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Is Aquaponics Beneficial in Terms of Fish and Plant Growth and Water Quality in Comparison to Separate Recirculating Aquaculture and Hydroponic Systems?

Faiqa Atique [1,2], Petra Lindholm-Lehto [3,*] and Juhani Pirhonen [1]

[1] Department of Biological and Environmental Science, University of Jyvaskyla, P.O. Box 35, FI-40014 Jyvaskyla, Finland; atiquefaiqa@gmail.com (F.A.); juhani.pirhonen@jyu.fi (J.P.)
[2] Institute of Bioeconomy, JAMK University of Applied Sciences, Tuumalantie 17, FI-43130 Tarvaala, Finland
[3] Aquatic Production Systems, Natural Resources Institute Finland, Survontie 9A, FI-40500 Jyvaskyla, Finland
* Correspondence: petra.lindholm-lehto@luke.fi

Abstract: Aquaponics is a technique where a recirculating aquaculture system (RAS) and hydroponics are integrated to grow plants and fish in a closed system. We investigated if the growth of rainbow trout (*Oncorhynchus mykiss*) and baby spinach (*Spinacia oleracea*) would be affected in a coupled aquaponic system compared to the growth of the fish in RAS or plants in a hydroponic system, all systems as three replicates. We also investigated the possible effects of plants on the onset of nitrification in biofilters and on the concentration of off-flavor-causing agents geosmin (GSM) and 2-methylisoborneol (MIB) in rainbow trout flesh and spinach. For the fish grown in aquaponics, the weight gain and specific growth rates were higher, and the feed conversion ratio was lower than those grown in RAS. In spinach, there were no significant differences in growth between aquaponic and hydroponic treatments. The concentration of GSM was significantly higher in the roots and MIB in the shoots of spinach grown in aquaponics than in hydroponics. In fish, the concentrations of MIB did not differ, but the concentrations of GSM were lower in aquaponics than in RAS. The onset of nitrification was faster in the aquaponic system than in RAS. In conclusion, spinach grew equally well in aquaponics and hydroponic systems. However, the aquaponic system was better than RAS in terms of onset of nitrification, fish growth, and lower concentrations of GSM in fish flesh.

Keywords: biological filtration; integrated aquaculture; muscle lipids; off-flavors; salmonids; soilless culture

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1. Introduction

Partly due to the tightened demands for environmental permissions, especially in the land-based aquaculture, recirculating aquaculture systems (RAS) are gaining popularity in producing fish for human consumption. The main advantage of RAS is highly decreased water use compared to traditional flow-through systems. Consequently, the nutrients released by the cultured animals are highly concentrated in the limited amount of effluent, which can offer cost-efficient opportunities for nutrient reuse and wastewater treatment [1]. In RAS, the maintenance of the microbial environment in biofilters is essential because the microbes responsible for nitrification convert harmful ammonia excreted by fish, first to nitrite and then to nitrate [2]. Exposure of fish to even low concentrations of ammonia and nitrite can be harmful and affect the fish welfare and survival, while nitrate is a rather safe compound for the fish at concentrations <100 mg/L [3]. The start-up of the nitrification process using intact biofilter media can take up to two months [2], after which the levels of ammonia and nitrite should remain at levels that are safe for fish [1]. Several studies have been conducted to increase the efficiency and speed up the onset of nitrification in RAS [2,4,5]. For example, the nitrification efficiency in RAS has been studied by investigating the biofilter configuration and relationship between the heterotrophic and

nitrifying bacteria, nitrification efficiency of the submerged biological filter, total ammonia nitrogen (TAN) concentrations and varying C/N ratios [4], biofilter media types and their effects on the efficiency of trickling filters [6,7] and the effects of the design of the biofilter on the oxidation of ammonia [2].

Hydroponics refers to the soilless cultivation of plants where the nutrients for the plant's growth are provided in a solution [8], and the plants get the nutrients from the water instead of soil [9]. Hydroponics is an efficient method for producing vegetables with minimal water and space [10,11]. Aquaponics refers to a system where RAS and hydroponics have been combined, and the RAS effluent with concentrated nutrients is utilized to grow plants [12,13]. The ammonia excreted by the fish is converted in the biofilter to nitrate, which is easily absorbed by the plants [14]. The fish feed contains macro- and micronutrients essential for fish growth but that are also important for the plants [15]. While absorbing the nutrients from the RAS wastewater, the plants also clean the water from compounds potentially harmful to the fish due to low water exchange [15]. However, plants differ in their demand for nutrients, and their availability in RAS effluent may not be enough for all plant species. To cope with this situation and provide enough nutrients for the plant's growth, some nutrients can be provided in a solution [12]. Aquaponics has been regarded as a sustainable and environmentally-friendly method for producing plants and fish [12,13]. In addition, it supports the idea of a circular economy as the wastes produced by fish are turned into a resource for the plants.

The presence of bacteria like Cyanobacteria, Actinomycetes, and Myxobacteria in RAS can produce off-flavor compounds geosmin (GSM) and 2-methylisoborneol (MIB) [16–18] which easily accumulate in fish flesh and cause earthy and musty flavor. GSM and MIB are semi-volatile terpenoid compounds that accumulate in the lipid-rich tissues of fish. The main route of uptake is through the gills, but also via the skin and gastrointestinal tract, and the uptake proceeds fast, typically within hours [18–20]. The concentrations of GSM and MIB in fish flesh seek equilibrium with their concentrations in water. However, factors such as water temperature and flow rate, fish age, size, and species, along with the exposure time, have been shown to affect their concentrations [18,19,21,22]. The removal of the off-flavor compounds from fish flesh is essential for it to be marketable. Depuration in clean water has been proved to be the only reliable method for off-flavor removal. Unfortunately, the removal of off-flavors is a slow process, and even in the optimal conditions, it can take from days to weeks [17,19,22]. The off-flavor compounds are typically removed by keeping the fish without feed in flow-through tanks until no off-flavor can be perceived by organoleptic testing. Other approaches have been examined to decrease the off-flavors in water and in fish, and reduce the time of depuration. These approaches include addition of peracetic acid and hydrogen peroxide (H_2O_2) [23], and the ozonation of circulating water or depuration water [18,24,25], and photocatalysis [26].

Due to the increasing demand for sustainable food production, including eco-friendly seafood and vegetables for the growing human population, more research is needed to understand the potential benefits of aquaponic systems. One of the problems with RAS is the long start-up time for a fully functioning biofilter. It appears that no attention has been paid to the possibility of using plants to shorten the duration of the onset of the nitrification process in RAS or to buffer the sharp increase of ammonia and nitrite caused by the maturing biofilter. On the other hand, in our unpublished organoleptic tests, rainbow trout (*Oncorhynchus mykiss*) reared in an aquaponic system tasted rather normal as compared to those reared in RAS, which possessed a very strong muddy taste. This suggests that the plants could potentially be used as absorbers of compounds causing off-flavors in fish, and bacteria from the genus *Streptomyces* have been found to be absent in aquaponics but not in RAS [27].

Consequently, our study hypothesized (1) that the onset of the nitrification process is faster in the aquaponics treatment compared to RAS, (2) that rainbow trout grown in an integrated system with baby spinach (*Spinacia oleracea*) have lower concentrations of off-flavor compounds compared to those reared in RAS, (3) that the plants in an aquaponic

system contain a higher concentration of GSM and MIB than plants grown in hydroponics, and (4) that the plants and fish grown in an aquaponic system grow equally well than in hydroponics and RAS, respectively.

2. Materials and Methods

2.1. Experimental Setup

A 42-day experiment was conducted from 4 May to 14 June 2021 at the Tarvaala Bioeconomy campus of the JAMK University of Applied Science, Finland, where three replicated RAS, aquaponic, and hydroponic systems were set up (3 + 3 + 3) in an industrial hall without temperature control. In the RAS and aquaponic systems, each of the six fish tanks was stocked with 20 rainbow trout of c. 90–110 g on 4 May, purchased from a RAS farm (Finnforel Ltd., Varkaus, Finland). Two hundred and fifty ml of filter starter (Easystart, Easy-Life International BV, Duiven, Netherlands) was added to each biofilter tank one week before (27 April) and six days after (10 May) the fish stocking. Each of the six deep-water culture (DWC) rafts (three rafts for aquaponics and hydroponics) were transplanted with 25 baby spinach plants on 5 May. Spinach seeds were germinated and grown in a greenhouse of the University of Jyvaskyla for three weeks before transplantation. The DWC tanks (W1 × L1 × D0.35 m) were made from high-density polyethylene containers, and the rafts were made of extruded polystyrene foam (XPS) Styrodur® with 25 drilled holes for 5 cm hydroponic pots filled with expanded clay. Each DWC was continuously aerated through air stones. In DWCs, the air temperature ranged from 15 to 20 °C. Light was provided to plants with LED lights (Kinwua bright, 215-watt, light intensity c. 1000 lux) for 16 h per day, and the scattering light from the DWCs provided illumination for the fish tanks which did not have separate lamps.

