

Microbial diversity in different compartments of an aquaponics system

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Abstract Aquaponics is a solution for sustainable production of fish and plants in a single semi-closed system, where nutrient-rich water from the aquaculture provides nutrients for plant growth. We examined the microbial communities within an experimental aquaponics system. Whereas the fish feces contained a separate community dominated by bacteria of the genus *Cetobacterium*, the samples from plant roots, biofilter, and periphyton were more similar to each other, while the communities were more diverse. Detailed examination of the data gave the first indications to functional groups of organisms in the different compartments of the aquaponic system. As other nitrifiers other than members of the genus *Nitrospira* were only present at low numbers, it was anticipated that *Nitrospira* may perform the nitrification process in the biofilm.

Keywords Community analysis · Metagenome · *Tilapia* · Lettuce · *Nitrospira*

Introduction

Aquaponic (AP) systems are sustainable multi-trophic-integrated quasi-closed-loop food production systems with low environmental impact considering that food is produced with low water consumption (Endut et al. 2011; Somerville et al. 2014; Goddek et al. 2016). AP combines a recirculating aquaculture system with a hydroponic unit. One of its most important features is the reliance on bacteria and their metabolic products. Bacteria serve as the bridge that connects the fish excrements, which is high in ammonium concentration, to the plant fertilizer, which should be a combination of low ammonium and high nitrate (Somerville et al. 2014). However, the total microbial community in different compartments of the AP systems has not been characterized yet using—omics technologies (Munguia-Fragozo et al. 2015). As the AP systems can have different subunits, i.e., fish tank, biofilter, drum filter, settler, or hydroponic, with each of them having different possible designs and different optimal conditions, the microbial communities in these components may differ drastically, and are, therefore, interesting to analyze, the ultimate goal being improved steering of the processes.

Until now, nitrifying bacteria are the best studied group of environmental importance in the AP systems (Tokuyama et al. 2004; Revsberg et al. 2006; Zou et al. 2016a, b). Within this group, the ammonium-oxidizing bacteria (AOB), who convert ammonium to nitrite, are of particular interest. This group of chemolithotrophic bacteria was restricted to two evolutionarily distinct lineages of the class Proteobacteria (Kowalchuk and Stephen

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2001), although also ammonium-oxidizing archaea are known (Brown et al. 2013). For the conversion of nitrite to nitrate, nitrite-oxidizing bacteria (NOB) are responsible, e.g., *Nitrospira* sp. or *Nitrobacter* sp. Recently, it was described that a *Nitrospira* sp. is also able to perform the complete nitrification from ammonium to nitrite (Daims et al. 2015). On the other hand, organisms that perform denitrification, ANAMMOX, or dissimilatory nitrate reduction are also known (Rurangwa and Verdegem 2015). This is a more heterogeneous group of organisms that may exert a substantial influence on the total nitrogen cycle in the AP.

As in most AP systems, only the water column has been sampled to characterize the microbial community or populations thereof, we intend to characterize the microbial diversity in the different compartments of the aquaponics system. For this, we have taken samples from three positions in the aquaponics system plus a sample of the fish feces. A community pattern based on amplicon sequencing of 16S rRNA genes was generated. Based on these, it was possible to gain insight in the functioning of the different compartments, such as fish tank, biofilter, and root system in the hydroponics unit.

Materials and methods

The AP system that was operated (Graber et al. 2014) consisted of one aquaculture unit connected to three hydroponic units, each of which consisted of three deep water culture basins. The system water flow is described in legend of Fig. 1. The experiment started in calendar week 47, 2013 by stocking 179 fish of 70 g size and inserting 648 lettuce seedlings. Before, another AP experiment had been conducted in the systems until mid-October; in the days before starting this experiment, the aquaculture system was kept in operation to maintain the biofilter function. The experiment entailed to grow *Tilapia* (*Oreochromis niloticus*, pink strain) using a commercially available extruded floating feed (Tilapia Vegi, Hokovit from Hofmann Nutrition AG, Bützberg, CH) in a semi-closed loop AP system with lettuce (*Lactuca sativa*, oak leaf variety Kiber from Rijk Zwaan) growing on floating rafts (Dryhydroponics, BV's-Gravenhage, NL). Five weeks before the beginning of the experiment, single pelleted lettuce was seeded into rock wool. System water was analyzed 3-weekly with HACH Lange LCK tests and a DR 3800 VIS Spectrophotometer (HACH Lange, Loveland, CO, USA), and HydroBuddy free software (Fernandez 2013) was used to calculate the

