JYU DISSERTATIONS 675

Faiqa Atique

The Effect of Plants on Microbes, Water Quality, and Fish Performance in an Aquaponic System

UNIVERSITY OF JYVÄSKYLÄ
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ABSTRACT

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Aquaponics is a way to utilize the nutrient-rich effluents from recirculating aquaculture systems (RAS) by combining hydroponics, i.e. soilless plant farming, with RAS. The plants in aquaponics can be grown as a single or mixed plant species together with fish. In this dissertation, I investigated if plant growth is affected in aquaponics compared to hydroponics and if the plants affected the growth and microbial communities in rainbow trout (*Oncorhynchus mykiss*) or water quality in aquaponics compared to RAS. The first experiment was conducted by pairing lettuce (*Lactuca sativa*) with mint (*Mentha spicata*), rucola (*Diplotaxis tenuifolia*), or wormwood (*Artemisia absinthium*) and growing them on nutrient-rich effluents from RAS. The growth of lettuce increased when mint or rucola was grown with lettuce. Specific microbial taxa in lettuce were detected and associated with increased biomass when grown with mint. The second experiment was conducted by growing baby spinach (*Spinacia oleracea*) and rainbow trout together. Baby spinach grew equally well in both aquaponics and hydroponics. Baby spinach had higher concentrations of off-flavor-causing compounds geosmin (GSM) and 2-methylisoborneol (MIB) in aquaponics compared to hydroponics. Rainbow trout had lower GSM in aquaponics compared to RAS. However, the concentration of GSM and MIB did not differ in the water of aquaponics and RAS. The third experiment investigated the effects of mint on the growth and microbial communities of rainbow trout. Microbial communities differed in the mucous and gut of rainbow trout in aquaponics compared to RAS. Water quality was better in aquaponics in terms of lower contents of ammonia, nitrite, and nitrate compared to RAS. In conclusion, plants in aquaponics improved fish growth due to better water quality. Plants grow equally well in aquaponics as in hydroponics and alter the microbial communities of rainbow trout in aquaponics.

Keywords: Aquaponics; baby spinach; feed conversion; hydroponics; mint; nitrification; recirculating aquaculture.

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LIST OF ORIGINAL PUBLICATIONS

The dissertation is based on the following original papers, which will be referred to in the text by their Roman numerals I–III.

The contribution of authors in different papers is presented in Table 1.

I Atique F., Juottonen H. & Kytöviita M.-M. Mint enhances lettuce biomass and provides microbes to co-cultured lettuce in a decoupled aquaponic system (Manuscript).

II Atique F., Lindholm-Lehto P. & Pirhonen J. 2022. Is aquaponics beneficial in terms of fish and plant growth and water quality in comparison to separate recirculating aquaculture and hydroponic systems? *Water* 14(9), 1947.

III Atique F., Juottonen H., Taipale S. & Kytöviita M.-M. Improved water quality, feed conversion, and modified microbiome of the rainbow trout (*Oncorhynchus mykiss*) in aquaponics as compared to recirculating aquaculture system (Manuscript).

TABLE 1 The contribution of different authors to papers I–III. FA = Faiqa Atique JP = Juhani Pirhonen, MMK = Minna-Maarit Kytöviita, HJ = Heli Juottonen, PLL = Petra Lindholm-Lehto, ST = Sami Taipale.

	I	II	III
Design and implementation of research	FA, MMK, HJ	FA, JP, PLL	FA, HJ, MMK
Data collection	FA	FA	FA, ST, HJ, MMK
Data analysis	FA, HJ, MMK	FA, PLL	FA, HJ, ST, MMK
Manuscript draft	FA	FA	FA
Editing and writing	FA, HJ, MMK	FA, PLL, JP	FA, HJ, ST, MMK

ABBREVIATIONS

ALA	alpha-linoleic acid
ANOVA	analysis of variance
ARA	arachidonic acid
DHA	docosahexaenoic acid
DIN	dissolved inorganic nitrogen
DWC	deep water culture
EPA	eicosapentaenoic acid
FCR	feed conversion ratio
GSM	geosmin
LIN	linoleic acid
MIB	2-methylisoborneol
MUFA	monounsaturated fatty acids
NFT	nutrient film technique
NMDS	non-metric multidimensional scaling
ω-3 PUFA	omega 3 polyunsaturated fatty acids
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PERMANOVA	permutational multivariate analysis of variance
RAS	recirculating aquaculture systems
RDA	redundancy analysis
rRNA	ribosomal RNA
SGR	specific growth rate
TAN	total ammonia nitrogen

1 INTRODUCTION

1.1 Challenges for food production

Food production depends on natural resources, such as land, freshwater, and the availability of nutrients and energy (Conijn *et al.* 2018). The world's food demand is increasing due to the expansion in the human population and economic development of the world (Merino *et al.* 2000, Davis *et al.* 2016). To meet the rising demand for food, farming practices are utilizing and exploiting these natural and scarce resources increasingly (Rockström *et al.* 2009, Van Vuuren *et al.* 2010). Increased wealth and awareness regarding food have shifted the dietary choices for more sustainable and environmentally friendly food products (Garnett 2011). In recent years the demand for meat has increased and inevitably the production of meat will cause an unsustainable load on natural resources (Goddek *et al.* 2019a). In the coming decades global food production will need to increase by more than 70 % to meet the millennium development goals including hunger elimination and ensuring environmental sustainability (Thomson 2009, Goddek *et al.* 2019a). The food production industry is tackling the challenges such as environmental pollution, loss of biodiversity, degradation of agricultural land, and scarcity of water resources in many of the most populated areas of the world. The current farming approaches are not sufficient to make improvements as required to meet the global food demands. The limited agricultural land, as well as the polluted and degraded environment, make it impossible to produce food in desired quality and quantities (Bajzelj *et al.* 2014).