Each of the six dual-drain fish tanks (500 L) was connected to a settling tank (500 L), bead filter (SuperBead small, Air-aqua BV, Staphorst, Netherlands, filled with 37.5 kg of beads), a moving bed biofilter filled with 300 L helix floating bio media (Sibo Fluidra, Doornhoek, Netherlands), and a UV light (AquaForte UV-C lamp 18 watt, Sibo Fluidra, Doornhoek, Netherlands). In the aquaponic systems, water was pumped from the DWC back to the fish tanks (i.e., coupled aquaponics). The oxygen saturation in the fish tanks was maintained at 80–85% throughout the experiment using air pumps and air stones. The water temperature depended on the hall temperature and increased during the experiment from 12 to 19 °C due to the lack of a temperature controller. The RAS and aquaponics water exchanges in the fish tank with tap water were done using the following percentages at each water change: first week 50% four times, second week 20–30% four times, third and fourth week 10% three times. No water was changed in the fifth week, and in the sixth week, 40–50% water of the system was changed twice in RAS while 20 to 30% in aquaponics. An equal amount of water was changed from RAS and aquaponics treatments (except week 6) which meant relatively more water change in RAS because the water volume for aquaponics was bigger (RAS + DWC). The fish were fed with dry pellets (EFICO Enviro 923 Advance 4.5 mm, Biomar, Brande, Denmark). According to the manufacturer, crude protein and fat contents of the diet were 43% and 51%, respectively. The fish were fed by hand twice per day for the first week and thereafter with automats three times per day. Feed intake was monitored every day, and the quantity of feed was changed depending upon the uneaten amount of feed on the tank bottom. Uneaten pellets were siphoned out of the tanks and counted. The number of uneaten pellets was converted to the weight of dry feed, knowing that 14 dry pellets equaled 1.00 g. The amount of daily feed intake was calculated as the difference between the fed and uneaten feed. The fish were not fed on the day of the harvest.

The water quality in fish tanks was recorded daily during week one and 3–4 times a week from week two to onward. The water quality was recorded for total ammonia nitrogen (TAN), nitrite, nitrate (API® Freshwater master test kits, Mars Fish Care Inc, Chalfont, PA, USA), pH, temperature (Digital PH/Temp Meter AD 12, ADWA instruments, Szeged, Hungary) and oxygen saturation (ExStik® DO600 dissolved oxygen, Extech,

Waltham, MA, USA). In the DWC, the humidity was checked with a humidity meter (Prego, Helsinki, Finland).

For the hydroponic plants, Substral® (Transmeri Ltd., Espoo, Finland) nutrient solution was used. The Substral solution was prepared according to the manufacturer's instructions (7 mL of Substral in 6 L of water), i.e., 408 mL of Substral was added to 350 L of water for each hydroponic DWC. This solution was added once in two weeks in hydroponics DWC when compensating for the evaporated water. The hydroponic plants were sprayed with the Substral solution (approximately 1 mL of Substral in 1.5 L of water) every day during the experiment, excluding the first week. Plants were also sprayed with water every day, excluding the first week.

For the aquaponic plants, modified micronutrients solution (Fe, B, Zn, Mo) and potassium were added in the form of a solution prepared by dissolving salts of $Fe\ (NO_3)_3 \times 9\ H_2O$ (101.2 g), $Mn\ (NO_3)_2 \times 4\ H_2O$ (36.52 g), $Zn\ (NO_3)_2 \times 6\ H_2O$ (2.7368 g), $Na_2MoO_4 \times 2\ H_2O$ (0.3533 g), $K_2B_4O_7 \times 4\ H_2O$ (28.26 g) in 1 L water [28]. This nutrient solution (10 to 15 mL) was added into the aquaponic system whenever water was added to the system and whenever plants showed any deficiency symptoms such as a change in leaf color or growth. The plants were also sprayed with water and this nutrient solution (1 mL in 1.5 L) every day, excluding the first week.

2.2. Sampling

The start point samples of spinach were taken just before the transplantation of spinach seedlings to the aquaponics system (5 May). The start point samples of fish were taken at the time of fish stocking (4 May). The endpoint samples were taken after six weeks on the day of the harvest of fish and spinach on (14–15 June). For the measurement of change in spinach biomass, 15 seedlings were sampled in the beginning, while at the end of the experiment, 20 plants were sampled from each DWC. The length and dry weight of the shoots and roots were recorded separately. The plants were dried at 60 °C for 72 h. For the GSM and MIB analyses, six fresh spinach seedlings were taken at the start, and three fresh spinach plants at the end from each tray, shoots, and roots were separated, cut into small pieces, and mixed into one homogeneous sample, i.e., one sample for each tray. The dry matter content of spinach was determined by the ISO 638:2008 standard method. The final samples from spinach shoots from each DWC were also analyzed for macronutrients (N, P, K, Ca, Mg, S) and micronutrients (Fe, Cu, Mn, Zn, B) at Eurofins Agroscience Services, Mikkeli, Finland. B, Ca, Cu, Fe, K, Mg, Mn, P, and Zn were measured with an ICP-OES method as reported by Eurofins. Nitrogen was determined with Kjeldahl-method while sulfur with ICP-OES method. Limits of detection (LOD) and limits of quantification (LOQ) for each nutrient are given in Supplementary Table S1.

For estimating the fish growth, the fish were weighed in the beginning (in batches) and at the end (individually) of the experiment. For the measurement of lipid content and off-flavors (GSM and MIB) in the fish muscle, three randomly selected individuals were sampled in the beginning. At the end, three individuals were sampled from each fish tank, i.e., nine fish per treatment. The sampled fish were killed with a sharp blow on the head, gutted, and filleted. From the lateral part of the fillet [29], 500 mg of muscle was taken from each fish, and the three samples from each tank were pooled. Water samples (500 mL) were taken from each DWC, each fish tank, and tap water at the beginning and the end of the experiment for the analysis of the off-flavor compounds (GSM and MIB) and anions (chloride, phosphate, sulfate, and nitrate, and nitrite). All samples were stored at −20 °C before the analyses.

2.3. Off-Flavor Analyses

The off-flavor-inducing compounds GSM (trans-1, 10–dimethyl–trans-9–decalol) and MIB (1-R-exo-1,2,7,7-tetramethyl-bicyclo [2.2.1] heptan-2-ol) were quantified by the method reported in Lindholm-Lehto [30]. In short, the sample extraction was performed by an automated SPME procedure (PAL3 autosampler, CTC Analytics, Zwingen, Switzerland) with an

SPME Arrow fiber made of DVB/carbon WR/PDMS (divinylbenzene/carboxene/polydimethyl siloxane). The pretreatment cycle included mixing, heating, adsorption and desorption of analytes, injection into the GC port, and conditioning of the fiber. The samples were analyzed by a GC-QQQ (7000 Series Triple Quadrupole mass spectrometer, Agilent, Santa Clara, CA, USA). It was operated with a Phenomenex Zebron ZB-5MSi (Torrance, CA, USA) capillary column (30 m × 0.25 mm × 0.25 µm) for the separation and with an electron ionization (EI) ion source, and MassHunter 10.0 software. The detection was performed in multiple reaction monitoring (MRM) mode. Levels of quantification (LOQ)s were (0.2 ng/L GSM; 0.4 ng/L MIB) for aqueous and (65 ng/kg GSM; 107 ng/kg MIB) for solid samples. The full method description and validation have been reported in [30].

2.4. Lipid Content

The total fat content was determined by the accredited in-house method JOK3008 which is based on AOAC Official Methods 920.39 (Fat (Crude) or ether extract in animal feed and) and 954.02 (Fat (crude) or ether extract in pet food; Association of Official Analytical Chemists, USA) and AACC method 30–25 (Crude fat in wheat, corn, and soy flour, feeds, and mixed feeds; Approved Methods of the American Association of Cereal Chemists, USA). The used equipment was Foss Soxtec/Hydrotec 8000™ System for total fat analysis, consisting of Soxtec™ 8000 extraction unit and Hydrotec™ hydrolysis unit (FOSS Analytical, Hillerød, Denmark). The test laboratory in Jokioinen, belonging to the Natural Resources Finland, holds FINAS (Finnish Accreditation Service) accreditation number T024 and follows the standard SFS-EN ISO/IEC 17025:2017. Muscle lipid contents have been reported as g/kg wet weight (ww).

2.5. Anions

Anion chloride (Cl^-), nitrite-N ($NO_2{}^-$), nitrate-N ($NO_3{}^-$), sulfate ($SO_4{}^{3-}$), and phosphate ($PO_3{}^{4-}$) were studied from the water samples taken at the end of the experiment. The pretreatment of samples by solid-phase extraction (SPE) has previously been reported in Lindholm-Lehto et al. [30,31]. The chromatographic analysis was conducted on Thermo Scientific Dionex Integrion HPIC ion chromatography equipment (Dionex, Sunnyvale, CA, USA) with the Cromeleon 7.2 software. The equipment consisted of a gradient pump (0–6000 psi), eluent generator (EDC 500 KOH), a guard column Dionex IonPacTM NG1 (2 × 50 mm), a pre-column (Dionex IonPacTM AG19 (2 × 50 mm–4 µm), and an analytical column Dionex IonPacTM AS-19 (2 × 250 mm–4 µm at 30 °C). The full description of the analysis method and validation data have been reported by Lindholm-Lehto et al. [30]. The LODs ranged between 0.018–0.131 mg/L and LOQs from 0.020 mg/L to 0.175 mg/L (Supplementary Table S2).

2.6. Calculations and Statistical Analyses

The specific growth rate (SGR) for each fish tank was calculated as Ln (W2) − Ln (W1) × 100/t, where W1 and W2 are the tank's average fish weights (g) in the beginning and at the end of the experiment, and t is the experimental period in days (42 d). Feed conversion ratio (FCR) was calculated as the weight of feed eaten (kg)/fish weight gain (kg). For analyzing the spinach biomass, dry weights were recorded at the start and the end of the experiment. Shoot and root lengths were recorded for each plant at the end of the experiment. The total individual plant weight (g) on each raft was calculated using the total end dry weight (root + shoot). The starting dry weight of spinach seedlings (0.003 ± 0.0005, n = 3) was negligible, and therefore biomass change during the experiment was not calculated separately.

