysis (observed ZOTU, Chao1, Shannon and InvSimpson), raw ZOTU counts were used and analyzed using *phyloseq* package v1.28.0 (McMurdie and Holmes, 2013). Sampling depth was addressed with a rarefaction curve using raw read counts and analyzed using the *vegan* package v2.5–6 (Oksanen et al., 2019). Further analyses were done using a rarefied dataset with the removal of the lowest 0.005 % ZOTUs. In the archaeal dataset, 25 samples with <1000 reads were removed from further analyses. However, in the bacterial dataset, samples with low counts plateaued, thus sample removal was unnecessary. Clustering was performed using the *microbiome* package v1.7.21 (Lahti and Shetty, 2017), network analysis was done using *phyloseq* and the error rate was determined using the *randomForest* package v4.6–14 (Liaw and Wiener, 2002). Classification plots, constrained analysis of principal coordinates (CAP) and multidimensional scaling (MDS) were used to analyze and describe shifts in the community using the *phyloseq* package. To test the significance of the observed Bray-Curtis dissimilarity between different sampling points, the ADONIS function in the *vegan* package was used.

3. Results and discussion

During the 12-week experiment, data was collected from three lettuce cycles (Supplementary file: Table S1). During one cycle, the fish gained on average 4.4 kg per system and 11.6 kg of lettuce biomass was produced. Bacterial and archaeal communities were analyzed using 16S rRNA gene amplicon sequencing. The Q_{30} of the bacterial and archaeal reads in the samples were on average 70.5 % and 81.8 %, respectively. A total of 3,165,652 (average per sample: 20556 ± 6744) bacterial and 843,060 (average per sample: 6435 ± 7639) archaeal reads were analyzed for this study.

3.1. Microbial diversity parameters

A total of 4323 and 417 ZOTUs were assigned to the bacterial and archaeal datasets, respectively. Based on rarefaction plots (Supplementary file: Fig. S1), the number of reads was the lowest in the FEC samples. In contrast, the samples from other compartments of the system had relatively high total numbers in both the archaeal and bacterial data sets. This trend was also observed for different alpha diversity measures (Supplementary file: Fig. S2A and Fig. S2B). Shannon indices revealed a more diverse community in the HTS, HPS, BFO and ROT samples, while the lowest values in both primer sets were for the FEC samples. When comparing the bacterial and archaeal datasets, there were 10 times as many ZOTUs observed in the former. In the archaeal dataset, only 40 ZOTUs were assigned to archaea, while the remaining ZOTUs were assigned to the bacterial community. After merging ZOTUs on the genus taxonomic level, reads were assigned to 575 bacterial taxa using a bacterial primer set and 7 archaeal taxa using an archaeal primer set. To allow for a more in-depth analysis of the genera present in different compartments, it was decided to focus on the ZOTUs that represent >0.005 % of the total sequencing depth, leaving 402 bacterial ZOTUs, with the number of archaeal ZOTUs remaining unchanged.

3.2. Microbial diversity in different compartments

Beta diversity was used to test whether each compartment of the aquaponic systems represented a unique microenvironment. The microbial community compositions were clustered by compartment (Fig. 2, Supplementary file: Fig. S3) with each compartment hosting different bacterial (Fig. 3A) and archaeal (Fig. 3B) populations. The most noticeable differences were observed between fish feces (FEC), water loop (BFO, FTW, HPS, HTS, ROT) and sludge (FRS, DIS, RSS) samples. In the archaeal dataset, the community was clustered into two groups: samples originating from aerobic (BFO, FTW, HPS, HTS, ROT) and sludge (FRS, DIS, RSS) compartments.

In most freshwater habitats, such as the aerobic compartments in this study (BFO, FTW, HPS, HTS), archaeal communities commonly present <10 % of the total rRNA phylotypes (Robertson et al., 2005). Using archaeal primers, the sludge samples (FRS, DIS, RSS) had high total reads, while the aerobic samples had low total reads, which were mostly assigned to the bacterial community (Fig. 3B, Supplementary file: Fig. S4 and Fig. S5). It is possible that low archaeal abundance in aerobic samples may have led to an increased mispriming rate during amplification (Boyle et al., 2009; Pausan et al., 2019).