The agricultural and aquaculture practices contribute to major water pollution (Graversgaard *et al.* 2018, Mavraganis *et al.* 2020). The pollution from plant farming must be addressed to achieve the sustainable development goals (Herrero *et al.* 2021) and new methods must be introduced to alleviate the pollution and minimize the use of land and water (Conijn *et al.* 2018, Goddek *et al.* 2019a, Herrero *et al.* 2021). Additionally, agricultural practices, including aquaculture operations are strictly regulated through environmental legislation in many countries globally. To tackle the challenges faced by the farming industry the existing farming approaches should transform into more

sustainable practices and thus, innovative food production methods will be needed (Godfray *et al.* 2010, Foley *et al.* 2011). The existing food production methods can be improved by nutrient recycling and waste management (Kahiluoto *et al.* 2014, Conijn *et al.* 2018). The agricultural practices can also be improved by taking advantage of endophytic (bacteria inside the plants) and epiphytic (bacteria on the surface of plants) bacteria (Harman *et al.* 2021). Moreover, the management of biological and chemical interactions in an ecosystem could play an important role in the improvement of agricultural productivity (Neher 1992, Umesha *et al.* 2018).

Aquaculture is the fastest-growing food production industry globally and the goal of aquaculture entrepreneurs is to reduce the nutrient load to secure the continuity of the industry (Varjopuro *et al.* 2000). In traditional, flow through aquaculture, the nutrient pollution from fish excrement and feed enters directly into natural water bodies resulting in thousands of tons of release of phosphorus and nitrogen into natural waters annually (Anon. 2013, Timmons *et al.* 2018). The environmental impact of aquaculture can be reduced by switching to closed, recirculating aquaculture systems (RAS) (Martins *et al.* 2010, Ebeling and Timmons 2012, Ahmed and Turchini 2021).

1.2 Recirculating aquaculture systems

RAS are classified as intensive aquaculture where fish is grown in a closed system with limited water exchange. RAS aim to recycle 90–99 % of water as compared to flow-through aquaculture (Badiola *et al.* 2012, Timmons *et al.* 2018). The percentage of water recirculation and the renewal rates of water in RAS depend upon the amount of feed fed to the fish and the system design. An extreme case is zero exchange RAS where new water is added only to compensate for the water loss due to evaporation and sludge removal (Vielma *et al.* 2022).

In RAS fish are produced indoors, which improves food safety compared to aquaculture outdoors. RAS products have a market advantage over traditional aquaculture as RAS production can ensure the desired volume of fresh fish products in a desired time frame (Goddek *et al.* 2019a). RAS consist of a series of tanks and filters, including fish tanks, a filter to remove solids, and a biofilter. In RAS water is reconditioned through mechanical and biological filtration, oxygenation, and aeration and then reused within the system. From the fish tank water is passed to a solids removal filter and then to a biological filter where ammonia-nitrogen is biologically converted to nitrate-nitrogen (Bartelme *et al.* 2017, Timmons *et al.* 2018, Preena *et al.* 2021). In general fish feed contains 30–60 % of protein and 4–10 % of nitrogen (Santos *et al.* 2022). Fish only assimilates 20–30 % of the feed which becomes part of fish biomass. The waste nitrogen in RAS originates from unassimilated feed or assimilated feed. The assimilated nitrogen is excreted by the fish in the form of ammonia through gills (Meriac *et al.* 2014, Santos *et al.* 2022). Ammonia is toxic for the fish and must be removed from the system or recycled within the system. The total ammonia nitrogen (TAN) of the system consists of ionized and unionized

ammonia. The unionized ammonia nitrogen is toxic to fish and should be maintained under 0.025 mg l^{-1} for cold-water fish species. The proportion of unionized ammonia of TAN rises with the increase in pH and temperature. In a typical recirculating aquaculture system (Fig. 1), the main conversion process for ammonia is the nitrification process. In nitrification, ammonia is oxidized to nitrite by bacteria from the genera *Nitrosomonas* and ammonia-oxidizing archaea (AOA), and then nitrite are oxidized to nitrate by the action of bacteria from the genus *Nitrobacter* and *Nitrospira* (Simeonidou *et al.* 2012, Bartelme *et al.* 2017).

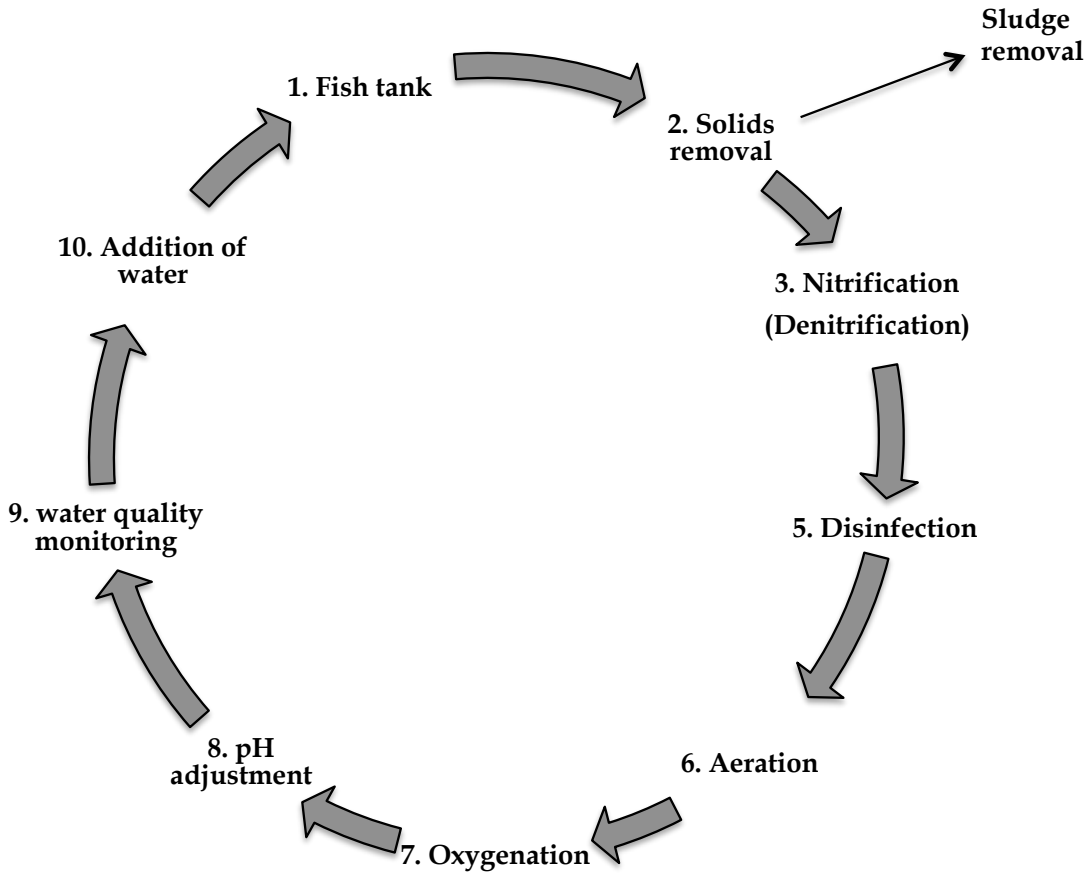

FIGURE 1 A typical recirculating aquaculture system showing different steps of water treatment.