Statistical analyses were run with IBM SPSS Statistics 26. Independent samples t-test was used to compare the means between treatments for fish and plant data analysis. The mean concentrations of macronutrients (g/kg) and micronutrients (mg/kg) in spinach shoots were also compared between the treatments by the independent samples t-test.

Homogeneity of variance was checked by Levene's test. The variances of the means of all variables were equal. The observational unit was always the tank or tray (i.e., $n = 3$).

The daily means of ammonia (TAN), nitrite, nitrate, and pH were compared between treatments by repeated measures ANOVA ($n = 3$). Mauchly's test of sphericity p-value was always < 0.15; thus, the Greenhouse-Geisser adjustment was applied. The selected anions (chloride, nitrite-N, nitrate-N, sulfate, and phosphate) were analyzed at the end of the experiment and compared between treatments by repeated measures ANOVA ($n = 3$). A Huynh–Feldt adjustment was applied because Mauchly's test of sphericity p-value was always one. The values for nitrate were Ln transformed before the statistical analysis. The values for nitrite were zero on the start and end day of the experiment and were not included in the analysis.

The MIB and GSM in spinach shoots and roots and lipid content in fish muscle between treatments were analyzed by independent t-test, while the start values were compared with the end values by one sample t-test. For assessing MIB and GSM in water samples and fish muscles repeated measures ANOVA was performed. A Huynh–Feldt adjustment was applied because Mauchly's test of sphericity p-value was always one.

3. Results

3.1. Fish Performance and Plant Growth

During the experiment, one fish died in one of the RAS tanks, but in aquaponics, there was no mortality. The SGR of the fish was significantly higher in aquaponics (1.95 ± 0.12) than in RAS (1.67 ± 0.08) (Table 1). The FCR in aquaponics was significantly lower (0.85 ± 0.08) than in RAS (1.06 ± 0.03) (Table 1). Weight gain was significantly higher for the fish grown in aquaponics than in RAS. Total feed consumed by individual fish did not differ between the treatments (Table 1).

Table 1. Initial and final wet weight, fish weight gain, specific growth rate (SGR), feed consumed, and feed conversion ratio (FCR) of rainbow trout (*Oncorhynchus mykiss*), grown in RAS and aquaponic systems for 42 days. In the aquaponics treatment rainbow trout was grown in a coupled aquaponic system with spinach (*Spinacia oleracea*).

	RAS		Aquaponics		Sig.
Initial weight (g)	107.7	±6.42	108.2	±1.26	ns
Final weight (g)	217.0	±7.24	245.3	±10.32	ns
Fish weight gain (g)	109.3	±3.05	137.1	±11.29	*
SGR	1.67	±0.08	1.95	±0.12	*
Feed consumed (g/fish)	112.0	±0.03	110.0	±0.01	ns
FCR	1.06	±0.03	0.86	±0.08	*

Values are means ± SD, $n = 3$. Statistical difference (Sig.) in the values between aquaponics and RAS treatments is shown by an asterisk * ($p < 0.05$), ns = not significant.

The mean dry weights for shoot, root, total dry weights, shoot to root ratio, mean shoot length, and root length of spinach were not significantly different between aquaponics and hydroponics treatments (Table 2).

3.2. Spinach Nutrient Analysis

The concentrations of macronutrients N ($p < 0.005$), P ($p < 0.05$), S ($p < 0.05$), and K ($p < 0.05$) were significantly higher in hydroponically grown spinach while Ca ($p < 0.0001$) and Mg ($p < 0.005$) were significantly higher in spinach grown in aquaponics. The micronutrients Fe ($p < 0.05$), Zn ($p < 0.05$), and B ($p < 0.0001$) were significantly higher in spinach grown in the aquaponics than in hydroponics, while Cu and Mn were at similar level in both systems (Table 3).

Table 2. Dry weights for shoots and roots, plant total dry weight, shoot and root length, and shoot to root ratio for weight and length of spinach (*Spinacia oleracea*) grown in hydroponic and aquaponic system for 42 days. In the aquaponics treatment spinach was grown in a coupled aquaponics system together with rainbow trout (*Oncorhynchus mykiss*).

	Hydroponics		Aquaponics	
Shoot weight (g)	0.88	±0.27	1.23	±0.34
Root weight (g)	0.18	±0.08	0.30	±0.16
Total weight (g)	1.07	±0.29	1.53	±0.48
Shoot length (cm)	12.15	±1.32	14.50	±1.69
Root length (cm)	29.23	±4.63	37.77	±5.73
Shoot to root ratio weight	5.54	±2.77	4.50	±1.46
Shoot to root ratio length	0.44	±0.02	0.40	±0.03

Values are means ± SD of one plant at the end of the experiment from three replicated rafts, $n = 3$, average start weight for total weight = 0.003 ± 0.0005. There were no statistically significant differences between the treatments.

Table 3. Micronutrients (mg/kg) Fe, Cu, Mn, Zn, B and macronutrients (g/kg) N, P, K, Ca, Mg, S in spinach (*Spinacia oleracea*) shoots grown in hydroponic and aquaponic system for 42 days. For aquaponics treatment spinach was grown in a coupled aquaponics system together with rainbow trout (*Oncorhynchus mykiss*).

	Aquaponics		Hydroponics		Sig.
Fe (mg/kg)	523.3	±75.05	143.3	±15.25	*
Cu (mg/kg)	37.30	±12.70	49.30	±9.60	ns
Mn (mg/kg)	403.3	±40.41	366.6	±246.84	ns
Zn (mg/kg)	526.6	±142.9	206.6	±55.07	*
B (mg/kg)	120.0	±0.00	32.30	±8.08	*
N (g/kg)	38.70	±3.00	57.50	±2.61	*
P (g/kg)	6.06	±1.10	9.40	±1.55	*
K (g/kg)	64.60	±5.68	83.30	±8.96	*
Ca (g/kg)	36.60	±3.51	7.26	±0.35	*
Mg (g/kg)	16.60	±1.52	5.80	±1.01	*
S (g/kg)	3.56	±0.41	5.63	±0.47	*

Values are means ± SD from three replicated rafts ($n = 3$). Statistical difference (Sig.) in the values between aquaponics and hydroponics treatments is shown by an asterisk * ($p < 0.05$), ns = not significant.

3.3. Onset of Nitrification

The mean concentration of total ammonia nitrogen (TAN) varied over days ($p < 0.05$) but not between treatments while the mean concentrations of nitrite ($p < 0.0001$), nitrate ($p < 0.0001$) and pH ($p < 0.0001$) differed significantly between treatments and over days. The maximum TAN concentration in the aquaponic treatment (2.00 ± 0.00 mg/L, $n = 3$) was reached on day 6 and it gradually decreased to zero by day 11. In RAS the maximum TAN (2.67 ± 0.58 mg/L, $n = 3$) was reached on day 9, and it decreased to 0 by day 18 (Figure 1a). From day 18 the concentration of TAN stayed at nearly zero in both treatments until the end of the experiment. The mean nitrite concentration decreased close to zero in the aquaponics treatment on day 11 while it took 39 days in RAS treatment (Figure 1b). The highest mean nitrite concentrations were recorded (4.83 ± 0.28 mg/L, $n = 3$) in aquaponics on day 7 but on day 11 in RAS treatment (Figure 1b).

During the experiment, the highest mean nitrate concentration was recorded on day 9 in aquaponics (81.67 ± 2.88 mg/L, $n = 3$) while on day 14 (80 mg/L) in RAS treatment (Figure 1c). The mean concentration of the nitrate followed a gradual decline and stayed lower in aquaponics compared to RAS treatment during the experiment until day 39 but became almost equal on day 42 (Figure 1c). The pH of the circulating water was significantly different between the treatments over the course of the experiment ($p < 0.0001$), and it gradually decreased during the experiment. The mean daily pH in the RAS treatment varied between 7.79 ± 0.00 ($n = 3$) and 6.43 ± 0.05 ($n = 3$) while in aquaponics it varied between 7.76 ± 0.05 ($n = 3$) and 6.83 ± 0.05 ($n = 3$) (Figure 1d).