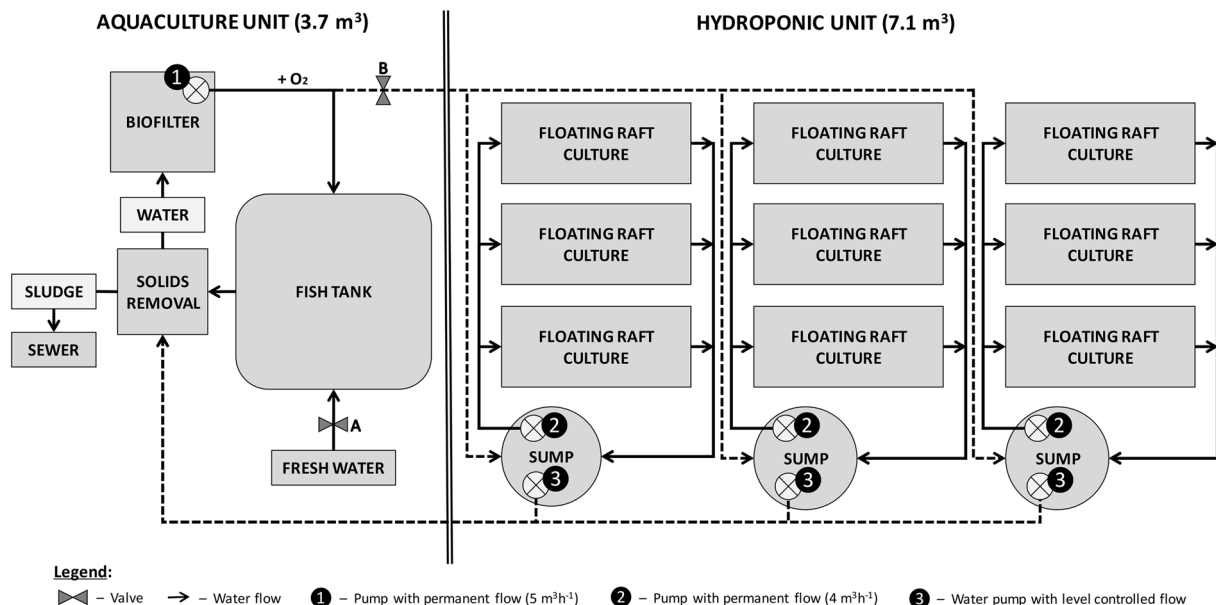


Fig. 1 Water flow in “Wädenswil aquaponic system” in December, 2013. Fresh water entered the system through valve A, controlled with the UF controller software (UrbanFarmers, Zurich, CH) and kept constant water level in the system. Water from the fish tank was continuously flowing to the solids removal unit (drum filter with 60-micron mesh size) and from here into a moving-bed biofilter. A heating element in the biofilter ensured a constant temperature of 28 °C in the system. A submerged circulation pump 1 was pumping water through oxygenation zone and low-pressure UV unit back to the fish tank. Water flow through valve B opened for 1 min every hour at

the beginning, and was increased to 1 min every 5 min on December 11, 2013. This provided an initial water supply of 0.60 m³ day⁻¹ and later of 7.1 m³ day⁻¹, or 8.5% and later 100% per day. Pump 2 was permanently pumping water to the hydroponic subunits and from there over the drainage point back to the sump. Pump 3 controlled by water level was pumping excess water back to the solids removal unit. Rinsing water from regular drum filter cleaning left the system to the sewers, thus about 100 l day⁻¹ or 0.9% day⁻¹ of the total system volume was removed

amount of needed nutrient supplements (Table 1) to meet the standard nutrient concentration for lettuce (Table 2). Electrical conductivity and pH were measured with Hach HQ40d Portable Multi-Parameter Meter.

Sampling

Samples were taken on December 17, 2013. The sample of fish feces was netted from the bottom of the fish tank, where the feces sedimented and accumulated after interrupting water circulation for 30 min. The sample representing the biofilm in the fish tank (periphyton) was scraped off directly from the tank side walls using a spatula. The moving-bed biofilter was sampled by removing 20 biochips (GEA, 2 H Random Media Typ BCN 011, size 11 mm, black polypropylene) with a pincer. The sample of lettuce roots was assembled by cutting one root hair from two random salads in each of the nine basins (total 18 roots). All samples were placed immediately into a 50 ml Falcon tube and transferred to the laboratory. There, all tubes were filled to 50 ml with ultrapure water.