The removal of nitrogenous compounds especially ammonia and nitrite from RAS is vital because these substances inhibit fish growth (Timmons *et al.* 2018, Preena *et al.* 2021). Nitrate is a relatively safe compound for fish (Timmons *et al.* 2018). The accumulation of nitrate at higher concentrations in RAS could decrease the fish growth depending upon the fish species and the exposure time of fish to nitrate (Davidson *et al.* 2014a, Monsees *et al.* 2017). The growth of rainbow trout is reported to be decreased at 100 mg l^{-1} nitrate levels while the growth of tilapia (*Oreochromis niloticus*) will be compromised at 400 mg l^{-1} nitrate (Davidson *et al.* 2014a, Mota *et al.* 2015, Monsees *et al.* 2017). Therefore, it is important to avoid excessive nitrate accumulation in RAS. The release of

nitrate from RAS into the environment can cause eutrophication and disrupt the ecosystem (Jian *et al.* 2022). Therefore, additional measures are needed to deal with nitrogen removal from RAS. Nitrate is not toxic for fish compared to ammonia and nitrite, but still effective control approaches should be taken up for the maximum removal of nitrogenous compounds from aquaculture systems. Nitrification is essential process to control the toxicity of nitrogenous compounds in RAS. Denitrifying biofilter has also been used to remove the nitrate from RAS (Singer *et al.* 2008, Joyce *et al.* 2019). The ammonia-oxidizing bacteria, nitrifying bacteria, and denitrifying bacteria play essential roles in nitrification and denitrification. Nitrification and denitrification can take place simultaneously in RAS but mostly the addition of an extra carbon source is required for effective denitrification (Van Rijn *et al.* 2006). To enhance the removal of nitrate from the RAS approaches such as denitrifying bioreactor, woodchip denitrification, and sludge denitrification (Suhr *et al.* 2013, Kiani *et al.* 2020, Pulkkinen *et al.* 2021) have been used but there is a need to investigate further innovative cost-effective methods to improve the nitrogen removal from the RAS (Martins *et al.* 2010).

In RAS, the onset of nitrification in biofilter is an important concern because the ammonia excreted by fish is converted to nitrate in nitrification. Hence, the system performance and water quality in RAS are dependent on nitrification (Pulkkinen *et al.* 2018). The start-up of nitrification in a biofilter is influenced by the presence of nitrifying bacteria, the size of the bacterial community, bacterial competition for space, and water quality (Li *et al.* 2022). During nitrification, peaks of metabolic products (TAN, nitrite, and nitrate) occur (Ida *et al.* 2006, Li *et al.* 2022). The concentrations of TAN, nitrite, and nitrate can increase exponentially in biofilter (Preena *et al.* 2021) until the biofilter becomes fully functional and the nitrifying bacteria become well established (Ebeling and Timmons 2012). The elevated concentrations of TAN and nitrite during the start-up of nitrification can exceed the tolerance limits of the fish. Therefore, it is important to keep the concentrations of TAN and nitrite under 1 mg l^{-1} preferably close to zero (Ebeling and Timmons 2012), and nitrate preferably under 100 mg l^{-1} for rainbow trout (Davidson *et al.* 2014a). The start-up of nitrification has been investigated by clean start-up (addition of fish), by the addition of chemicals, or by the addition of bacterial inoculates (Grommen *et al.* 2002, Kuhn *et al.* 2010, Pulkkinen *et al.* 2018) but so far no attention has been paid to use plants to assist the biofilter maturation and hence, nitrification start-up in aquaculture. The roots of plants provide additional surface area and act as a biofilter to support nitrification (Vaillant *et al.* 2004). The abundance of microbes in the system may play a role in initiating and speeding up the nitrification activity. A recent study reported that the nitrifying microbial communities present in the hydroponic component of aquaponics may play a larger role in the nitrogen cycle of the system than was previously thought (Schmautz *et al.* 2022). Plants grown together with fish have been reported to contain nitrifying bacterial communities in abundance depending upon the plant species (Hu *et al.* 2015). Therefore, the plants may assist in the rapid maturation of the biofilter and hence affect the onset of nitrification. Moreover, plants can help to level up the peaks of TAN, nitrite, and nitrate during the nitrification process by utilizing nitrogenous compounds for their growth

(Hachiya and Sakakibara 2016). Nitrate and ammonium are regarded as primary sources of nitrogen for most plants (Imsande 1986, Bloom *et al.* 2002) but the preferred source of nitrogen is nitrate (Olsson and Falkengren-Grerup 2000, Vaillant *et al.* 2004). The preference of plants to utilize nitrate (Wongkiew *et al.* 2018) is useful in managing the pH of the RAS towards the acceptable range of most of the fish species. Absorption of nutrients by plants is an electrically neutral process but the absorption of nutrients can affect pH by releasing proton or hydroxide ions into the medium. The absorption of NH^{4+} releases a proton while the absorption of nitrate releases a hydroxide ion (Van Rooyen and Nicol 2022). The absorption of ammonium ions decreases the pH of the water which not only interacts with the absorption of other essential nutrients (Riley and Barber 1971) but also reduces plant and fish growth (Goddek *et al.* 2019b). This is particularly relevant to the RAS because pH tends to decrease during the nitrification process due to alkalinity consumption and acid production (Chen *et al.* 2006) which can affect the growth of fish negatively. Therefore, in RAS pH is managed by the addition of sodium bicarbonate, calcium carbonate, or hydroxide (Martins *et al.* 2017).

1.3 Hydroponics

Hydroponics is a soilless crop farming (Sharma *et al.* 2018). Soilless culture systems are one way to avoid soil-borne diseases.