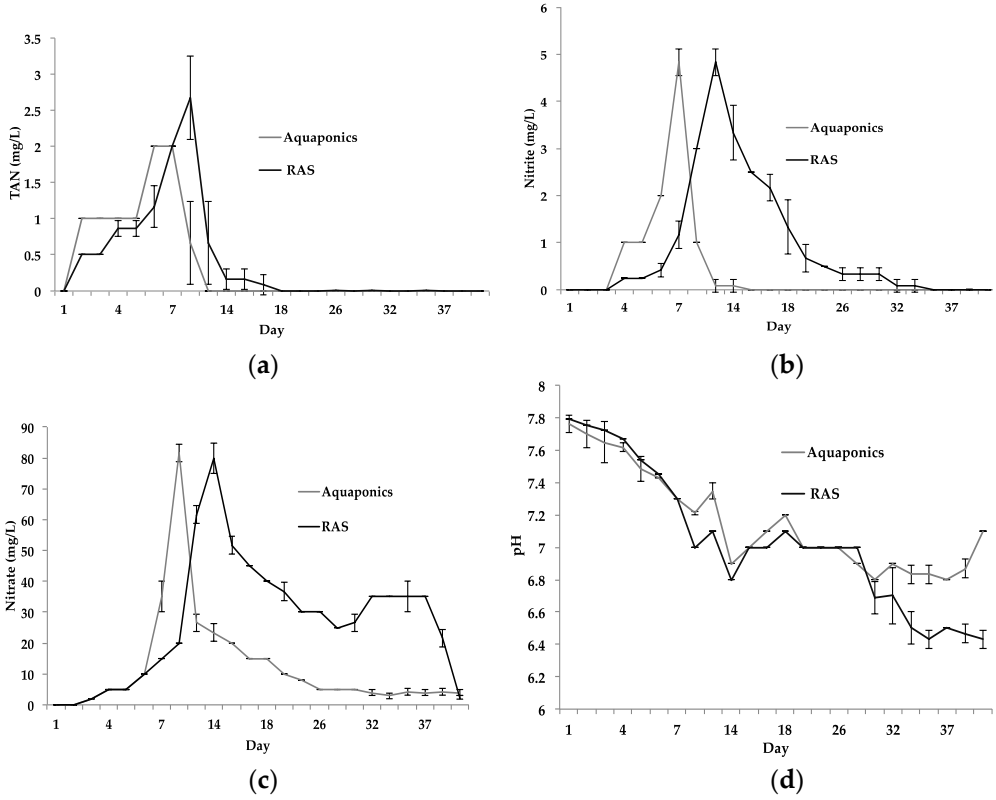

Figure 1. The mean concentration (mg/L) ± SD (n = 3) of (**a**) total ammonium nitrogen (TAN) (**b**) nitrite, (**c**) nitrate, and (**d**) pH in RAS and aquaponics treatments during the 42-day experiment. For the aquaponics treatment spinach was grown in a coupled aquaponics system together with rainbow trout (*Oncorhynchus mykiss*).

3.4. Water Quality

Selected anions (chloride, nitrite-N, nitrate-N, sulfate, and phosphate) were analyzed and quantified at the end of the experiment. Additionally, the concentrations in tap water were analyzed containing 7.7 mg/L Cl^-, 0.21 mg/L NO_3-N, 0.75 mg/L SO_4^{2-}, and below limits of detections for NO_2-N and PO_4^{3-} (Supplementary Table S2). There was no significant difference ($p > 0.05$) in the concentrations (mg/L) of chloride, phosphate, sulfate, or nitrate between hydroponics and aquaponics treatments (Table 4).

Table 4. Concentrations of chloride Cl^-, nitrate-N NO_3-N, sulfate SO_4^{2-}, and phosphate PO_4^{3-} (mg/L) in water samples taken on the last day (day 42) of the experiment in aquaponics and hydroponics deep water culture units. For aquaponics treatment spinach (*Spinacia oleracea*) was grown in a coupled aquaponic system together with rainbow trout (*Oncorhynchus mykiss*).

Element (mg/L)	Aquaponics		Hydroponics	
Chloride	24.80	±14.68	14.24	±6.19
Phosphate	0.10	±0.11	8.07	±6.20
Sulfate	69.06	±49.74	85.32	±57.03
Nitrate-N	3.44	±1.96	4.51	±2.74

Values are means ± SD, n = 3. There were no statistically significant differences between the treatments (independent sample t test, $p > 0.05$). Nitrite-N was below the LOD (0.13 mg/L) in both treatments.

The concentration (mg/L) of chloride was higher ($p < 0.05$) in the aquaponics circulating water than in RAS water, but the concentration of other anions did not differ between the treatments (Table 5).

Table 5. Concentrations of chloride Cl^-, nitrate-N NO_3-N, sulfate SO_4^{2-}, and phosphate PO_4^{3-} (mg/L) in water samples taken on the last day (day 42) of the experiment in aquaponics and RAS from fish tanks. For aquaponics treatment spinach (*Spinacia oleracea*) was grown in a coupled aquaponics system together with rainbow trout (*Oncorhynchus mykiss*).

Element (mg/L)	Aquaponics		RAS		Sig.
Chloride	22.53	±4.34	8.79	±1.17	*
Phosphate	0.30	±0.11	0.43	±0.20	ns
Sulfate	73.10	±38.71	31.77	±21.67	ns
Nitrate	4.11	±1.16	2.00	±1.08	ns

Values are means ± SD, $n = 3$. Statistically significant difference (Sig.) in the values between aqua-ponics and RAS treatments is shown by an asterisk *, ns = not significant (independent samples t test, $p < 0.05$). Nitrite-N was below the LOD (0.13 mg/L) in both treatments.

3.5. Off-Flavors

The concentrations of off-flavors GSM and MIB ranged from 2 to 8 ng/L (GSM) and from 13 to 36 ng/L (MIB) in water (Table 6). GSM concentrations decreased significantly during the experiment for hydroponics and aquaponics DWC (Table 6) but did not differ for RAS and aquaponics fish tank water (Table 6). In the case of MIB, the concentrations increased slightly during the experiment, excluding the hydroponic DWC water but without statistical significance (Table 6). MIB was 9.5 ng/L in the inlet water, while GSM remained below the limit of detection (<LOD). The concentrations of GSM were significantly lower ($p < 0.05$) in fish muscle grown in aquaponics compared to fish grown in RAS (Table 6). The concentration of GSM decreased ($p < 0.05$) in fish muscle after six weeks for the fish grown in aquaponics (Table 6).

Table 6. Concentrations of off-flavor geosmin (GSM ng/L) and 2-methylisoborneol (MIB ng/L) in water from deep water culture (DWC) hydroponics (no fish tanks) and aquaponics (with baby spinach and rainbow trout), and water from fish tanks (aquaponics and RAS), and in rainbow trout muscle (ng/kg) (aquaponics and RAS) in the beginning (5 May) and at the end (14 June) of the 6-week experiment.

Off-Flavors	Aquaponics				Hydroponics (DWC) or RAS			
	Start		End		Start		End	
GSM DWC	5.60	±1.60	3.62	±1.37 ^{A}	6.88	±1.49	3.72	±2.52 ^{A}
MIB DWC	13.08	±2.70	21.05	±14.83	23.81	±3.83	17.07	±9.64
GSM Tank	6.29	±3.13	7.97	±1.29	6.62	±2.50	4.97	±2.82
MIB Tank	24.32	±2.35	36.21	±13.35	20.77	±8.56	28.28	±4.83
GSM Muscle	493.6	±99.10	376.0	±24.99 ^{Aa}	493.6	±99.10	466.3	±39.40 ^{a}
MIB Muscle	1758.1	±298.5	1611.5	±298.6	1758.1	±298.5	1473.8	±240.3

Values are means ± SD, $n = 3$ for end samples and 2 for start samples. The superscript letter "^{A}" indicates statistically significant difference ($p < 0.05$) between the sampling points and "^{a}" between the treatments (aquaponics vs. RAS).

GSM and MIB were also detected in the shoots and roots of the spinach both in aquaponics and hydroponics in the start and after six weeks of the experiment. The concentrations of both GSM and MIB decreased in the shoots of both systems ($p < 0.05$) and in the roots of spinach grown in hydroponics (MIB $p < 0.001$) after six weeks (Table 7). In roots, however, the concentrations of GSM and MIB increased during the experiment for the aquaponics treatment, but without significant difference ($p > 0.05$, Table 7). While comparing between treatments, the MIB in shoots and GSM in roots for aquaponics were statistically higher than in hydroponics (Table 7).

Table 7. Concentrations (ng/L) of off-flavors geosmin (GSM) and 2-methylisoborneol (MIB) in spinach grown in aquaponics and hydroponics in the beginning (5 May) and after six weeks (14 June) of the experiment.

Off-Flavors		Aquaponics			Hydroponics	
		Start	End		End	
MIB (ng/L)	shoot	1079.4	704.4	± 73.08 ^{Aa}	278.00	±158.2 ^{Aa}
	root	1260.6	1496.6	± 998.2	300.50	± 80.48 ^{A}
GSM (ng/L)	shoot	134.4	8.68	± 2.50 ^{A}	7.87	± 2.24 ^{A}
	root	212.8	3579.8	± 1682.8 ^{a}	191.80	± 48.78 ^{a}

Values are means ± SD, $n = 3$, $p < 0.05$, The superscript letter "^{A}" indicates statistically significant difference ($p < 0.05$) between the sampling points and "^{a}" between the treatments (aquaponics vs. RAS).

The lipid content (%) remained similar in fish muscle in RAS and aquaponics. The slight increase was observed from the start value of 6.0% to 7.5 ± 0.9 % in RAS and to 6.3 ± 1.7 % in aquaponics, but without a statistically significant difference ($p > 0.05$) between the systems.

4. Discussion

The fish species used in this study, rainbow trout, has been listed as one of the most invasive species in the world [32], and it is also on the blacklists of invasive species in some European countries [33]. However, the capacity of rainbow trout to establish self-sustaining populations in Europe is quite limited despite popular stockings for recreational purposes [33]. Farming rainbow trout on land in RAS and aquaponics decreases the potential risk of fish escapes as compared to, e.g., rearing in cages. From the environmental protection point of view, aquaponics can also be regarded as a method complying with the best management practices [34].