3.2.1. Biofilter material (BFO)

The biofilter is one of the most studied compartments of recirculating aquaculture and aquaponic systems, and the communities in the BFO samples resembled those previously studied (Bartelme et al., 2017; Eck et al., 2019; Schmautz et al., 2017). The members of the *Alphaproteobacteria,* a well-known class of oligotrophs, represented >35 % of the bacterial population. At the family level, the highest proportion of sequences in the biofilter samples were dominated by groups of *Planctomycetaceae* (mean relative abundance of 9.3 %), *Hyphomicrobiaceae* (8.6 %), *Cytophagaceae* (6.2 %), *Chitinophagaceae* (5.9 %) and *Sphingomonadaceae* (5.7 %), with approximately 4.8 % of the reads not assigned.

At the genus level, *Hyphomicrobium*, *Novosphingobium*, *Meiothermus* and *Nitrospira* were the most abundant taxa (>4 %) in the BFO samples. *Hyphomicrobium* is a methylotrophic bacterium commonly found in soil and aquatic habitats and can grow in the presence of a range of carbon compounds (Moore, 1981; Oren and Xu, 2014; Poindexter, 2006). While most species proliferate aerobically, some can also do so under anaerobic conditions and may be involved in the denitrification process (Kloos et al., 1995; Moore, 1981). *Meiothermus* is a member of the UV radiation resistant phylum *Deinococcus-Thermus* (Rosenberg, 2014), members of which have also previously been found in other aquaponic systems using UV treatment (Eck et al., 2019; Schmautz et al., 2017). A bacterium commonly found in biofilms of human-made aquatic systems, *Pedomicrobium* (Sly et al., 1988), was also identified.

In contrast to the highly diverse bacterial community, the archaeal community in the biofilter was dominated by just four families of the phylum Euryarchaeota, *Methanobacteriaceae* (67.1 %), *Methanosarcinaceae* (21.5 %) and *Methanoregulaceae* (4.6 %), which may be involved in methanogenesis (Oren, 2014a, 2014b, 2014c), and an additional archaeon from the order *Thermoplasmatales Incertae Sedis* (5.8 %), commonly found in water systems (Reysenbach and Brileya, 2014).