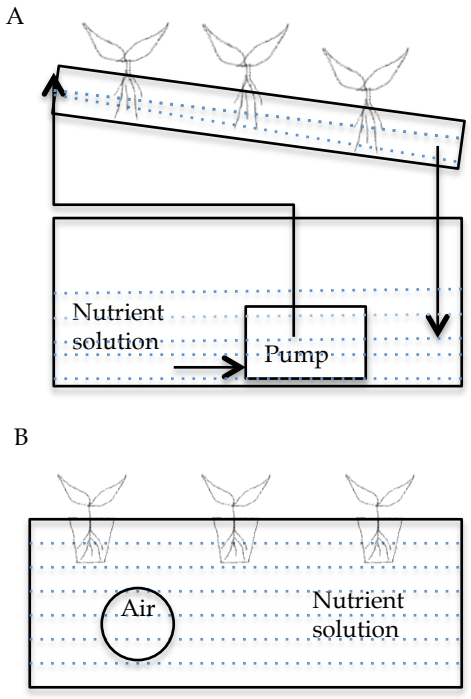

FIGURE 2 Conceptual difference between two hydroponics systems. A) nutrient film technique (NFT) B) deep-water culture (DWC).

Soilless farming can provide an advantage to using substrates other than soil

and make it easy to deal with pathogens with better management of environmental conditions and pest control (Sharma *et al.* 2018, Goddek *et al.* 2019b). In hydroponics, the growth of crops is not dependent on soil quality, but oxygenated nutrients solution can be supplied to plants to fulfil their growth requirements.

There are different types of hydroponics. The most common hydroponic systems are nutrient film technique (NFT) and deep-water culture (DWC). In NFT nutrients are provided to roots by using a thin film of oxygenated nutrient solution flowing in a narrow channel where some part of the roots are in contact with nutrients (Fig. 2 A). DWC is a way of growing plants on floating rafts placed inside a tray filled with a nutrient solution where water is oxygenated. The plants are inserted in the rafts and suspended in a way that most of the roots are in the water and absorbing the nutrients from the solution (Fig. 2 B) (Sharma *et al.* 2018, Timmons *et al.* 2018, Goddek *et al.* 2019b).

1.4 Aquaponics

Aquaponics is integrated farming of aquatic organisms (e.g. RAS) and plants (e.g. hydroponics) where most of the nutrients for the growth of plants are derived from the waste originating from feeding the aquatic organisms (Palm *et al.* 2018). Aquaponics involves microbiological processes to recycle and reuse nutrients from aquaculture effluents and save resources (Baganz *et al.* 2022).

Aquaponics can be of two types depending upon if the hydroponics and RAS are operated as one closed system (coupled aquaponics) or as two separate systems (decoupled aquaponics). The basic principle of coupled aquaponics is that the nutrient-rich effluents from RAS are circulated to hydroponics where plants absorb nutrients from the water, and then water is recirculated back to RAS (Fig. 3 A). In decoupled aquaponics, nutrient-rich effluents from RAS are directed to the hydroponics but not circulated back to the RAS (Fig. 3 B).

The nutrient-rich effluents from RAS are concentrated in nitrogen and phosphorus (Van Rijn 2013, Buzby and Lin 2014). Moreover, the effluents from RAS contain dissolved oxygen and dissolved organic matter and contain approximately 99 % of the nutrients required by plants for their growth (Skar *et al.* 2015). Hence, the nutrient-rich effluents from RAS can be used as a nutrient solution in hydroponics. The hydroponics component of the aquaponics should be designed by considering the maximum removal of nutrients from RAS. In the context of nutrient removal, the NFT hydroponic systems are less efficient compared to DWC in aquaponics (Lennard & Leonard 2006). Integration of hydroponics with RAS not only mitigates the discharge of nutrient-rich RAS effluents to the environment but also grows valuable plants by utilizing the nutrients from the RAS effluents and promotes circular economy. On the other side, it also reduces the application of mineral fertilizers for the growth of plants and hence, the depletion of natural mineral resources (Schmautz *et al.* 2016, Eck *et al.* 2019). Optimally, aquaponics can be a sustainable, environmentally safe, and water-efficient food production method (Al-Hafedh *et al.* 2008, Palm *et al.* 2015).

Aquaponics is regarded as a resource-efficient and sustainable technique but there are some limitations to implementing this technology on a commercial scale successfully. These systems are complex to manage compared to other agricultural practices and require intensive control and monitoring. Special management skills are required to manage these systems successfully and the availability of technical expertise is crucial. The ratio of available nutrients for plant growth in aquaponics is a challenge (Goddek *et al.* 2015). Several other factors such as power outages, pipe leakage, and equipment failure could result in fish mortality or plant losses. The setup and operational costs for aquaponics are higher compared to other agricultural practices which is a big limitation for start-ups (El-Essawy *et al.* 2019). Aquaponic farming has not yet been established as a profitable operation (Turnsek *et al.* 2020).