Amplicon sequencing and bioinformatics

Bacteria from biofilter chips and lettuce roots were obtained by adding 50 ml sterile ultrapure water to the tube and vigorous vortexing for 2 min, followed by 5 min

in an ultrasonic bath (Sonorex; Baudelin). Afterwards, biochips and roots were removed with a pincer, and collection of bacteria was done by centrifugation (5000 rpm, 10 min). The pellet was used for DNA extraction. The periphyton sample and the fish feces sample were used directly. All samples were extracted with the MoBio PowerSoil DNA isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to manufacturer's instructions. Sequencing on an Illumina MiSeq (2×300 bp) was done by GATC AG (Konstanz, D) according to the InView™ Microbiome Profiling protocol. Amplicons generated using the primers 27F (5'-AGAGTTTGA TCCTGGCTCAG-3') and 534R (5'-ATTACCGCGGCT GCTGG-3') were targeting the variable regions V1-V3. Reads were analyzed using the program MGX (Sebastian Jaenicke, Stefan P. Albaum, Burkhard Linke, Jens Stoye, and Alexander Goesmann: manuscript in preparation). As the reverse reads were not useful due to a technical failure during sequencing, only the forward reads (from primer 27F) were used for the assignment to the phylogenetic groups. Data were assigned down to genus level using the RDP Classifier (Wang et al. 2007), and analyzed at different taxonomic levels where applicable or required (Tables S1–S3). Diversity measures (Shannon index H' and Shannon evenness E) were calculated as described by Hill et al. (Hill et al. 2003). Data have been made available under the study accession PRJEB15038 at EBI.

Table 1 Nutrients supplementations and supplied nutrients added to the aquaponic system on weekly basis

Nutrient supplementation	Company	Supplied nutrients
Iron DTPA	Ökohum GmbH, Herrenhof, CH	Fe
Multi Micro Mix	Ökohum GmbH, Herrenhof, CH	Mn, Cu, Zn, Mo, B
Krista SOP	Yara UK Limited, Grimsby, UK	K, S
Krista MKP	Yara UK Limited, Grimsby, UK	P, K
YaraLiva® Calcinit	Yara UK Limited, Grimsby, UK	N, Ca
EPSO TOP®	K + S Kali GmbH, Kassel, D	Mg, S

Table 2 Targeted values for each measured parameter in the “Wädenswil aquaponic system”, the corresponding HACH Lange LCK tests, and water analysis results around the sampling date

Parameter	Test method	Target value	Water analysis values		
			Nov. 20, 2013	Dec. 13, 2013	Jan 16, 2014
PO ₄ -P	HACH Lange test 349	35 mg l ⁻¹	15.6 mg l ⁻¹	12.1 mg l ⁻¹	12.9 mg l ⁻¹
NO ₃ -N	HACH Lange test 339	120 mg l ⁻¹	135 mg l ⁻¹	116.2 mg l ⁻¹	84.4 mg l ⁻¹
NO ₂ -N	HACH Lange test 341	1 mg l ⁻¹	0.02 mg l ⁻¹	9 mg l ⁻¹	0.5 mg l ⁻¹
NH ₄ -N	HACH Lange test 304	0 mg l ⁻¹	19 mg l ⁻¹	0.28 mg l ⁻¹	0.18 mg l ⁻¹
K	HACH Lange test 328	150 mg l ⁻¹	175 mg l ⁻¹	77.8 mg l ⁻¹	78 mg l ⁻¹
Fe	HACH Lange test 321	3 mg l ⁻¹	0.1 mg l ⁻¹	0.26 mg l ⁻¹	0.01 mg l ⁻¹
Ca	HACH Lange test 327	200 mg l ⁻¹	N.A	140.8 mg l ⁻¹	111.8 mg l ⁻¹
Mg	HACH Lange test 326	40 mg l ⁻¹	33.8 mg l ⁻¹	45.4 mg l ⁻¹	38.1 mg l ⁻¹
EC	Probe	<1500 μS cm ⁻¹	1686 μS cm ⁻¹	1471 μS cm ⁻¹	1194 μS cm ⁻¹
pH	Probe	6.5	5.61	6.78	7.24

Results and discussion

At the time of sampling, the AP system was running smoothly, but based on the water quality measurements closest to the sampling date (Table 2), the system was still adapting the microbial community to the changed operation mode. This could explain the high values for ammonium on Nov. 20 and for nitrite on Dec. 11. As the tanks were not cleaned between the previous and current experiment, the walls of the fish tanks were covered with a layer of biofilm (periphyton), which was green-brownish in color and approximately 0.5 mm thick. The lettuce was in its 9th week of growth, was not diseased, and had no pests. After sampling for this study, the system was continued to operate for another 5 weeks.