In the present study, rainbow trout grown in an integrated system with spinach had higher SGR (1.95 ± 0.12) compared to fish grown in RAS (1.67 ± 0.08). Pulkkinen et al. [2] reported an SGR of 1.58 ± 0.03 for RAS-grown rainbow trout of a similar size and temperature, which indicates good growth of fish and thus good rearing conditions in both of our systems. Rainbow trout has already earlier been shown to be a suitable species for aquaponic systems [15]. The feed conversion ratio of rainbow trout in the aquaponic system was lower (0.85 ± 0.08) than in RAS (1.06 ± 0.03). As the amount of feed consumed was almost equal in both treatments, this, in turn, was seen as higher growth rates of the fish in aquaponics. In experiments where feed intake is monitored, FCR for juvenile rainbow trout fed dry pellets is typically about one, and more commonly below one [2,35,36]. A plausible explanation for the improved production parameters in the aquaponic system was the difference in water quality. For example, the onset of nitrification was faster in aquaponics than in RAS, and the overall level of nitrate was lower in the aquaponics treatment (12.30 ± 0.83) than in RAS (26.98 ± 1.04). On the other hand, Davidson et al. [3] did not find any difference in the final weight or FCR between rainbow trout reared in "low" (30 mg/L) or "high" (91 mg/L) nitrate in RAS. However, the average final biomass and density were significantly higher in the "low" nitrate treatment, affected by higher mortality in the "high" treatment. However, the FCR was rather high in both treatments (about 1.3) [3]. FCR-values below one are also reported, e.g., for Murray cod (*Maccullochella peelii peelii*; 0.85) [37] and Nile tilapia (*Oreochromis niloticus*; 0.93) [38] reared in aquaponics.

Managing water quality is crucial for the growth and survival of the fish [39]. During protein catabolism, ammonia is excreted by fish through the gills into the water and it is first oxidized to nitrite and then to nitrate. Nitrite is produced as an intermediate product during the nitrification process and can be oxidized into nitrates if the biofilter is well established [1]. Long-term exposure of fish to nitrite and TAN can be lethal if they exceed the acceptable concentration, which should be less than 1 mg/L but preferably close to 0 [1], and salmonids are more sensitive to nitrite than many other species [40]. The acceptable limit for unionized ammonia nitrogen for cold-water fish is 0.025 mg/L, and the proportion

of unionised ammonia of TAN increases with the increase in pH and temperature [1]. The concentrations of TAN, nitrite, and pH should be adjusted if they are outside the acceptable limits [1].

Aquaponics systems are beneficial in terms of low water use, nutrient recycling, and improving the quality of the recirculating water [41,42]. In the present study, we found that the first step of nitrification (oxidation of TAN to nitrite) started slightly faster in aquaponics treatment than in RAS. TAN levels rose at the beginning of the experiment in both treatments and then gradually decreased, and there was no significant difference between treatments in TAN concentrations. The daily mean concentration of TAN decreased below 1 mg/L (0.67 $\pm$ 0.58) on day 9 in aquaponics treatment while on day 11 in RAS. For the second step of nitrification (oxidation of nitrite to nitrate), the peak concentration of nitrite (4.83 $\pm$ 0.14 mg/L) in aquaponics was reached on day 7, while it took 11 days longer in RAS (4.83 $\pm$ 0.28 mg/L). After day 11, the concentration of nitrite became nearly zero in aquaponics, while in RAS similar concentration was attained in 34 days. Before the nitrification process was properly established, TAN and nitrite were much above the recommended levels [1]. However, these levels did not seem to induce mortality or other negative effects on fish, as seen as good growth and low FCR. This suggests that rainbow trout has a relatively high short-term tolerance for TAN and nitrite. The lower concentrations of TAN, nitrite, and especially nitrate in aquaponics treatment clearly showed that the aquaponic systems worked as expected [43]. This was demonstrated by the plants absorbing all these dissolved nutrients from the water.

Fischer et al. [27] studied the water quality and productivity of the spring onion (*Allium fistulosum*), lemongrass (*Cymbopogon citratus*), and largemouth bass (*Micropterus salmoides*) juveniles in both RAS and aquaponics. They reported no significant difference in TAN, nitrite, and pH between the treatments. However, nitrate increased steadily in both treatments, although still at a higher level in RAS than in the aquaponic system. In our experiment, the nitrate level remained lower in the aquaponics treatment compared to the RAS treatment. The sudden drop in the nitrate level after day 37 in RAS can be partly linked to water changes as 40–50% water of the system was changed twice in RAS treatment during week six due to the decrease in pH; however, water change cannot be the only reason for this unexpected drop of nitrate level below 5 mg/L (Figure 1c, Table 4).

In our study, the pH remained nearly neutral and only slightly different between treatments until day 30. After day 30, there was a sudden decline in pH in RAS treatment. The reason could be linked to the lack of alkalinity management and the absence of water change in week 5. In the aquaponics treatment, the pH of the system remained after the first experimental week rather steadily close to seven, and no drop in pH was observed after week five as in RAS (Figure 1d). Nitrification is an acid-forming process that can destroy the alkalinity of the water and result in a pH decrease [1]. Alkalinity of water is also affected by feed input, water exchange rates, hydraulic retention time and nitrifying activity [44,45]. Alkalinity adjustment is needed in RAS, which is usually done by the addition of sodium bicarbonate or diluted NaOH [1,44], but in aquaponics, compounds containing sodium should not be used [1]. Water exchanges also help maintaining the water quality for RAS by preventing the accumulation of harmful compounds in the system [45]. One reason for the absence of pH drop in our aquaponic system could be that the plants buffered the pH of the system [42]. During the process of absorbing nitrates from the surrounding water, the plants exchange H^+ and OH^- between the medium and roots. Plants excrete the anion and uptake cation leaving the root medium alkaline [46,47]. On the other hand, the stability of pH in aquaponics can also be related to anoxic parts in the biofilter causing denitrification and, thus, recovery of alkalinity [1].

The tap water analysis of the present study showed very low concentrations of sulfate. Similarly, the concentration of nitrate-N was also very low in tap water. In the area of the inlet water uptake, the sulfate concentrations for sulfate are typically very low [48], and for nitrate, they remain below 5 mg/L [48]. The guidelines of Norwegian authorities

recommend that water nitrite levels should be below 0.1 mg/L but there are no reference values for freshwater chloride concentration [49].

The chloride concentration was significantly higher in aquaponics tank water than in RAS, which can be linked to the smaller relative water exchange in aquaponics compared to RAS treatment. Chloride from the fish feed possibly accumulated in the systems during the experiment. The minor increase in phosphate likely originates from the fish metabolism as fish feed contains phosphorus [50,51]. Both chloride and phosphate remained clearly below the recommended limit value of 3 mg/L for salmonids [51]. Additionally, the plants in the aquaponic system absorb phosphate from circulating water [42] but likely because of the low level of phosphate in both aquaponics and RAS, the difference between the two systems was not significant (Table 5).

The plants can perform well at variable concentrations of nutrients in soilless systems [52]. In our study, the concentrations of macronutrients, i.e., nitrate, chloride, phosphate, and sulphate, were within the range required for the growth of plants in soilless systems [52–55]. Chloride is a beneficial nutrient for plant growth which is required in small quantities, and less than 70 mg/L is generally safe for all plants [52]. Higher chloride concentration can affect the absorption of nitrogen and other nutrients. Phosphate is also needed in small quantities for the plant's growth. Phosphorus plays an important role in root development and flower quantity [54]. A study documented that the Kale plant (*Brassica oleracea*) performed well at 10-times lower concentrations (0.1 mM) of phosphate without compromising its growth in hydroponics [55]. Sulfur is an important nutrient for plants and required in small quantities. Plant absorbs sulfur in the form of sulfate. The sulfur requirement can vary for different plants depending upon the plant species. Generally the recommended concentration of sulfur in hydroponics nutrient solution range from 48 to 336 mg/L [12,53]. In the present study the sulfate concentration ranged from 69.06 $\pm$ 49.74 (aquaponics) to 85.32 $\pm$ 57.03 (hydroponics). The concentrations of nutrients are typically lower in aquaponics water compared to standard hydroponics solution, but most leafy vegetables can grow at lower concentrations than in standard hydroponic solution [56]. However, most plants require nutrient supplementation in aquaponics to deal with their nutritional requirements [56]. The nutrient requirements of plants vary with developmental stage, environmental conditions, plant species, and variety [12,56].

In the present study, we added micronutrients (Fe, Cu, Mn, Zn, B) to the aquaponic systems to comply with the plant's growth requirements. The modified nutrient solution was prepared by considering safe nutrient limits for the plants and rainbow trout [28,57]. The amount of Fe, Zn, and B were higher in spinach leaves grown in aquaponics than in hydroponics, most likely due to the addition of micronutrient solution and spraying with this solution. The amounts of Ca and Mg were also higher in the spinach grown in the aquaponics treatment, which can be linked to the Ca and Mg in the fish feed. The amount of macronutrients N, P, S, and K were higher in hydroponically grown spinach which was due to the added nutrients into the hydroponic system in the form of the solution and spray. According to the manufacturer, the Substral solution contained nitrogen (N) 6%, phosphorus (P) 1.3%, potassium (K) 5%, sulphur (S) 0.6% and chloride (Cl) less than 0.5%. The concentrations of macronutrients in hydroponics nutrient solution explain the higher amounts of macronutrients in spinach grown in hydroponics. In our study, the concentrations of macronutrients in spinach shoots were comparable to those reported by Maneejantra et al. [58], except that magnesium was lower in spinach grown in hydroponics and calcium higher in aquaponics. As to micronutrients, the concentration of iron was higher (523.3 mg/kg $\pm$ 75.05) in spinach grown in aquaponics compared to the reported 267 mg/kg [59] for spinach purchased from the vegetable market. The concentration of iron can vary in different parts of plants. In general, iron concentrations in leaves in most plants range between 0.1 to 5000 mg/kg [60]. The concentration of zinc was lower in aquaponics (526.6 mg/kg $\pm$ 142.9) and hydroponics (206.6 mg/kg $\pm$ 55.7) compared to the concentration reported in another study for spinach (3230 mg/kg) [59].