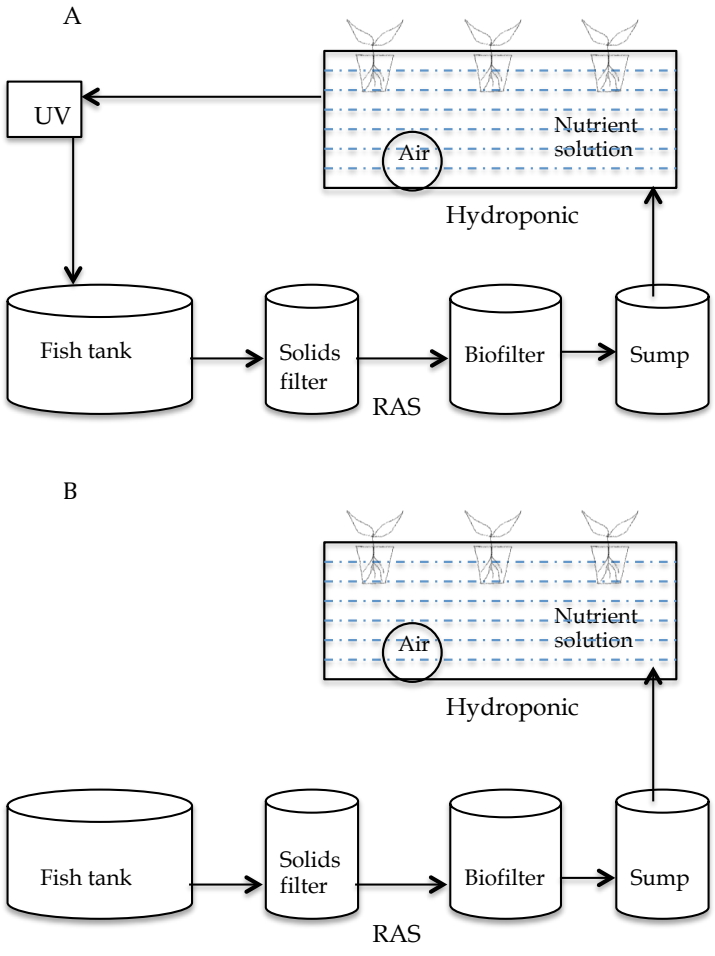

FIGURE 3 Conceptual difference between A) coupled and B) decoupled aquaponics. Starting from the fish tank the arrows show the direction of nutrient-rich effluents from recirculating aquaculture (RAS) to hydroponics. UV = ultraviolet light and Air = air stone.

For the successful establishment of an aquaponic system and to improve the growth and quality of fish and plants in aquaponics further research is required (Tyson *et al.* 2011, Junge *et al.* 2017). The research on aquaponics lags behind the research on hydroponics and RAS. Little attention has been paid to companion

planting in aquaponics (Maucieri *et al.* 2017). Companion planting is a way to grow plants by mixing plant species deliberately to benefit plants (Tringovska *et al.* 2015). In aquaponics, the removal of nutrients from the RAS effluents is a point of interest, and companion planting may help to increase the nutrient removal due to the complementarity of nutrient use by different plant species (Silvertown and Law 1987, Mahdi *et al.* 1989, Pii *et al.* 2015). Companion planting may improve plant productivity (Grunert *et al.* 2020, Lin *et al.* 2021) in aquaponics. Plants can sense their companions through chemical signals, and physical contact (Gagliano *et al.* 2012) when grown together. Many plants are good companions and facilitate each other (Marler *et al.* 2021) by enhancing growth (Plath *et al.* 2011) and by nitrogen fixation (Fustec *et al.* 2010). Another overlooked aspect in aquaponics is the removal of off-flavor-causing compounds by using plants (Schmautz *et al.* 2017, Fischer *et al.* 2021).

1.5 Microbes in aquaponics

In aquaponics, the recycling of nutrients is driven by microbes which help to convert fish waste into plant biomass. Thus, making the function of the microbial ecosystem paramount (Skar *et al.* 2015). The microbial communities play an important role in different components of the aquaponics system (Schmautz *et al.* 2017). For example, the biofilter in the RAS component plays an important role in the nitrification process while the hydroponics component contains microbes associated with plant roots. Moreover, the solid waste in the system is decomposed by microbes (Leonard *et al.* 2000, Joyce *et al.* 2019). The microbial processes such as the biological degradation of solids can increase the biological oxygen demand and chemical oxygen demand in the water (Rojas-Tirado *et al.* 2018). The performance of the aquaponics system, the production rates of plants, and the growth and welfare of the fish ultimately depend upon the microbiota of the system. Microbes perform the important role of fundamental biological filtration of water to provide the required nutrients for plant growth (Kasozi *et al.* 2021). Therefore, microbes in aquaponics may affect the system performance, water quality, and the growth and quality of the plants and fish (Goddek *et al.* 2019b, Joyce *et al.* 2019, Kasozi *et al.* 2021).

Interaction between bacteria can also influence the system's performance. One important bacteria-to-bacteria interaction in aquaponics is the relationship between the heterotrophic and autotrophic nitrifying bacteria in the biofilter. In biofilter, heterotrophic bacteria are fast growers, and their populations could develop in hours while autotrophic nitrifying bacteria are slow growers and their populations take days to develop (Qi *et al.* 2022). The autotrophic nitrifying bacteria utilize carbon dioxide derived from fish respiration while heterotrophic bacteria utilize organic carbon from fish feed and fish excreta (Joyce *et al.* 2019, Preena *et al.* 2021). When the C/N ratio in the biofilter or rearing water is high such as 1 or 2 the nitrification rates has been reported to decrease by 70 % (Michaud *et al.* 2006). At a high C/N ratio, heterotrophic bacteria compete with autotrophic nitrifying bacteria for oxygen and space (Navada *et al.* 2020). The dynamics and the maximum biomass of heterotrophic

and nitrifying bacteria over time are estimated by the supply of organic matter (Blancheton *et al.* 2013). The competition between heterotrophic and nitrifying bacteria is a major operational challenge in RAS because reduced nitrifying populations will lead to the elevation of ammonium and nitrite concentrations which is a potential risk for fish well-being (Blancheton *et al.* 2013). The heterotrophic bacterial population in biofilter can be controlled by controlling the organic matter reaching the biofilter or by disinfection routines of circulating water (Blancheton *et al.* 2013). However, the moderate presence of heterotrophic bacteria in RAS is regarded beneficial in relation to maintaining the bacterial quality of the water (Michaud *et al.* 2006). The co-occurrence of nitrifying and heterotrophic bacteria allows carbon and ammonia oxidation simultaneously (Elenter *et al.* 2007). Heterotrophs are important for the formation of biofilms as they produce extracellular polymeric substance (EPS) which is crucial for biofilm formation and cannot be produced by nitrifiers (Zhu *et al.* 2016). Additionally, heterotrophs produce proteases and deaminases which are important in relation to the decomposition of proteins from fish feed and fish faeces to ammonia (Itoi *et al.* 2006, Blancheton *et al.* 2013). The heterotroph competition with other bacteria reduces the growth of pathogens in the system (Michaud *et al.* 2006). In aquaponics, water can be treated with ozone or ultraviolet (UV) radiations to kill the pathogens (Summerfelt *et al.* 2009) but they also kill the beneficial bacteria in the system and disturb the balance between microbial communities of the aquaponics as well. The imbalance of the microbial system may lead to low growth and survival of the fish and plants (Attramadal *et al.* 2012, Joyce *et al.* 2019).