Table 3 Summary of metagenomics data. For each sample, the total number of reads, the amount of reads assigned to RDP, the percentage assigned, and the diversity measures Shannon index and Shannon evenness are given

	Biofilter	Plant roots	Periphyton	Fish feces
Total # reads	104,325	102,729	56,702	156,932
# reads assigned to RDP	90,459	75,247	45,247	102,140
Percentage assigned	86.7%	73.2%	80.5%	65.1%
Diversity measures				
Shannon index (H')	3.93	3.93	3.82	1.18
Shannon evenness (E)	0.63	0.63	0.61	0.19

Microbial diversity parameters

Between 65.1% (fish feces) and 86.7% of the total reads could be assigned to any taxonomic level in the RDP database (Table 3). Based on a rarefaction plot (Fig. 2), it can be seen that the asymptotic number of genera in the samples was lowest in the fish feces sample, while the remaining samples were relatively comparable for the estimated total genus numbers. This trend was also observed when comparing the diversity at phylum level (Fig. 3), where the fish feces sample is largely dominated by a single phylum (Fusobacteria). The three other samples contained a more homogeneous community, with a dominance of Proteobacteria in all samples. Shannon indices and evenness were calculated as described before (Hill et al. 2003) (Table 3). When the data were analyzed at genus level, the Shannon indices were quite equal for the biofilter ($H' = 3.93$), plant root ($H' = 3.93$), and periphyton ($H' = 3.82$) samples, while the value is lower for the fish feces ($H' = 1.18$). Shannon evenness (E) was calculated to be 0.63, 0.63, 0.63, and 0.19 for biofilter, plant root, periphyton, and fish feces, respectively. Again here, it is clearly visible that the diversity in fish feces is much less than in the other samples, whereas the evenness confirms the presence of the dominance of the Fusobacteria.

Diversity of bacteria from the fish gut

Of the total number of reads obtained, only 65.1% of the reads from the fish feces sample were assigned to 11 phyla,

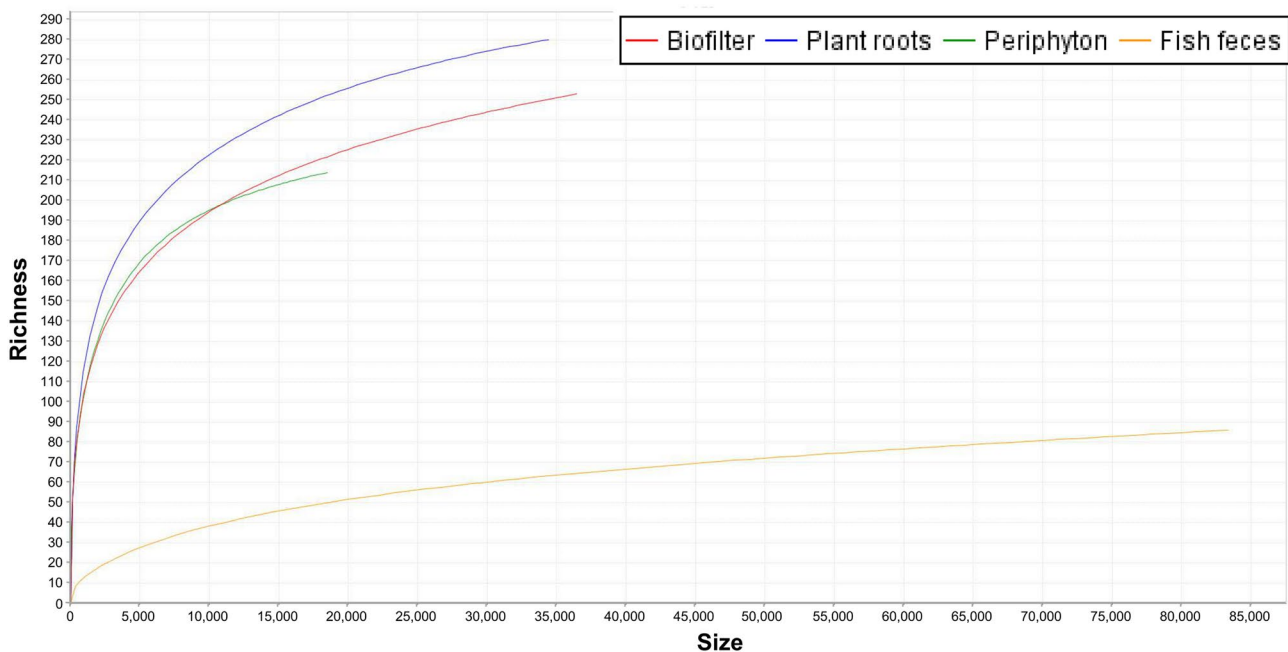
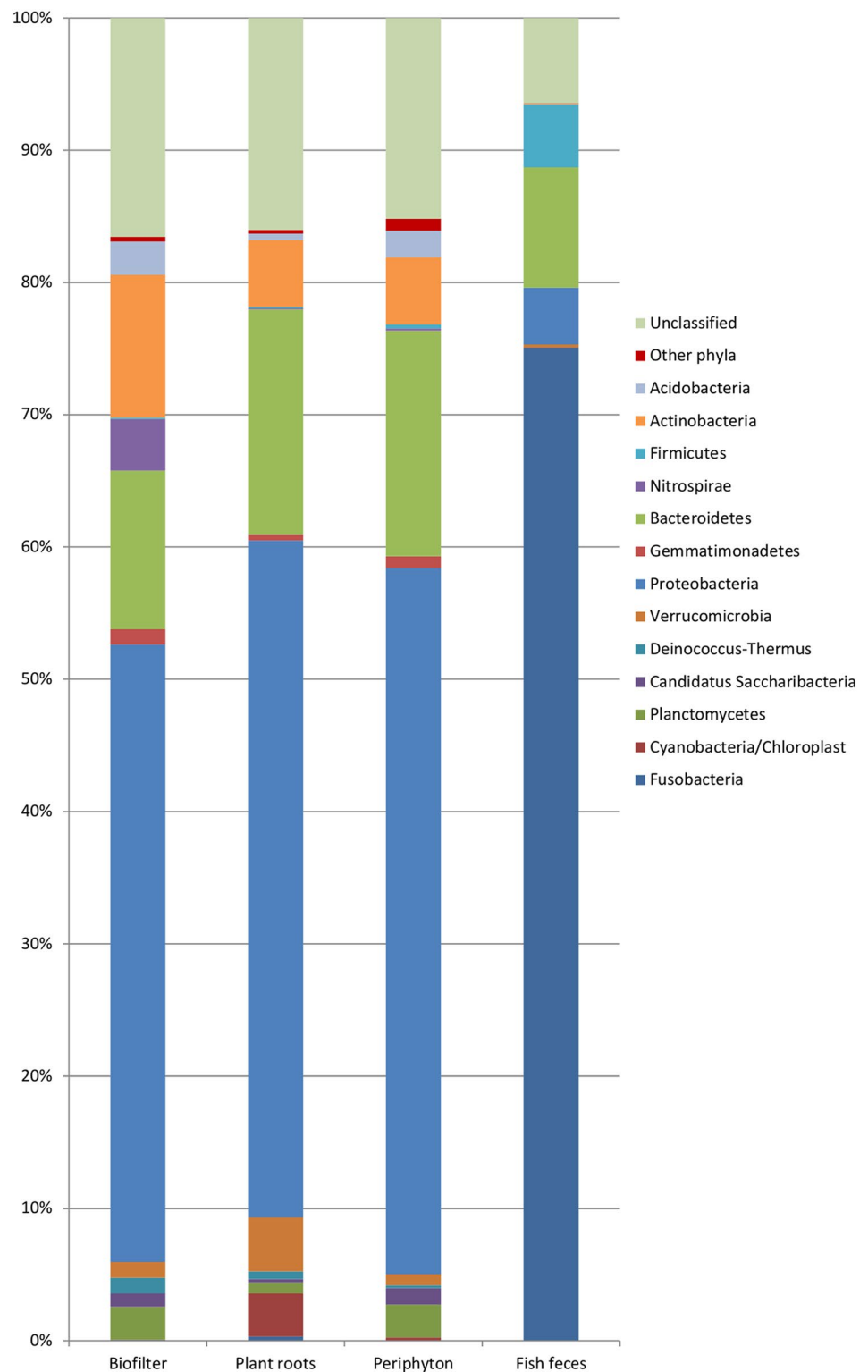