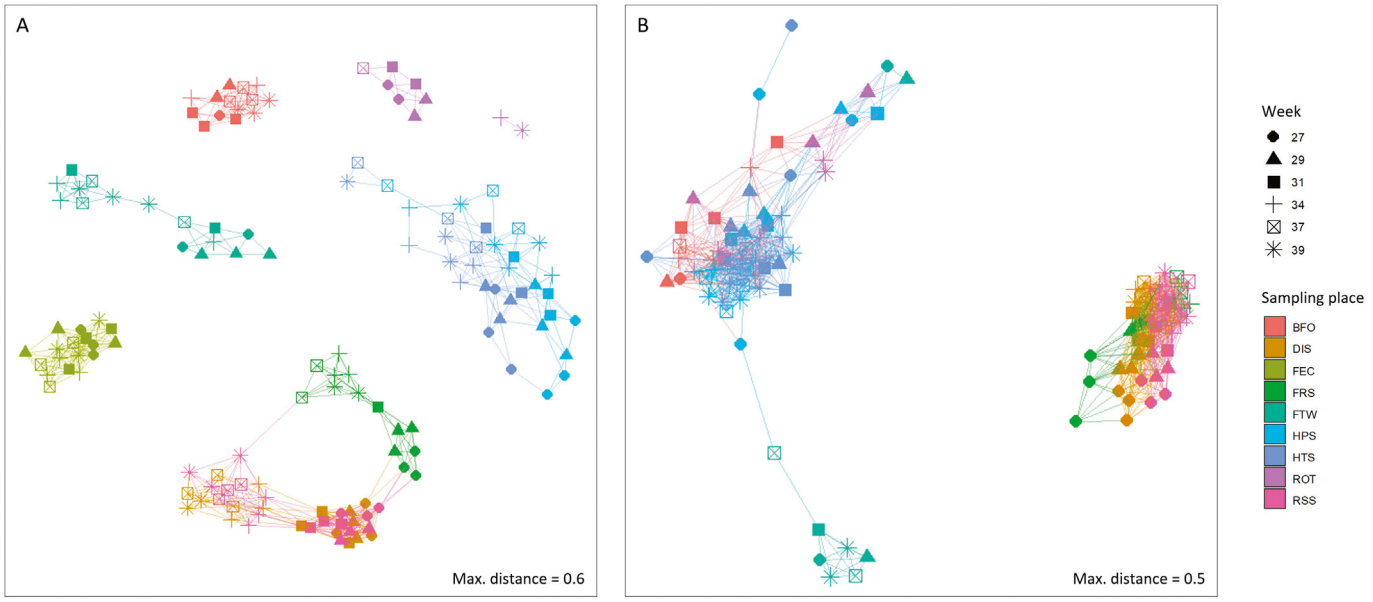

Fig. 2. Network clustering using Bray-Curtis distance between different compartments in the aquaponic system (FTW, fish tank wall surface; HPS, sump surface; HTS, hydroponic table surface; BFO, biofilter material; ROT, roots; FEC, fish feces; FRS, settled fresh sludge; DIS, digested sludge; RSS, supernatant of digested sludge returned back to the system), where bacterial primer set (A) could predict the origin of the sample with <5 % error rate and archaeal primer set (B) with a more prominent 38 % error rate. The connection between nodes signifies the distance between samples.