In aquaponics, water quality is influenced by the plant metabolites which are known to attract specific microbes in the surrounding medium (Khashi u Rahman *et al.* 2019). Microbes of the plants may influence the water quality and growth of fish or plants (Schmautz *et al.* 2017). Moreover, if the plants are grown as companions in hydroponics it could further influence the microbial communities of the aquaponic system (Srivastava *et al.* 2017, Horner *et al.* 2019, Grunert *et al.* 2020). The companion plants may alter the microbial communities of the neighbour plants due to plant-plant and plant-microbial interactions (Srivastava *et al.* 2017, Horner *et al.* 2019, Grunert *et al.* 2020). The dynamics of microbes in one compartment of the system can affect the functioning of another part of the system because of these interactions. However, there is currently limited information on plant and fish-associated bacteria in aquaponic systems. The microbes from fish and plants may raise food safety concerns for each other (Sawyer 2021). Therefore, research on plants and fish-associated bacteria in aquaponics is an important area (Schmautz *et al.* 2017, Tunçelli *et al.* 2023).

1.6 Off-flavor-causing compounds in RAS and fish quality

Several compounds are reported to produce earthy and musty flavor in fish (Lovell *et al.* 1986, Cotsaris *et al.* 1995). Geosmin (GSM) and 2-methylisoborneol (MIB) are the most commonly found off-flavor-causing compounds in RAS

water where they are produced mainly due to the presence of bacteria like Cyanobacteria, Actinomycetes, and Myxobacteria (Dickschat *et al.* 2005, Schrader and Summerfelt 2010, Lukassen *et al.* 2017, Lindholm-Lehto *et al.* 2019). Off-flavor-causing compounds from RAS water accumulate in fish mainly through the gills, skin, and gastrointestinal tract (Howgate 2004, Davidson *et al.* 2014b, Lindholm-Lehto *et al.* 2019). These off-flavor-causing compounds are not dangerous to fish, but their removal from fish is important. The earthy and musty flavor of fish reduces the consumer acceptability of the fish and causes a financial threat to the RAS industry (Badiola *et al.* 2012). The removal of these compounds is a time-consuming process and the only reliable method is the depuration of fish in clean water (Hathurusingha and Davey 2014, Davidson *et al.* 2020). Other suggested methods for the removal of off-flavor-causing compounds from fish are the addition of peracetic acid (CH_3CO_3H) or hydrogen peroxide (H_2O_2) (Suurnäkki *et al.* 2020), ozonation of circulating water (Fotiou *et al.* 2015, Spiliotopoulou *et al.* 2018) and photocatalysis (Xue *et al.* 2016) but these methods are expensive or time-consuming. Bacteriophage-based treatment for the removal of off-flavor compounds from RAS water may be effective because some bacteriophages can inhibit the growth of off-flavor-producing bacteria (Jonns *et al.* 2017, Almeida *et al.* 2019). Bacteriophage-based treatment may be one of the approaches to deal with off-flavor compounds in RAS water, but further research is required to find more reliable, cost-effective, and timesaving approaches to deal with the removal of off-flavor-causing compounds. Plants have been used to treat wastewater (Endut *et al.* 2009, Enduta *et al.* 2011) but so far, no attention has been paid to removing off-flavor-causing compounds from RAS water through plants.

1.7 Growth of plants and nutrients in aquaponics

In aquaponics, the plant growth is comparable to the plant growth in hydroponics (Pantanella *et al.* 2012, Delaide *et al.* 2016, Fischer *et al.* 2021) and even better than in soil farming (Albadwawi *et al.* 2022). The mineral content of aquaponically grown plants has been reported same or even higher as compared to hydroponics (Schmautz *et al.* 2016, Eck *et al.* 2019). Most herbs can grow on the concentration level of most of the nutrients present in RAS effluents (Graber and Junge 2009, Delaide *et al.* 2016, Bittsanszky *et al.* 2016), but plants have different requirements for nutrients depending upon the plant species or growing stage (Zekki *et al.* 1996, Eck *et al.* 2019). Nutrients can be added into the hydroponics compartment of aquaponics for plants with a higher need for nutrients (Goddek *et al.* 2015, Bittsanszky *et al.* 2016), but the nutrient addition must be within the tolerance limits of fish, plants, and microbes in the system. However, in aquaponics, it is hard to monitor and control the composition of nutrients in the RAS water because the amount of nutrients is dependent on the biological breakdown of organic matter (Bittsanszky *et al.* 2016).

The plants in aquaponics obtain carbon from atmospheric CO_2 fixation (Timmons and Ebeling 2013). The fish feed provides phosphorus in circulating water which can be available to plants in aquaponics (Cerozi and Fitzsimmons 2017). The nitrification process provides ammonia, nitrite, and nitrate in the system which can be taken by plants (Tyson *et al.* 2004, Palm *et al.* 2018). Another source of nutrients in aquaponics is water which is added or exchanged during the maintenance of the system (Delaide *et al.* 2017). Depending upon the source of water added some nutrients such as magnesium, calcium, sulphur, and trace elements can be available to plants (Eck *et al.* 2019, Lennard and Goddek 2019). Thus, the effluents from fish production can be used as a source of nutrients for plant growth (Fig. 3).

1.8 Fatty acids in fish and plants

The main human concerns related to aquaponic food are food safety and food nutritional quality (Suárez-Cáceres *et al.* 2022, Tunçelli *et al.* 2023). Fatty acids are an important nutritional constituent of human diets due to their health benefits. Fishes have high contents of polyunsaturated fatty acids (Castell *et al.* 1972, Yu and Sinnhuber 1975) and are a good source of fatty acids for humans (Aslan *et al.* 2007). The proportion of different fatty acids is affected by the diet consumption of fish (Einen *et al.* 1999, Johansen *et al.* 2001, Taipale *et al.* 2022). Some plants such as mint (*Mentha spicata, Mentha piperita*, *Mentha veridis, Mentha pulegium*), soya (*Glycine max)*, and olive (*Olea europaea*) are good sources of fatty acids (Maffei 1992, Gargouri *et al.* 2004, Bellaloui *et al.* 2013, Hernández *et al.* 2021, El Menyiy *et al.* 2022, Alameen *et al.* 2023). Moreover, root exudates of some plants contain fatty acids (Zhang *et al.* 2020, Senff *et al.* 2022). Fish grown with mint have been reported to contain higher fatty acid contents compared to fish grown in tanks without mint (Alameen *et al.* 2023). Thus, selecting plant species in aquaponics may be a way to improve the fatty acid composition of fish.