Fig. 2 Rarefaction at genus level present in the different compartments of the aquaponics unit

Fig. 3 Classification of reads from biofilter, plant roots, periphyton, or fish feces to level phylum indicated as percentage of population. Phyla that were represented in all samples below 0.5% of total community (Table S1) are displayed under “other phyla”, which contain the phyla Armatimonadetes, Parcubacteria, Chloroflexi, Spirochaetes, Hydrogenedentes, Chlamidiae, candidate division WPS-2, and SR1



with 6.4% of the reads not assigned to any phylum. The majority (93.2% of the assigned reads) was assigned to the dominant four phyla (Fusobacteria, Bacteroidetes, Firmicutes, and Proteobacteria), similar to what was found for rainbow trout or carp (Etyemez and Balcázar 2015; Li

et al. 2015), but in different relative amounts. The Fusobacteria were mainly represented by members of the genus *Cetobacterium*, a common inhabitant of fish guts (Tsuchiya et al. 2008), and constituted around 75% of the total bacterial community. In addition, the fish feces also contained

known gut bacteria-like members of the genera *Bacterioides*, *Clostridium*, and *Aquaspirillum* (Table S3). In contrast to the other samples, relative high numbers of *Enterobacteriales* (Fig. S1), mainly *Plesiomonas*, were found in the fish feces sample, whereas other Gammaproteobacteria, such as *Xanthomonadales* or *Pseudomonadales*, dominating in the other samples, were found only in low numbers. Although a small number of the genus *Aeromonas* (0.25% of total community), which contain potential fish pathogens, were found in the feces sample, it should be noted that during the operational time, fish were healthy. It can be proposed that the number of *Aeromonas* is kept below the threshold value for disease establishment (Bruhn et al. 2005; Pang et al. 2015), or the reads assigned to *Aeromonas* do represent a species that is not pathogenic to fish. The fish gut is an ecosystem that is quite different from its environment, while it does not influence the microbial communities in the aquaponics system.