Even if the dry weight of spinach at the end of the experiment did not significantly differ statistically between aquaponic and hydroponic treatments, in aquaponics, the total plant weight was 43% higher (40% for shoot, 70% for root) than in hydroponics. The nutritional content in spinach leaves varied and depended upon the quantity of nutrients supplied and present in the circulating water. In a hydroponics system, the addition of Substral nutrient solution, compensation of evaporated water with Substral solution, and daily spraying of plants with this solution provided the nutrients that were essential for the spinach growth while in aquaponics treatment, the plants were getting nutrients with the circulating water. In addition, the micronutrient solution for aquaponics spinach fulfilled the expected needs of micronutrients. The color of spinach was vibrant green with no signs of yellowing. Our results are supported by the earlier studies showing that the aquaponic systems can produce the same or higher yield compared to hydroponics [27,61,62]. A study on spinach grown with stellate sturgeon (*Acipenser stellatus*) in an aquaponics system showed that the growth, quantity, and quality of aquaponically grown spinach were similar to the field-grown spinach [63].

The concentrations of off-flavors were relatively low for GSM (2–8 ng/L) and MIB at moderate levels (15–35 ng/L) in the water of the studied systems. These are typical for a RAS (5–25 ng/L GSM, 50–130 ng/L MIB, [64] and 128 ng/L GSM, 94 ng/L MIB) [23] although each RAS is a unique system. The off-flavor concentrations in the inlet water of a fish farm often increase in the spring and summer due to increased microbial activity, typical for warmer seasons [65], although moderate concentrations of off-flavors have been observed in the winter [66,67]. Concentrations detected in the inlet water could partly explain the increase of MIB in the water samples. Low concentrations of GSM were detected in fish muscle (400–500 ng/kg), likely remaining below the typical limit of sensory detection of 700–900 ng/kg for GSM and MIB [68,69]. However, even lower sensory detection limits have been suggested (250 ng/ kg GSM) [70]. Despite the low accumulation of GSM, concentrations of 1200–1800 ng/kg MIB were detected, which were clearly above the sensory detection limit (700–900 ng/kg) [68,69]. However, there were no statistically significant differences between the concentrations of MIB or GSM in RAS and aquaponics water, which suggests that spinach cannot be used to significantly improve water quality in aquaponic systems with respect to preventing off-flavor accumulation in water and fish. However, this does not rule out the possibility that some other plant species could be used for this purpose, which would warrant further investigation.

Water uptake of plant roots proceeds via hydraulic conductance. The roots control the movements of water in the root–soil interface by specific transporter proteins on root-cell membranes, hydraulic conductivity, and cell-wall structure [71]. Compounds are transferred in a plant via hydraulic conductivity. Lipophilic molecules cannot move freely in an aqueous cellular environment [72], leading to lower transfer of lipophilic compounds in shoots, including GSM and MIB. Besides the uptake of water by the roots, it is possible that microbes on the root biofilm also produce off-flavor compounds. GSM is known to form in the roots of several root crops, including beet (*Beta vulgaris*) and spinach [73]. Although GSM and MIB are lipophilic compounds (K_{ow} 3.57 for GSM and 3.31 for MIB), they are still sparsely soluble in water (solubility GSM 160 mg/L, MIB 305 mg/L) and therefore transferrable to the plant shoots in minor proportions [19]. This was supported by the results of this study, and higher concentrations of MIB (with higher solubility) were detected in the shoots. All this may explain the high concentrations observed in the roots and much lower concentrations in the shoots of spinach, especially in aquaponics.

In this study, both GSM and MIB were found in the roots and shoots of spinach. This may suggest that bacteria producing the off-flavor compounds can occur or be absorbed in roots at high concentrations and be transported even to plant shoots. Several studies have examined differences in the bacterial composition between RAS and aquaponic systems with some key differences [74,75]. Fischer et al. [27] reported substantially more bacterial diversity in the aquaponic system than in RAS and detected bacteria closely associated with plant roots, including Rhodobacterales, Rhizobiales and Folman et al. [76] in *Lysobacter*

sp. Additionally, Fischer et al. [27] detected *Streptomyces* in RAS but not in the aquaponic system, and both GSM and MIB are known to be secondary metabolites of *Streptomyces* [77]. So far, the functional significance of GSM is unknown in bacteria and plants. Maher and Goldman [73] studied GSM concentrations in beet and found 43000–17300 ng/kg. This was higher than the concentrations found in this experiment, although differences between different vegetables can be expected. Murungi et al. [78] detected GSM in spinach roots, but they did not quantify its content.

Fischer et al. [27] detected bacteria *Streptomyces* only in RAS reared largemouth bass (*Micropterus salmoides*) but not in aquaponics with lemongrass and spring onion. *Streptomyces* is known to be associated with off-flavor production, but Fischer et al. [27] did not measure the off-flavor concentrations. The results of our study showed no significant difference in off-flavors in water between RAS and aquaponics. However, the concentration of GSM was lower in fish flesh reared in aquaponics. The occurrence of off-flavors is an important issue because off-flavors require additional process solutions, time, and high amounts of clean water for their removal, which leads to decreased profitability of RAS production. Furthermore, any off-flavors above the sensory detection limit decrease the consumer acceptance of raised fish and make them unmarketable.

5. Conclusions

We got support for our first hypothesis about the onset of nitrification process, as it was faster in the aquaponics treatment than in RAS. The second hypothesis regarding the concentration of off-flavor compounds in fish flesh was supported only partly, as the GSM concentration was lower in rainbow trout flesh grown in aquaponics but the concentration of MIB was at a similar level in fish reared in aquaponics and RAS. The third hypothesis about the concentration of off-flavor compounds being higher in the plants grown in aquaponics than in hydroponics was supported partly, as the concentration of GSM was higher in the roots and MIB was higher in the shoots of spinach grown in aquaponics than in hydroponics. In the fourth hypothesis we assumed similar growth for the plants and fish. Spinach grew equally well in both aquaponics and hydroponics treatments, but the aquaponics system was better in terms of fish growth with improved FCR, likely because of the better water quality in the aquaponic system.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14091447/s1, Table S1: Limits of detection (LOD), limits of quantification (LOQ) for micronutrients (mg/kg dm) Fe, Cu, Mn, Zn, B and macronutrients (g/kg dm) N, P, K, Ca, Mg, S for ICP-OES and ICP-OES methods.; Table S2: Limits of detection (LOD), limits of quantification (LOQ), and linearity (R^2) of selected standard solutions (1–100 mg/L) for HPIC analysis of anions.

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Institutional Review Board Statement: The fish used in this study did not experience at any moment pain, distress or suffering that would be comparable to the introduction of a needle into the body. Therefore no experimental animal permit was needed according to the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The laboratory has been accepted by the Regional State Administrative Agency on 27 March 2018 for keeping fishes for experimental purposes (permission number ESAVI/5100/04.10.05/2018).