1.9 Aims of the dissertation

The main aim of the dissertation was to investigate the effect of plants on the growth of rainbow trout (*Oncorhynchus mykiss*), water quality, and microbial communities in aquaponics, and to compare the growth of plants in aquaponics to hydroponics.

Companion planting has been used in agriculture, but it has gained little attention in aquaponics. When plants are grown in proximity they can benefit each other or alter the plant-associated bacteria (Marler and Callaway 2021). Therefore, the first aim was to investigate if companion plants affect the growth and microbes in other plants (I). For this purpose, lettuce (*Lactuca sativa*) was used as the target plant and it was grown with three companion plant species

namely mint (*Mentha spicata*), rucola (*Diplotaxis tenuifolia*) or wormwood (*Artemisia absinthium*).

Nitrification is an essential process for the conversion of toxic ammonia excreted by fish to nitrate in RAS. The nitrification start-up process can take more than two months (Pulkkinen *et al.* 2019). During nitrification, the concentrations of TAN, nitrite, and nitrate can exceed the recommended tolerance limits of fish and can affect fish growth and survival negatively (Preena *et al.* 2021). Plants in aquaponics absorb ammonia and nitrate from circulating water and they may affect the process of nitrification (Schmautz *et al.* 2017). Therefore, the second aim was to investigate if plants affect the nitrification start-up in aquaponics when rainbow trout was grown with mint or baby spinach (*Spinacia oleracea*) (II, III).

The off-flavor-causing compounds (GSM and MIB) in RAS water have been a problem because they can accumulate in fish in a very short time and make it unmarketable (Dickschat *et al.* 2005, Schrader and Summerfelt 2010, Lukassen *et al.* 2017, Lindholm-Lehto *et al.* 2019). Several techniques have been practiced to remove off-flavor-causing compounds from RAS water but they are time-consuming or costly (Hathurusingha and Davey 2014, Davidson *et al.* 2020). Plants have been used to treat wastewater, but no attention has been paid to using plants to remove off-flavor-causing compounds from RAS water. Therefore, the third aim was to investigate if plants have the potential to remove off-flavor-causing compounds from RAS water (II). The removal of GSM and MIB was investigated by using baby spinach grown together with rainbow trout (II).

Mint is known for its root exudates (Surendran *et al.* 2017) and may affect the nutritional quality of fish. The root exudates are secreted by healthy plants in the growing medium and they attract specific microbes (Baetz and Martinoia 2014). The root exudates of plants contain several chemical compounds including organic acids, fatty acids, carbohydrates, and amino acids (Surendran *et al.* 2017). Growing fish with mint may affect the microbial communities or the nutritional quality of fish. Therefore, the fourth aim was to investigate if mint altered the microbial communities or fatty acid contents of rainbow trout (III).

2 MATERIAL AND METHODS

2.1 Experimental design and sampling

2.1.1 Experimental design (I)

In study I, an experiment on lettuce growth, companion planting, and microbial communities in lettuce was performed in a decoupled aquaponic system. The system consisted of one RAS unit with fifteen rainbow trout (initial weight ± SD: 228 ± 15 g) and four hydroponic DWC systems (Fig. 4).

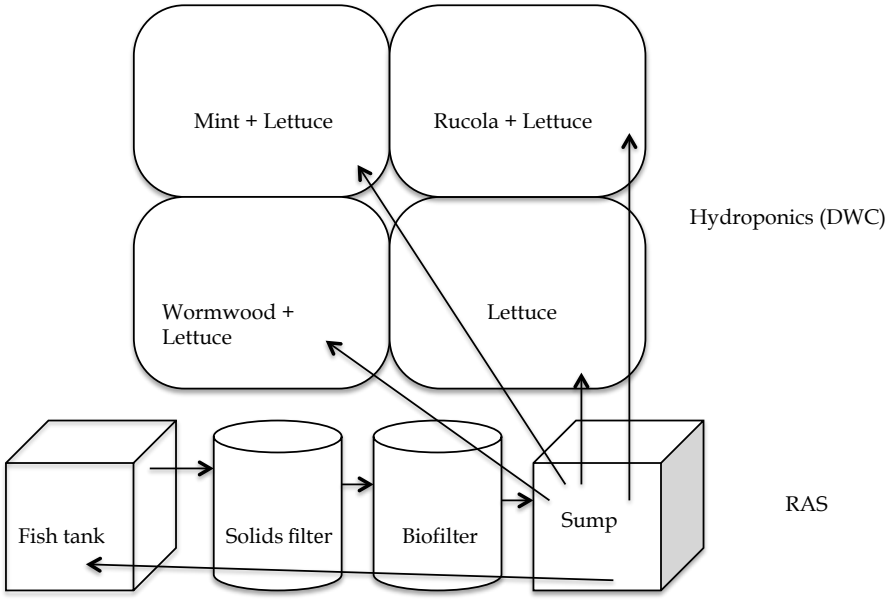

FIGURE 4 A schematic diagram of the decoupled recirculating aquaponics system used in study I. In the experiment nutrient-rich effluents from recirculating aquaculture system (RAS) were used to grow lettuce and companion plants in four isolated deep-water culture (DWC) hydroponic systems. The arrows represent the water flow starting from the fish tank to other tanks and DWCs.

The RAS unit comprised of a fish tank (1 m^3), swirl separator solids filter (500 l), fixed bed biofilter (500 l), and sump tank (500 l). The total water volume in the

RAS unit was approximately 2500 l. The water was directed from the fish tank to the swirl separator solids filter to filter solid particles. From the swirl separator water was directed to a fixed bed biofilter and lastly to the sump tank. In the sump tank water was aerated before supplying to DWCs. Water was also aerated in the fish tank, biofilter, and in each DWC. DWC consisted of a high-density polystyrene tank (1 × 1 × 0.35 m) with floating rafts made of expanded polystyrene sheets of 5 cm thickness with 5 cm drilled holes to hold plant baskets. At the start of the experiment, 135 l water from the RAS unit was given to each DWC but later 100 l water was given daily. Additional micronutrient solution (Ingestad 2006) and macronutrients were supplied to each DWC every day. The four DWC units were isolated from each other and there was no mixing of water between DWCs. The hydroponic system contained 15 lettuce seedlings and 15 seedlings of its companion plants i.e. mint, rucola, or wormwood. When lettuce was a companion plant the DWC contained 30 lettuce plants (15 + 15). The experiment lasted for 30 days, and the experiment was repeated three times to serve as a replicate in time. LED lights were used to provide 16 hours of light to plants. The humidity was maintained above 50 % during the whole experiment. Plants were harvested on the 30th day of each time replicate. Fish were not used in any procedure but only RAS nutrient-rich effluents were used to grow plants.