Combination of diversity data to compartments

The samples from biofilter, plant roots and periphyton largely contained the same genera, but the abundance of each varied within the individual samples. Whereas this could be an effect of the single sample, a more structural difference may also be inferred. Whereas the biofilter and periphyton contained larger fractions of *Rhizobiales*, the smaller fractions on plant roots could be caused by the larger number of root-associated bacteria-like members of the *Burkholderiales*, *Flavobacteriales*, or *Pseudomonadales* (Fig. S1). Actinobacteria were more represented in the biofilter, while members of the *Sphingomonadales* and *Xanthomonadales* were more abundant in the periphyton. *Methylophilales*, obligate aerobic Betaproteobacteria that can use C1 compounds as carbon source (Garrity et al. 2005), were nearly exclusive present on plant roots. It is thus obvious that bacteria, often detected in plant rhizosphere systems, were also present in the rockwool containing the lettuce roots in the hydroponic unit.

Indications to nitrogen cycling in the aquaponics system

The bioconversion of nitrogen compounds is of largest importance to aquaponics systems. The primary input of nitrogen into the system is via the fish food, which is nearly quantitatively eaten by the fish (Schmautz 2015). Their excrements contain large amounts of ammonium, which can accumulate in aquaculture systems over time. As in the aquaponics systems, plants prefer nitrate over ammonium (Hu et al. 2015), and there is a clear role for the bacterial communities in the system to convert ammonium to nitrate. Only by a good coordination of the food inputs, fish biomass per volume, microbial communities, and plant-growth

stage, the levels of nitrogen compounds and especially ammonium can be kept constant. This implies a healthy microflora that can perform the nitrification process.

The biofilter sample contained large numbers of *Nitrospira* (3.9% of total community) that were found only in low numbers in the periphyton or the plant roots. On the other hand, only small percentages of *Nitrosomonadales* (0.64%) and *Nitrobacter* (0.11%) were found in the same samples (Table S3). Whereas the second group of organisms are commonly tested for in aquaponics systems and mainly held responsible for nitrification (Rurangwa and Verdegem 2015; Zou et al. 2016a), *Nitrospira* has only recently been described as total nitrifier (Daims et al. 2015), being able to directly convert ammonium to nitrate in the system. The dominance of *Nitrospira* is thus a novelty in such systems and might be correlated with a difference in the basic setup of the aquaponics system (Graber et al. 2014). It must be noted that the increased presence does not necessarily correlate to a larger activity of these organisms in the system, as the metabolic activities were not measured. The periphyton contained larger numbers of the putative denitrifiers *Dokdonella* and *Thermomonas* (Tian et al. 2015) than on plant roots or in the biofilter (Table S3). This potential of denitrification would partially explain the loss of nitrogen in the system that is only possible by measurement of the complete nutrient balance (Graber et al. 2014; Schmautz 2015). In addition, a large number of unclassified planctomycetes were found in the data set of the three samples (Fig. 3). As most ANAMMOX species are not yet cultivated as pure cultures (Oshiki et al. 2016), they will not appear in the RDP list based on its inclusion policy (Wang et al. 2007). Whether ANAMMOX bacteria can be involved in nitrogen cycling in AP systems that should be examined in more detail.

Organisms involved in biosynthesis of off-flavors

One concern in aquaculture is the production of off-flavors, such as geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) or 2-methylisoborneol (1,2,7,7-tetramethyl-exobicyclo[2.2.1]-heptan-2-ol), by the microbial communities (Rurangwa and Verdegem 2015). These compounds are secondary metabolites produced by *Streptomyces* in recycling aquaculture systems (Schrader and Summerfelt 2010). Mostly, accumulation of organic solids and phosphate influences the number of off-flavor-producing bacteria. However, the metagenome data showed that there were no *Streptomyces* present in the current data set (Table S3), while off-flavors were not detected from the aquaponics system, indicating that the Wädenswil aquaponics system, which included a solid removal unit, was working well. In addition, low phosphate levels were measured in the

system, which is mainly due to incorporation of phosphate in plant biomass.

Chloroplast DNA

The plant root sample contained reads for the chloroplasts from land plants (Streptophyta; 2.8%) (Table S3). This is due to the use of a primer set that does not exclude these sequences, and those reads mainly represent remainders of the lettuce roots in the DNA extraction. In addition, reads for chloroplasts from Bacillariophyta (diatoms) were found mostly associated with plant roots (0.3%). Chlorophyta (green algae) chloroplasts were found both at low percentages in the samples of plant roots and in the periphyton. All in all, it can be concluded that there is a small community of eukaryotic, chloroplast-containing microorganisms in the system. However, as the system water was not sampled systematically, it cannot be excluded that more eukaryotes were present in the system.