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References

1. Timmons, M.B.; Guredat, T.; Vinci, B.J. *Recirculating Aquaculture*, 4th ed.; Ithaca Publishing Company LLC: Ithaca, NY, USA, 2018.
2. Pulkkinen, J.T.; Eriksson-Kallio, A.M.; Aalto, S.L.; Tiirola, M.; Koskela, J.; Kiuru, T.; Vielma, J. The effects of different combinations of fixed and moving bed bioreactors on rainbow trout (*Oncorhynchus mykiss*) growth and health, water quality and nitrification in recirculating aquaculture systems. *Aquac. Eng.* **2019**, *85*, 98–105. [CrossRef]
3. Davidson, J.; Good, C.; Welsh, C.; Summerfelt, S.T. Comparing the effects of high vs. low nitrate on the health, performance, and welfare of juvenile rainbow trout *Oncorhynchus mykiss* within water recirculating aquaculture systems. *Aquac. Eng.* **2014**, *59*, 30–40. [CrossRef]
4. Michaud, L.; Blancheton, J.P.; Bruni, V.; Piedrahita, R. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquac. Eng.* **2006**, *34*, 224–233. [CrossRef]
5. Menéndez, S.; Barrena, I.; Setien, I.; González-Murua, C.; Estavillo, J.M. Efficiency of nitrification inhibitor DMPP to reduce nitrous oxide emissions under different temperature and moisture conditions. *Soil Biol. Biochem.* **2012**, *53*, 82–89. [CrossRef]
6. Krüner, G.; Rosenthal, H. Efficiency of nitrification in trickling filters using different substrates. *Aquac. Eng.* **1983**, *2*, 49–67. [CrossRef]
7. Lekang, O.I.; Kleppe, H. Efficiency of nitrification in trickling filters using different filter media. *Aquac. Eng.* **2000**, *21*, 181–199. [CrossRef]
8. Jensen, M.H. Hydroponics worldwide. In *International Symposium on Growing Media and Hydroponics*; Papdopoulos, A.P., Ed.; ISHS ActaHortic: Windsor, ON, Canada, 1997; Volume 481, pp. 719–730. [CrossRef]
9. Aires, A. Hydroponic production systems: Impact on nutritional status and bioactive compounds of fresh vegetables. In *Vegetables: Importance of Quality Vegetables to Human Health*; Asaduzzaman, M., Asao, T., Eds.; IntechOpen: London, UK, 2018; pp. 55–56. [CrossRef]
10. Asao, T. *Hydroponics: A Standard Methodology for Plant Biological Researches*; BoD–Books on Demand; IntechOpen: London, UK, 2012. [CrossRef]
11. Sharma, N.; Acharya, S.; Kumar, K.; Singh, N.; Chaurasia, O.P. Hydroponics as an advanced technique for vegetable production: An overview. *J. Soil Water Conserv.* **2018**, *17*, 364–371. [CrossRef]
12. Goddek, S.; Joyce, A.; Kotzen, B.; Burnell, G.M. *Aquaponics Food Production Systems: Combined Aquaculture and Hydroponic Production Technologies for the Future*; Springer International Publishing: Cham, Switzerland, 2019. [CrossRef]
13. Wirza, R.; Nazir, S. Urban aquaponics farming and cities-a systematic literature review. *Rev. Environ. Health* **2020**, *36*, 47–61. [CrossRef]
14. Yep, B.; Zheng, Y. Aquaponic trends and challenges–a review. *J. Clean. Prod.* **2019**, *228*, 1586–1599. [CrossRef]
15. Skar, G.S.L.; Liltved, H.; Rye, K.P.; Høgberget, R.; Björnsdottir, R.; Homme, J.M.; Oddsson, S.; Paulsen, H.; Drengstig, A.; Savidov, N.; et al. *Aquaponics NOMA (Nordic Marine)–New Innovations for Sustainable Aquaculture in the Nordic Countries*; Nordic Innovation: Oslo, Norway, 2015.
16. Dickschat, J.S.; Bode, H.B.; Mahmud, T.; Müller, R.; Schulz, S. A novel type of geosmin biosynthesis in myxobacteria. *J. Org. Chem.* **2005**, *70*, 5174–5182. [CrossRef]
17. Hathurusingha, P.I.; Davey, K. A predictive model for taste taint accumulation in recirculating aquaculture systems (RAS) farmed-fish–demonstrated with geosmin (GSM) and 2-methylisoborneol (MIB). *Ecol. Modell.* **2014**, *291*, 242–249. [CrossRef]
18. Lindholm-Lehto, P.C.; Vielma, J.; Pakkanen, H.; Alén, R. Depuration of geosmin-and 2-methylisoborneol-induced off-flavors in recirculating aquaculture system (RAS) farmed european whitefish *Coregonus lavaretus*. *J. Food Sci. Technol.* **2019**, *56*, 4585–4594. [CrossRef]
19. Howgate, P. Tainting of farmed fish by geosmin and 2-methyl-iso-borneol: A review of sensory aspects and of uptake/depuration. *Aquaculture* **2004**, *234*, 155–181. [CrossRef]
20. Davidson, J.; Schrader, K.; Ruan, E.; Swift, B.; Aalhus, J.; Juarez, M.; Wolters, W.; Burr, G.; Good, C.; Summerfelt, S.T. Evaluation of depuration procedures to mitigate the off-flavor compounds geosmin and 2-methylisoborneol from Atlantic salmon *Salmo salar* raised to market-size in recirculating aquaculture systems. *Aquac. Eng.* **2014**, *61*, 27–34. [CrossRef]
21. Davidson, J.; Summerfelt, S.; Espmark, Å.M.O.; Mota, V.C.; Marancik, D.; Earley, R.L.; Snead, A.; Good, C. Effects of ozone on post-smolt atlantic salmon (*Salmo salar*) performance, health, and maturation in freshwater recirculation aquaculture systems. *Aquaculture* **2021**, *533*, 736208. [CrossRef]

22. Davidson, J.; Grimm, C.; Summerfelt, S.; Fischer, G.; Good, C. Depuration system flushing rate affects geosmin removal from market-size atlantic salmon *Salmo salar*. *Aquac. Eng.* **2020**, *90*, 102104. [CrossRef]
23. Suurnäkki, S.; Pulkkinen, J.T.; Lindholm-Lehto, P.C.; Tiirola, M.; Aalto, S.L. The effect of peracetic acid on microbial community, water quality, nitrification and rainbow trout (*Oncorhynchus mykiss*) performance in recirculating aquaculture systems. *Aquaculture* **2020**, *516*, 734534. [CrossRef]
24. Fotiou, T.; Triantis, T.M.; Kaloudis, T.; Hiskia, A. Evaluation of the photocatalytic activity of TiO_2 based catalysts for the degradation and mineralization of cyanobacterial toxins and water off-odor compounds under UV-A, solar and visible light. *Chem. Eng. J.* **2015**, *261*, 17–26. [CrossRef]
25. Spiliotopoulou, A.; Rojas-Tirado, P.; Chhetri, R.K.; Kaarsholm, K.M.S.; Martin, R.; Pedersen, P.B.; Pedersen, L.-F.; Andersen, H.R. Ozonation control and effects of ozone on water quality in recirculating aquaculture systems. *Water Res.* **2018**, *133*, 289–298. [CrossRef]
26. Xue, Q.; Liu, Y.; Zhou, Q.; Utsumi, M.; Zhang, Z.; Sugiura, N. Photocatalytic degradation of geosmin by Pd nanoparticle modified WO_3 catalyst under simulated solar light. *Chem. Eng. J.* **2016**, *283*, 614–621. [CrossRef]
27. Fischer, H.; Romano, N.; Jones, J.; Howe, J.; Renukdas, N.; Sinha, A.K. Comparing water quality/bacterial composition and productivity of largemouth bass *Micropterus salmoides* juveniles in a recirculating aquaculture system versus aquaponics as well as plant growth/mineral composition with or without media. *Aquaculture* **2021**, *538*, 736554. [CrossRef]
28. Ingestad, T. Mineral nutrient requirements of *pinus silvestris* and *Picea abies* seedlings. *Physiol. Plant.* **2006**, *45*, 373–380. [CrossRef]
29. Priyantha Indrajith, H.; Davey, K. Experimental validation of a time-dependent model for chemical taste taint accumulation as geosmin (GSM) and 2-methylisoborneol (MIB) in commercial RAS farmed barramundi (*Lates calcarifer*). *Ecol. Model.* **2016**, *340*, 17–27. [CrossRef]
30. Lindholm, L. Developing a robust and sensitive analytical method to detect off flavour compounds in fish. *Environ. Sci Pollut. Res.* **2022**. [CrossRef] [PubMed]
31. Lindholm-Lehto, P.; Pulkkinen, J.; Kiuru, T.; Koskela, J.; Vielma, J. Water quality in recirculating aquaculture system using woodchip denitrification and slow sand filtration. *Environ. Sci. Pollut. Res.* **2020**, *27*, 17314–17328. [CrossRef]
32. Lowe, S.; Browne, M.; Boudjelas, S.; De Poorter, M. *100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database*; The Invasive Species Specialist Group (ISSG): Auckland, New Zealand, 2000.
33. Stanković, D.; Crivelli, A.J.; Snoj, A. Rainbow trout in Europe: Introduction, naturalization, and impacts. *Rev. Fish. Sci. Aquac.* **2015**, *23*, 39–71. [CrossRef]
34. Clay, J.W. The role of better management practices in environmental management. In *Environmental Best Management Practices for Aquaculture*; Tucker, C.S., John, A.H., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, 2008; pp. 55–72.
35. Salgado-Ismodes, A.; Taipale, S.; Pirhonen, J. Effects of progressive decrease of feeding frequency and re-feeding on production parameters, stomach capacity and muscle nutritional value in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **2020**, *519*, 734919. [CrossRef]
36. Tunçelli, G.; Pirhonen, J. Effects of weekend starvation and the duration of daily feeding on production parameters of rainbow trout *Oncorhynchus mykiss*. *Aquaculture* **2021**, *543*, 737028. [CrossRef]
37. Lennard, W.A.; Leonard, B.V.A. Comparison of three different hydroponic sub-systems (gravel bed, floating and nutrient film technique) in an aquaponic test system. *Aquacult. Int.* **2006**, *14*, 539–550. [CrossRef]
38. Palm, H.W.; Nievel, M.; Knaus, U. Significant factors affecting the economic sustainability of closed aquaponic systems. Part III: Plant units. *AACL Bioflux* **2015**, *8*, 89–106.
39. Yildiz, H.Y.; Robaina, L.; Pirhonen, J.; Mente, E.; Domínguez, D.; Parisi, G. Fish welfare in aquaponic systems: Its relation to water quality with an emphasis on feed and faeces—a review. *Water* **2017**, *9*, 13. [CrossRef]
40. Lewis, W.M., Jr.; Morris, D.P. Toxicity of nitrite to fish: A review. *Trans. Am. Fish. Soc.* **1986**, *115*, 183–195. [CrossRef]
41. Rakocy, J.; Masser, M.P.; Losordo, T. Recirculating Aquaculture Tank Production Systems: Aquaponics-Integrating Fish and Plant Culture. SRAC, publication number 454. 2006. Available online: https://www.researchgate.net/publication/284496499_Recirculating_aquaculture_tank_production_systems_Aquaponics-Integrating_fish_and_plant_culture (accessed on 8 March 2022).
42. Makhdom, S.; Shekarabi, S.P.H.; Shamsaie Mehrgan, M. Biological nutrient recovery from culturing of pearl gourami (*Trichogaster leerii*) by cherry tomato (*Solanum lycopersicum*) in aquaponic system. *Environ. Sci. Pollut. Res.* **2017**, *24*, 20634–20640. [CrossRef] [PubMed]
43. Buzby, K.M.; Lin, L.-S. Scaling aquaponic systems: Balancing plant uptake with fish output. *Aquac. Eng.* **2014**, *63*, 39–44. [CrossRef]
44. Loyless, J.C.; Malone, R.F.A. Sodium bicarbonate dosing methodology for pH management in freshwater–recirculating aquaculture systems. *Progress. Fish-Cult.* **1997**, *59*, 198–205. [CrossRef]
45. Espinal, C.A.; Matulić, D. Recirculating aquaculture technologies. In *Aquaponics food Production Systems*; Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M., Eds.; Springer International Publishing: Cham, Switzerland, 2019; pp. 35–76. [CrossRef]
46. Touraine, B.; Grignon, N.; Grignon, C. Charge balance in NO_3^--fed soybean: Estimation of K^+ and carboxylate recirculation. *Plant Physiol.* **1988**, *88*, 605–612. [CrossRef]
47. Jackson, L.E.; Schimel, J.P.; Firestone, M.K. Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. *Soil Biol. Biochem.* **1989**, *21*, 409–415. [CrossRef]