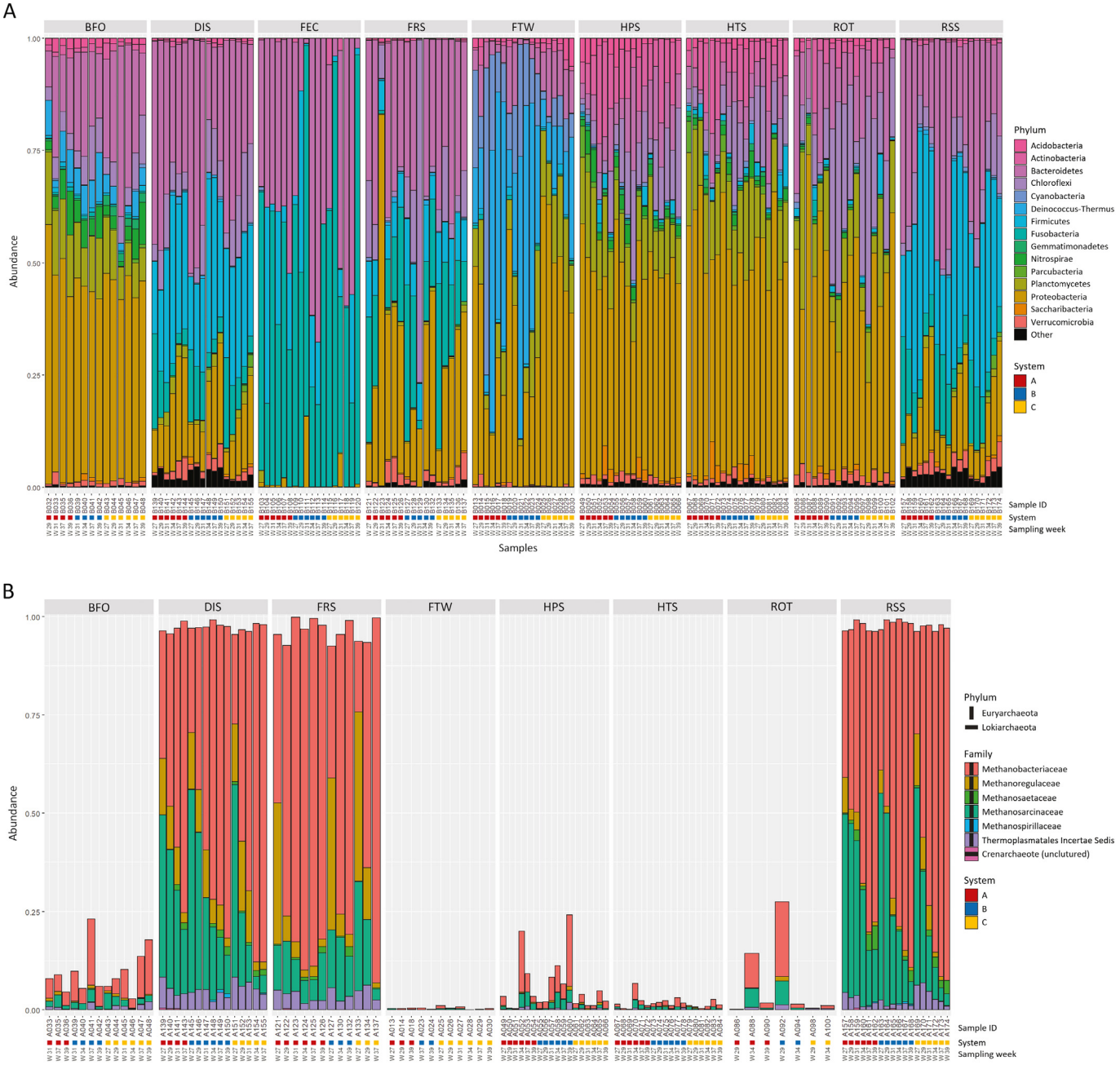

Fig. 3. Classification of bacterial (A) and archaeal (B) community in different compartments in the aquaponic system (FTW, fish tank wall surface; HPS, sump surface; HTS, hydroponic table surface; BFO, biofilter material; ROT, roots; FEC, fish feces; FRS, settled fresh sludge; DIS, digested sludge; RSS, supernatant of digested sludge returned back to the system) as a percentage of the total population.

3.2.2. Fish tank surface (FTW)

Members of *Deinococcus-Thermus* phylum represented >27 % (*Deinococcaceae,* genus unknown – 22.5 % and *Meiothermus* – 5.4 %) of the total bacterial community in the FTW samples. The abundance of *Cyanobacteria* (13.8 %) was dominated by *Synechococcus*, an obligate photoautotroph (Vilibić and Šantić, 2008). At the family level, the highest proportion of sequences in the FTW samples belonged to the bacterial groups *Rhodobacteraceae* (11.6 %), *Alcaligenaceae* (10.9 %) and *Planctomycetaceae* (8.9 %). Since the fish tank was the only compartment of the system directly exposed to the sunlight, finding photosynthetic organisms such as *Synechococcus*, *Rhodobacter* and *Chloracidobacterium* could be foreseen. Organisms commonly found in freshwater systems, such as *Pirellula*, *Limnohabitans* and *Lysobacter* (Dedysh et al., 2020; Gómez Expósito et al., 2015; Kasalický et al., 2013) were also observed. In contrast to the highly diverse bacterial community, the archaeal community was found to be dominated by the same families as already observed in the BFO samples. The only difference was that the member of *Thermoplasmatales Incertae Sedis* was not detected on the surface of the fish tank.

3.2.3. Fish feces (FEC)

The microbial community in the fish feces was different to the other samples, with *Fusobacteria*, *Bacteroidetes* and *Firmicutes* representing >97 % of all of the assigned bacterial reads. A substantial proportion of sequences belonged to the genus *Cetobacterium* (50.1 %), a common bacterium found in the fish gut (Tsuchiya et al., 2008). Furthermore, undescribed members of the families *Porphyromonadaceae* and *Macellibacteroides*, as well as *Clostridium* and *Turicibacter,* all common bacteria found in the intestinal tract (Auchtung et al., 2016; Larsen et al., 2014;

Sakamoto, 2014), were also observed. Archaeal community counts were too low to identify specific archaeal taxa present in the FEC samples. This was likely due to a lack of archaea in the fish feces.