Plant root communities: potential for inherent biocontrol?

Plant protection in AP is a challenge to the practitioners, as plant protection agents that may be harmful to fish in the concentrations applied cannot be used (Bittsánszky et al. 2015). Therefore, AP systems need to be operated under plant protection based on biocontrol or at least integrated pest management (Rakocý 2007). The beneficial effect of biocontrol strains can be either directly expressed in a plant-growth-promoting effect in the absence of pathogens, or indirectly by protecting the plant against soil-borne diseases (Lugtenberg and Kamilova 2009). A large group of these are *Pseudomonas* spp., being able to produce antimicrobial compounds, to compete for space and nutrients, or to enhance induced resistance and/or parasitism (Haas and Défago 2005; Flury et al. 2016). A relative high proportion of reads (2.2%) from the plant roots was assigned to the genus *Pseudomonas*, indicating that the lettuce plants may have selected for a community that is able to perform inherent biocontrol on its roots. In addition, other genera known as rhizosphere bacteria, including *Acidovorax*, *Sphingobium*, or *Flavobacterium*, were also enriched on plant roots, showing that this rhizosphere-based selection is not only limited to the pseudomonads. The role of these bacteria on the plant roots, including a potential for biocontrol of plant pathogens (Sirakov et al. 2016), is not known yet.

Conclusion

From the different functionalities in the system, it was expected that each of the characterized niches would

contain a different bacterial community. The fish feces sample appeared to be a separate ecosystem, while the others were more comparable. Nevertheless, each of the samples had its own specialized group of organisms that may be linked to the microbial functions in that niche sample. A more thorough characterization of microbial communities, when possible down to the species level, linked with biochemical and system parameters, including more sampling sites, will be required to be able to link up the community with its physiological role in the AP systems.

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Compliance with ethical standards

Conflict of interest Author Andreas Graber declares that, at the time of the experiments, he was part-time Director R&D at the ZHAW spin-off company UrbanFarmers, but that the research published in this manuscript was performed as part of his research portfolio at ZHAW. The other authors declare to have no conflict of interest.

References

- Bittsánszky A, Gyulai G, Junge R, Schmutz Z, Komives T (2015) Plant protection in ecocycle-based agricultural systems: aquaponics as an example. In: International Plant Protection Congress (IPPC) 2015, Berlin, Germany, doi:10.13140/RG.2.1.4458.0321
- Brown MN, Briones A, Diana J, Raskin L (2013) Ammonia-oxidizing archaea and nitrite-oxidizing nitrospiras in the biofilter of a shrimp recirculating aquaculture system. FEMS Microbiol Ecol 83:17–25
- Bruhn JB, Dalsgaard I, Nielsen KF, Buchholtz C, Larsen JL, Gram L (2005) Quorum sensing signal molecules (acylated homoserine lactones) in Gram-negative fish pathogenic bacteria. Dis Aquat Org 65:43–52
- Daims H, Lebedeva EV, Pjevac P, Han P, Herbold C, Albertsen M, Jehmlich N, Palatinszky M, Vierheilig J, Bulaev A, Kirkegaard RH, von Bergen M, Rattei T, Bendinger B, Nielsen PH, Wagner M (2015) Complete nitrification by *Nitrospira* bacteria. Nature 528:504–509
- Endut A, Jusoh A, Ali N, Wan Nik WB (2011) Nutrient removal from aquaculture wastewater by vegetable production in aquaponics recirculation system. Desalin Water Treatm 32:422–430
- Etyemez M, Balcázar JL (2015) Bacterial community structure in the intestinal ecosystem of rainbow trout (*Oncorhynchus mykiss*) as revealed by pyrosequencing-based analysis of 16 S rRNA genes. Res Vet Sci 100:8–11
- Fernandez D (2013) HydroBuddy: An open source nutrient calculator for hydroponics and General agriculture, v1.50. <http://sciencein-hydroponics.com>