48. Valvira. Talousvesiasetuksen Soveltamisohje. Osa III. Enimmäisarvojen Perusteet. 2020. Available online: https://www.valvira.fi/documents/14444/6739502/Talousvesiasetuksen_soveltamisohje_osa_3.pdf/b9faedd0-cd83-fd94-09e2-452e7e7ee123 (accessed on 8 March 2022). (In Finnish).
49. Gutierrez, X.; Kolarevic, J.; Sæther, B.-S.; Bæverfjord, G.; Takle, H.; Medina, H.; Terjesen, B. *Effects of Sub-Lethal Nitrite Exposure at High Chloride Background during the Parr Stage of Atlantic Salmon*; Aquaculture Europe: Rhodes, Greece, 2011; pp. 1080–1081.
50. BioMar Finland | BioMar. Available online: https://www.biomar.com/fi/finland/ (accessed on 8 March 2022).
51. Davidson, J.; Good, C.; Welsh, C.; Brazil, B.; Summerfelt, S. Heavy metal and waste metabolite accumulation and their potential effect on rainbow trout performance in a replicated water reuse system operated at low or high system flushing rates. *Aquac. Eng.* **2009**, *41*, 136–145. [CrossRef]
52. Zaman, M.; Shahid, S.A.; Heng, L. *Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques*; Springer International Publishing: Cham, Switzerland, 2018. [CrossRef]
53. Trejo-Téllez, L.I.; Gómez-Merino, F.C. Nutrient solutions for hydroponic systems. In *Hydroponics—A Standard Methodology for Plant Biological Researches*; Asao, T., Ed.; IntechOpen: London, UK, 2012; pp. 1–22. [CrossRef]
54. Maucieri, C.; Nicoletto, C.; van Os, E.; Anseeuw, D.; Havermaet, R.V.; Junge, R. Hydroponic Technologies. In *Aquaponics Food Production Systems: Combined Aquaculture and Hydroponic Production Technologies for the Future*; Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M., Eds.; Springer International Publishing: Cham, Switzerland, 2019; pp. 77–110. [CrossRef]
55. Van Rooyen, I.L.; Nicol, W. Inferential control of the phosphate concentration in hydroponic systems via measurement of the nutrient solution's pH-buffering capacity. *Sci. Hortic.* **2022**, *295*, 110820. [CrossRef]
56. Bittsanszky, A.; Uzinger, N.; Gyulai, G.; Mathis, A.; Junge, R.; Villarroel, M.; Kotzen, B.; Komives, T. Nutrient supply of plants in aquaponic systems. *Ecocycles* **2016**, *2*, 17–20. [CrossRef]
57. FAO: Aquaculture Feed and Fertilizer Resources Information System. Rainbow Trout Nutritional Requirements. Available online: https://www.fao.org/fishery/affris/species-profiles/rainbow-trout/nutritional-requirements/en/ (accessed on 8 March 2022).
58. Maneejantra, N.; Tsukagoshi1, S.; Lu, N.; Supaibulwatana, K.; Takagaki, M.; Yamori, W. A Quantitative analysis of nutrient requirements for hydroponic spinach (*Spinacia oleracea* L.) production under artificial light in a plant factory. *J. Fertil. Pestic.* **2016**, *7*, 170–174. [CrossRef]
59. El-Sayed, S.M. Use of spinach powder as functional ingredient in the manufacture of UF-Soft cheese. *Heliyon* **2020**, *6*, e03278. [CrossRef] [PubMed]
60. Ancuceanu, R.; Dinu, M.; Hovaneț, M.V.; Anghel, A.I.; Popescu, C.V.; Negreș, S. A survey of plant iron content-a semi-systematic review. *Nutrients* **2015**, *7*, 10320–10351. [CrossRef] [PubMed]
61. Pantanella, E.; Cardarelli, M.; Colla, G.; Rea, E.; Marcucci, A. Aquaponics vs. hydroponics: Production and quality of lettuce crop. *Acta Hortic.* **2012**, *927*, 887–893. [CrossRef]
62. Delaide, B.; Goddek, S.; Gott, J.; Soyeurt, H.; Jijakli, M.H. Lettuce (*Lactuca sativa* L. var. *sucrine*) growth performance in complemented aquaponic solution outperforms hydroponics. *Water* **2016**, *8*, 467. [CrossRef]
63. Petrea, S.M.; Cristea, V.; Dediu, L.; Liu, F.; Contoman, M.; Lupoae, P.; Antache, A.; Bandi, A.C. A study of nitrogen dynamics in an integrated stellate sturgeon and spinach aquaponic system with different crop densities. *Bull. Univ. Agric. Sci. Vet. Med. Cluj-Napoca Anim. Sci. Biotechnol.* **2014**, *71*, 218–226. [CrossRef]
64. Burr, G.S.; Wolters, W.R.; Schrader, K.K.; Summerfelt, S.T. Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, *Salmo salar*, cultured in a recirculating aquaculture system. *Aquac. Eng.* **2012**, *50*, 28–36. [CrossRef]
65. Ludwig, F.; Medger, A.; Börnick, H.; Opitz, M.; Lang, K.; Göttfert, M.; Röske, I. Identification and expression analyses of putative sesquiterpene synthase genes in *Phormidium* sp. and prevalence of geoA-like genes in a drinking water reservoir. *Appl. Environ. Microbiol.* **2007**, *73*, 6988–6993. [CrossRef]
66. Watson, S.B.; Ridal, J. Periphyton: A primary source of widespread and severe taste and odour. *Water Sci. Technol.* **2004**, *49*, 33–39. [CrossRef]
67. Wang, R.; Li, D.; Jin, C.X.; Yang, B.W. Seasonal occurrence and species specificity of fishy and musty odor in Huajiang reservoir in winter, China. *Water Resour. Ind.* **2015**, *1*, 13–26. [CrossRef]
68. Persson, P.-E. Sensory properties and analysis of two muddy odour compounds, geosmin and 2-methylisoborneol, in water and fish. *Water Res.* **1980**, *14*, 1113–1118. [CrossRef]
69. Young, W.F.; Horth, H.; Crane, R.; Ogden, T.; Arnott, M. Taste and odour threshold concentrations of potential potable water contaminants. *Water Res.* **1996**, *30*, 331–340. [CrossRef]
70. Grimm, C.; Lloyd, S.W.; Zimba, P. Instrumental versus sensory detection of off-flavors in farm-raised channel catfish. *J. Aquac.* **2004**, *236*, 309–319. [CrossRef]
71. Ryan, P.R.; Delhaize, E.; Watt, M.; Richardson, A.E. Plant roots: Understanding structure and function in an ocean of complexity. *Ann. Bot.* **2016**, *118*, 555–559. [CrossRef]
72. Li, N.; Xu, C.; Li-Beisson, Y.; Philippar, K. Fatty acid and lipid transport in plant cells. *Trends Plant Sci.* **2016**, *21*, 145–158. [CrossRef] [PubMed]
73. Maher, L.; Goldman, I.L. Bidirectional recurrent half-sib family selection for geosmin concentration in table beet. *Crop Sci.* **2017**, *57*, 2564–2574. [CrossRef]
74. Wongkiew, S.; Park, M.-R.; Chandran, K.; Khanal, S.K. Aquaponic systems for sustainable resource recovery: Linking nitrogen transformations to microbial communities. *Environ. Sci. Technol.* **2018**, *52*, 12728–12739. [CrossRef]

75. Bartelme, R.P.; Smith, M.C.; Sepulveda-Villet, O.J.; Newton, R.J. Component microenvironments and system biogeography structure microorganism distributions in recirculating aquaculture and aquaponic systems. *mSphere* **2019**, *4*, e00143-19. [CrossRef]
76. Folman, L.B.; Postma, J.; van Veen, J.A. Characterisation of *Lysobacter enzymogenes* (Christensen and Cook 1978) strain 3.1T8, a powerful antagonist of fungal diseases of cucumber. *Microbiol. Res.* **2003**, *158*, 107–115. [CrossRef]
77. Rurangwa, E.; Verdegem, M.C.J. Microorganisms in recirculating aquaculture systems and their management. *Rev. Aquac.* **2015**, *7*, 117–130. [CrossRef]
78. Murungi, L.K.; Kirwa, H.; Coyne, D.; Teal, P.E.A.; Beck, J.J.; Torto, B. Identification of key root volatiles signaling preference of tomato over spinach by the root knot nematode *Meloidogyne incognita*. *J. Agric. Food Chem.* **2018**, *66*, 7328–7336. [CrossRef] [PubMed]