3.2.4. Hydroponic compartments (HPS and HTS) and root samples (ROT)

Samples originating from the hydroponic components, HTS, HPS and ROT, mainly contained the same microbial communities. However, the relative abundances of bacteria differed. In total, >44 % of the reads originated from the phylum *Proteobacteria*. Surface samples from the HTS and HPS were dominated by the bacterial groups *Planctomycetaceae* and *Methylobacteriaceae* (>5.7 % of the bacterial population), both commonly present in aquatic and soil systems (Kelly et al., 2014; Lage and Bondoso, 2014). Approximately 10 % of the HPS and HTS bacterial reads could not be assigned to a family.

The ROT samples were dominated by bacterial representatives of *Herpetosiphonaceae* (13.4 %), *Planctomycetaceae* (9.5 %) and *Comamonadaceae* (5.7 %), and 3 % of the reads could not be assigned. The ROT samples were dominated by *Herpetosiphon*, a chemoheterotrophic bacterium present in freshwater, marine and soil environments (Hanada, 2014; Quinn and Skerman, 1980), representing >13.4 % of the total reads. The same genus was also found in the FRS samples, with *Pirellula*, *Rhizobium* and an undescribed member of the family *Caldilineaceae* being the most abundant taxa (>4 %). *Sphingobium, Ideonella* and *Flavobacterium*, commonly found in soil, aquatic systems and plant-associated habitats (Glaeser and Kämpfer, 2014; Noar and Buckley, 2009; Waśkiewicz and Irzykowska, 2014), were also present. While commonly found in the rhizosphere, *Pseudomonas* (Dennert et al., 2018), were only present in low abundance. In contrast to the highly diverse bacterial community, the archaeal community was found to be dominated by a similar archaeal community composition to that found in the biofilter.

3.2.5. Fresh (FRS) and digested sludge (DIS and RSS)

Fresh sludge (FRS) sampled in the outflow from radial flow settler and samples from the anaerobic digester (DIS and RSS) showed an clear change in microbial community composition during the experimental period in terms of both archaeal and bacterial communities (Fig. 3). Furthermore, changes in numbers of present taxa were also observed between the first sampling in calendar week 27 and the last sampling in calendar week 39 (Supplementary file: Fig. S6). The steady increase in biodiversity over the experimental period can be explained by the slow and gradual development of anaerobic communities, as reported by Goux et al. (Goux et al., 2016).

The archaeal community in the DIS samples was dominated by the archaeal families *Methanobacteriaceae*, *Methanosarcinaceae* and *Methanoregulaceae*. Relative abundances were recorded at 27 %, 47 %, and 15 % at the first sampling and 79 %, 10 % and 2 % at the last sampling for each family, respectively. A similar pattern was observed in the RSS samples. While FRS samples showed an increase in *Methanobacteriaceae* and a decrease in *Methanoregulaceae*, the abundance of *Methanosarcinaceae* was stable over time. In the anaerobic digestion process, two groups of microorganisms are classified depending on the primary metabolic pathway they use to produce biogas. Acetoclastic methanogens, members of the family *Methanosarcinaceae,* produce methane from acetic acid, while hydrogenotrophic methanogens, members of the family *Methanobacteriaceae*, produce methane from hydrogen and carbon dioxide (Liu et al., 2016; Song et al., 2010). The dominance of one group over the other is related to the environmental conditions. Conditions such as increased levels of ammonium, highly volatile fatty acid (VFA) concentrations and high-solids environments are favorable for hydrogenotrophic organisms, while the presence of acetate, lower ammonium concentrations and lower salt concentration environments promote the growth of often dominated acetoclastic methanogens (Franke-Whittle et al., 2014; Liu et al., 2016; Stams et al., 2019). As only the supernatant was removed from the anaerobic digester during the experimental period, solids accumulated in the anaerobic digester over time. The resulting increase in ammonium, solids and potentially also VFAs may explain the dominance shift from acetoclastic methanogens to hydrogenotrophic methanogens.