- Flury P, Aellen N, Ruffner B, Péchy-Tarr M, Fataar S, Metla Z, Dominguez-Ferreras A, Bloemberg G, Frey J, Goesmann A, Raaijmakers JM, Duffy B, Höfte M, Blom J, Smits THM, Keel C, Maurhofer M (2016) Insect pathogenicity in plant-beneficial pseudomonads: phylogenetic distribution and comparative genomics. *ISME J* 10:2527–2542
- Garrity GM, Bell JA, Lilburn T (2005) Order III. Methylophilales *ord. nov.* In: Brenner DJ, Krieg NR, Staley J, Garrity GM (eds) *Bergey's Manual of Systematic Bacteriology*—second edition. Volume two: the proteobacteria. Part C: The Alpha-, Beta-, Delta-, and epsilonproteobacteria. Springer, New York, pp 770–773
- Goddek S, Espinal CA, Delaide B, Jijakli MH, Schmautz Z, Wuertz S, Keesman KJ (2016) Navigating towards decoupled aquaponic systems: a system dynamics design approach. *Water* 8:303
- Graber A, Antenen N, Junge R (2014) The multifunctional aquaponic system at ZHAW used as research and training lab. In: Maček Jerala M, Maček MA (eds) *Conference VIVUS: Transmission of Innovations, Knowledge and Practical Experience into Everyday Practice*, Strahinj, Slovenia
- Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* 3:307–319
- Hill TCJ, Walsh KA, Harris JA, Moffett BF (2003) Using ecological diversity measures with bacterial communities. *FEMS Microbiol Ecol* 43:1–11
- Hu Z, Lee JW, Chandran K, Kim S, Brotto AC, Khanal SK (2015) Effect of plant species on nitrogen recovery in aquaponics. *Bioresour Technol* 188:92–98
- Kowalchuk GA, Stephen JR (2001) Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Annu Rev Microbiol* 55:485–529
- Li T, Long M, Gatesoupe F-J, Zhang Q, Li A, Gong X (2015) Comparative analysis of the intestinal bacterial communities in different species of carp by pyrosequencing. *Microb Ecol* 69:25–36
- Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* 63:541–556
- Munguia-Fragozo P, Alatorre-Jacome O, Rico-Garcia E, Torres-Pacheco I, Cruz-Hernandez A, Ocampo-Velazquez RV, Garcia-Trejo JF, Guevara-Gonzalez RG (2015) Perspective for aquaponics systems: “omic” technologies for microbial community analysis. *Biomed Res Int* 2015:480386
- Oshiki M, Satoh H, Okabe S (2016) Ecology and physiology of anaerobic ammonium oxidizing bacteria. *Environ Microbiol* 18:2784–2796
- Pang M, Jiang J, Xie X, Wu Y, Dong Y, Kwok AHY, Zhang W, Yao H, Lu C, Leung FC, Liu Y (2015) Novel insights into the pathogenicity of epidemic *Aeromonas hydrophila* ST251 clones from comparative genomics. *Sci Rep* 5:09833
- Rakocy J (2007) Ten guidelines for aquaponic systems. *Aquaponics J* 46:14–17
- Revsberg NP, Risgaard-Petersen N, Schramm A, Nielsen LP (2006) Nitrogen transformations in stratified aquatic microbial ecosystems. *Ant Leeuwenhoek* 90:361–375
- Rurangwa E, Verdegem MCJ (2015) Microorganisms in recirculating aquaculture systems and their management. *Rev Aquacult* 7:117–130
- Schmautz Z (2015) Mass balance and nutrient cycling in aquaponics. Master thesis, Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia, p 86
- Schrader KK, Summerfelt ST (2010) Distribution of off-flavor compounds and isolation of geosmin-producing bacteria in a series of water recirculating systems for rainbow trout culture. *N Am J Aquacult* 72:1–9
- Sirakov I, Lutz M, Graber A, Mathis A, Staykov Y, Smits THM, Junge R (2016) Potential for combined biocontrol activity against fungal fish and plant pathogens by bacterial isolates from a model aquaponic system. *Water* 8:518
- Somerville C, Cohen M, Pantanella E, Stankus A, Lovatelli A (2014) Small-scale aquaponic food production: integrated fish and plant farming. In: *FAO Fisheries and Aquaculture Technical Paper Food and Agriculture Organization of the United Nations, Rome, Italy*, p 262
- Tian H-L, Zhao J-Y, Zhang H-Y, Chi C-Q, Li B-A, Wu X-L (2015) Bacterial community shift along with the changes in operational conditions in a membrane-aerated biofilm reactor. *Appl Microbiol Biotechnol* 99:3279–3290
- Tokuyama T, Mine A, Kamiyama K, Yabe R, Satoh K, Matsumoto H, Takahashi R, Itonaga K (2004) *Nitrosomonas communis* strain YNSRA, an ammonia-oxidizing bacterium, isolated from the reed rhizosphere in an aquaponics plant. *J Biosci Bioeng* 98:309–312
- Tsuchiya C, Sakata T, Sugita H (2008) Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Lett Appl Microbiol* 46:43–48
- Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261–5267
- Zou Y, Hu Z, Zhang J, Xie H, Guimbaud C, Fang Y (2016a) Effects of pH on nitrogen transformations in media-based aquaponics. *Bioresour Technol* 210:81–87
- Zou Y, Hu Z, Zhang J, Xie H, Liang S, Wang J, Yan R (2016b) Attempts to improve nitrogen utilization efficiency of aquaponics through nitrifies addition and filler gradation. *Environ Sci Pollut Res Int* 23:6671–6679