The bacterial community in the DIS samples was dominated by the phyla *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Fusobacteria* with relative abundances of 47 %, 19 %, 7 % and 19 % respectively at the beginning, and 27 %, 28 %, 22 % and 3 % respectively at the end of the experimental period. The same pattern was observed for the bacterial community in the FRS and RSS samples. The dominance of *Bacteroidetes*, *Firmicutes* and *Proteobacteria* in anaerobic digesters is known (Liu et al., 2016; Nsanzumukiza et al., 2018; Shin et al., 2019; Sun et al., 2015) since bacteria from these groups perform hydrolytic and acidogenic functions within the anaerobic digestion processes. *Fusobacteria*, originating from fish feces, on the other hand, has been associated with acidogenic and fermentative functions in sludge originating from aquaculture (Saito et al., 2017) and piggery slurries (Pampillón-González et al., 2017). The shift in community composition during the experimental period showed an increase in *Proteobacteria* and *Firmicutes* population and, at the same time, a reduction in *Bacteroidetes* and *Fusobacteria*. The change in bacterial community composition may be partially explained by possible VFA accumulation in the digester. Accumulation of VFA suggests that fermentation activity, in which *Fusobacteria* are involved, was followed by the promotion of *Proteobacteria*, which are dominant among VFA-utilizing communities in the sludge (Ariesyady et al., 2007; Guo et al., 2015).

Results showed that the bacterial communities were distinct in the different compartments (ADONIS $R^2 = 0.64, p < 0.001$). The same was shown for the archaeal dataset (ADONIS $R^2 = 0.73, p < 0.001$). Overall, it was possible to predict the origin of the bacterial dataset samples with an error rate of <5 %, while the error rate for the archaeal primer set was higher (38 %). Differing community structures in the compartments could be clearly observed through MDS of both datasets (Supplementary file: Fig. S7).

3.3. Influence of the environmental parameters on the microbial community

To assess the influence of environmental conditions on the microbial community, the measured abiotic parameters with the most relevant nutrients were correlated with a 16S rRNA gene bacterial community and plotted in a 2-dimensional matrix (Fig. 4). The fish feces samples had to be excluded from this analysis since these samples were not chemically analyzed. The results explain the >39 % variation in the community. Ammonium, phosphate and electrical conductivity exhibited a strong influence in the anaerobic compartments, while dissolved oxygen, nitrate and sulfate had a strong influence in the aerobic compartments. Combining environmental parameters with microbial data revealed significant differences between aerobic and anaerobic compartments, as previously observed (Schmautz et al., 2021a).

3.4. Nitrogen cycle-related taxa

One of the most studied processes in the nitrogen cycle is nitrification. Ammonium oxidizing bacteria, ammonium oxidizing archaea and nitrite oxidizing bacteria are all involved in nitrification (Hu and He, 2017) and COMAMMOX bacteria, i.e. *Nitrospira* spp., can perform complete nitrification (Daims et al., 2015). In this study, the BFO samples contained large numbers of *Nitrospira* (4.9 %), with the same genus also found in the hydroponic compartment samples HPS (2.4 %) and HTS (1.6 %). This would support the assumption that the nitrification process also occurs in these parts of the aquaponic system (Schmautz et al., 2021b, Schmautz et al., 2021a). An unknown genus of the *Nitrosomonadaceae* family, comprising the genera *Nitrosomonas*, *Nitrosolobus* and *Nitrosospira*, all known to be organisms capable of nitrification (Prosser et al., 2014), was most abundant in HPS (2.4 %), whereas their abundance in the BFO samples was lower (1.5 %). Furthermore, a possible ammonia-oxidizing archaeon from the phylum *Thaumarchaeota* (Pester et al., 2011; Stieglmeier et al., 2014) was present in very low numbers in the BFO and HTS samples.

Potential nitrogen-fixing *Rhizobium* and *Devosia* (Fischer, 1994; Rivas et al., 2002) were found on the lettuce roots, while *Bradyrhizobium* (Fischer, 1994) but there were higher counts in the HTS samples. Next to well-known nitrogen fixing bacteria, *Novosphingobium,* a bacterium associated with plant and freshwater habitats (Glaeser and Kämpfer, 2014) and

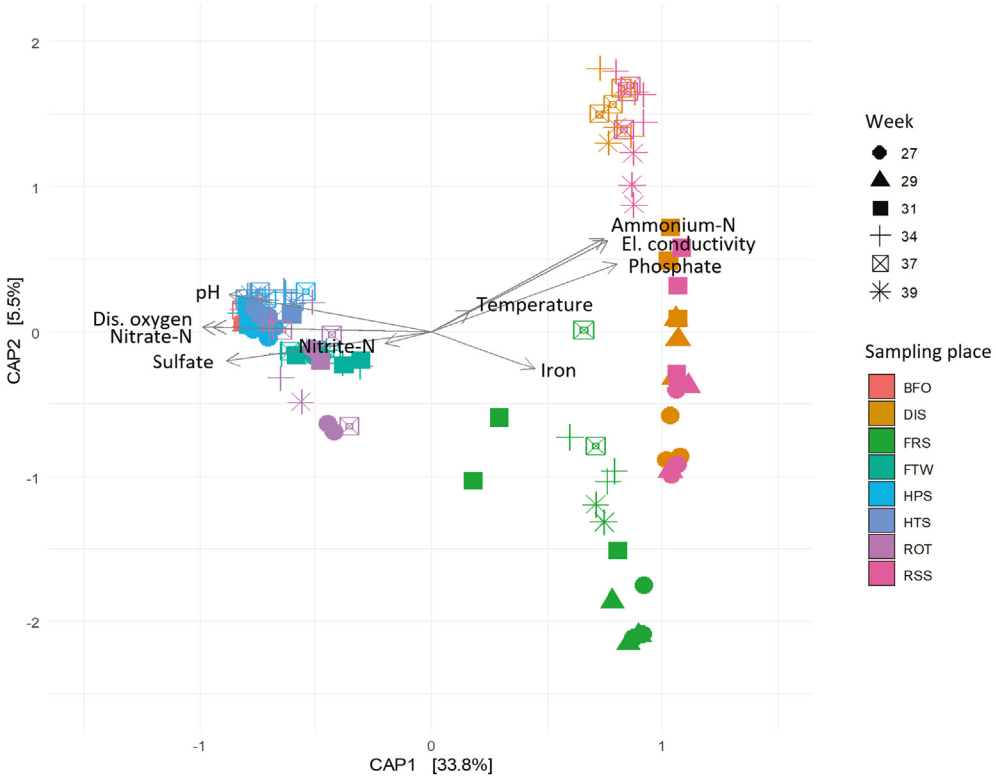

Fig. 4. Constrained Analysis of Principal Coordinates of the bacterial community structure in different compartments in the aquaponic system (FTW, fish tank wall surface; HPS, sump surface; HTS, hydroponic table surface; BFO, biofilter material; ROT, roots; FEC, fish feces; FRS, settled fresh sludge; DIS, digested sludge; RSS, supernatant of digested sludge returned back to the system) over the 12-week experimental period based on Bray-Curtis distance with the abiotic parameters and most relevant water chemistry parameters.

for which some strains are known to fix nitrogen (Addison et al., 2007), was found in high abundance in the BFO samples (5.4 %). A nitrogen-fixing archaeon from the family *Methanosarcinaceae* (Oren, 2014b) was present in high abundance in all of the anaerobic samples.

Denitrification, anaerobic ammonium oxidation (ANAMMOX) and dissimilatory nitrate reduction to ammonium (DNRA) are nitrate respiration pathways in the microbial nitrogen cycle. While denitrification and ANAMMOX pathways produce dinitrogen and nitrous oxide gas, thus leading to loss of dissolved nitrogen from water, DNRA retains dissolved nitrogen by recycling nitrate to ammonium (Bu et al., 2017; Song et al., 2014). Denitrification is carried out by various facultative heterotrophic bacteria and archaea, such as *Azospira*, *Vogesella, Clostridium, Acidovorax*, *Flavobacterium*, *Hydrogenophaga* and *Thermomonas* (Adrados et al., 2014; Bellini et al., 2013; Caskey and Tiedje, 1980; He et al., 2018; Hoshino et al., 2005; Mergaert et al., 2003; Rossi et al., 2015), which were all observed in the sludge samples. Despite the relatively large phylogenetic distance, all ANAMMOX organisms belong to the same order, *Brocadiales* (van Niftrik and Jetten, 2012), of which *Candidatus* Brocadia was observed in the FTW samples. In environments such as the anaerobic digester, with high salinity, high carbon to nitrate ratios, high concentrations of sulfide and elevated temperature, microorganisms favor the DNRA pathway over denitrification (Bu et al., 2017; Dong et al., 2011; Giblin et al., 2010; Schmidt et al., 2011; Song et al., 2014). Microorganisms capable of the DNRA pathway, such as *Desulfovibrio*, *Ignavibacterium* and undescribed members of the families *Draconibacteriaceae*, *Holophagaceae*, and *Caldilineaceae* (Han et al., 2020; Pandey et al., 2020; Song et al., 2014) were observed in sludge samples with higher ammonium concentrations.

4. Conclusions

The findings reported above have shown distinct differences in community composition between compartments of aquaponic systems, providing insights into spatial and temporal distribution, which is likely the result of the specific plant variety, fish stocking, feed composition, environmental conditions and nutrient abundances (Schmautz et al., 2021a; Schreier et al., 2010). Higher microbiome diversity was observed in the aerobic loop of the system. Furthermore, anaerobic communities were also detected in the aerobic parts of the system. This can be explained by the connected water loop and analysis method, which was limited to determining the presence of specific microorganisms and their localized distribution within the system, disregarding their activity. Thus, future research dealing with the isolation and quantitative assessment of active microbial populations and gene expression for specific biological processes, such as the nitrogen cycle-related pathways, methanogenesis and sulfate reduction (Schreier et al., 2010), is necessary. Additionally, the importance of archaea, although not significant within this study, needs to be investigated in different setups of aquaponic systems.

CRediT authorship contribution statement

Zala Schmautz: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft; Jean-Claude Walser: Data curation, Formal analysis, Visualization, Writing – review & editing; Carlos A. Espinal: Investigation, Methodology, Writing – original draft; Florentina Gartmann: Investigation, Writing – review & editing; Ben Scott: Investigation, Writing – review & editing; Joël F. Pothier: Software, Writing – review & editing; Emmanuel Frossard: Conceptualization, Supervision, Writing – review & editing; Ranka Junge: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing; Theo H.M. Smits: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Finances

This work was supported by the Swiss National Science Foundation (SNF project 310030_169665) and by the Department of Life Sciences and Facility Management of the Zurich University of Applied Sciences.

Data availability

Data will be made available on request.

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.

Acknowledgements

The authors would like to thank Rahel Wanner (Zurich University of Applied Sciences, Wädenswil, Switzerland), Anja Jerak, Ajda Kranjc Požar and Jena Jamšek (University of Ljubljana, Slovenia), Joel Miguel Pérez Cortes and Susana Berenice Zarzoza Mora (Chapingo Autonomous University, Mexico State, Mexico) for their support during the experiments and laboratory work, as well as Misha Teale and Darren Mace (Zurich University of Applied Sciences, Wädenswil, Switzerland) for their critical review of the manuscript. The authors would also like to thank the group at Genetic Diversity Centre (ETH Zurich, Switzerland) for access to analytical equipment and, especially, Silvia Kobel for technical support.

Appendix A. Supplementary data

Supplementary information file (Supplementary_information.pdf) includes Supplementary methods, Supplementary tables and Supplementary figures. Supplementary files (Bact_181113_ReadReport.xlsx and Arch_181210_ReadReport.xlsx) include a report on the removed reads during the bioinformatical analyses. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.158426.

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