2.1.2 Sampling (I)

The total dry weight (root + shoot) of lettuce and companion plant was measured at the start and end of each time replicate. For analysing bacterial community three (100 g) fresh weight samples of five pooled plants from each plant species were collected. Three water samples per replicate were collected by filtering 50 ml of RAS water through a Millipore membrane filter (0.22 µm pore size, Ø 47 mm).

2.1.3 Experimental design (II and III)

For II and III, the experiments were performed in a coupled aquaponic system. In II when baby spinach was grown with rainbow trout (initial weight 108 ± 1.3 g) three aquaponic, three RAS, and three hydroponic systems were set up. In three aquaponic systems water was circulated from RAS to hydroponics and then back to RAS. Water was disinfected with UV light (II) (Fig. 5). In III when the mint was grown with rainbow trout (initial weight 54.8 ± 0.9 g) the experimental facility consisted of three identical RAS and three identical aquaponic systems. The DWCs were connected to RAS and water was circulated from RAS to DWC and then back to RAS without disinfecting the water with UV light (III) (Fig. 5). In III the three systems were considered aquaponics when connected DWC contained mint seedlings while three systems were considered as RAS when connected to empty DWC units. Each RAS was comprised of a dual drain fish tank (500 l) connected with a settling tank (500 l) bead filter, a moving bed biofilter (300 l) with helix floating bio media, and a sump tank (500 l). DWC units (1 × 1 × 0.35 m) were made of high-density polyethylene tanks while the rafts were made of rigid XPS styrodur

containing 5 cm drilled holes for holding plant baskets. For growing plants fifteen hours of light was provided to all of the DWC units. Oxygen level was maintained at 80–85 %. Water exchange (10–50 %) with tap water was carried out 2–3 times a week. Ten to fifteen ml of modified micronutrient solution (Fe, B, Zn, Mo, Ingestad 2006) was supplied to all DWCs two to three times a week whenever water was added to the system. The fish were fed dry pellets. Daily feed intake was estimated as the difference between the feed given and the uneaten feed collected from the tank bottom. Water temperature during the experiments depended on the temperature of the experimental facility hall because of the absence of a temperature controller.

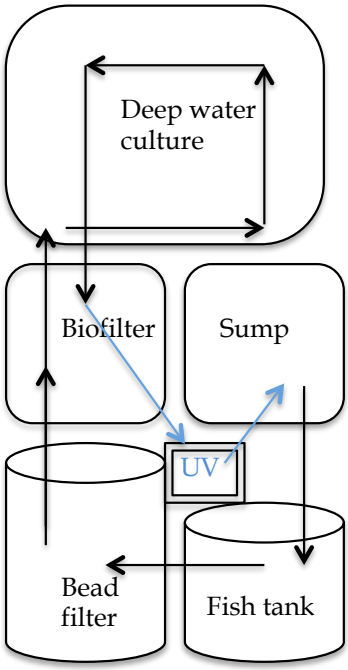

FIGURE 5 A schematic diagram of the coupled recirculating aquaponic system used in II and III. The arrows represent the water flow starting from the fish tank to other tanks.

2.1.4 Sampling (II and III)

The samples for baby spinach and rainbow trout in II were taken as follows. For measuring the biomass of spinach 15 seedlings at the start and 20 spinach plants at the end of the experiment were sampled. The length and dry weight of spinach were measured. The shoot length was measured from the above-water part of the spinach to the end of the leaf tip and the root length was measured from the underwater part of the spinach to the tip of the longest root. The samples for analysing GSM and MIB were collected as six fresh spinach seedlings at the start and three fresh spinach plants at the end from each DWC. For quantifying lipid content, GSM, and MIB in the fish muscle three fish at the start and three fish from each tank at the end were sampled. Samples (500 mg) were collected from the lateral part of the fillet as described by Hathurusingha and Davey (2016). The collected fish samples were pooled to make one pooled sample from the start samples and one sample per fish tank at the end. Water

samples (500 ml) were collected from each DWC, each fish tank, and tap water for the analysis of the GSM and MIB.

In III samples for mint and rainbow trout were taken as follows. Mint samples for the biomass assessment were collected at the start and end of the experiment. Start samples were collected just before the transplantation of seedlings to the aquaponic system. Five seedlings were pooled for each start sample and three start samples were collected. Three end samples were collected at the time of harvest by pooling five plants for each sample. To measure the relative growth of mint, the dry weight of shoots and roots was recorded. For analysing microbial communities in mint, two samples of (100 mg) fresh weight were collected from five pooled mint plants at the start and end of the experiment separately from root and shoot. For analysing fatty acid content in fish, three muscle samples (500 mg) were collected from the lateral part of the fish. For investigating the microbial communities in the fish mucous, anterior, and posterior gut (200 mg) samples were collected from three fish at the start and three fish from each fish tank at the end. The gut samples included the contents of the intestine. Anterior gut samples were collected from the proximal part of the intestine while posterior gut samples were collected from the distal part of the intestinal tract. For examining the microbial communities in water one (50 mL) water sample at the start and two (50 ml) water samples at the end of the experiment from each fish tank were filtered through a Millipore membrane filter (0.22 μm pore size, Ø 47 mm).

2.2 Water quality measurements

Water quality measurements were performed for TAN, nitrite, nitrate, and pH. In study I, TAN, nitrite, nitrate, and pH were measured by using a Tetra® test kit, Melle, Germany. Water temperature in the fish tank was recorded with a temperature meter (OWAY Technology Co, LTD, Guangdong, China), and oxygen saturation was measured by ATC digital oxygen sensor (Shenzhen Yago Technology Limited, Guangdong, Shenzhen, China). In II and III water quality for TAN, nitrite, and nitrate was recorded by API® Freshwater master test kit (Mars Fish Care Inc, Chalfont, PA, USA) while pH and temperature were recorded by Digital pH/Temperature Meter (AD 12, ADWA instruments, Szeged, Hungary. Oxygen saturation was recorded by oxygen meter (ExStik® DO600 Extech, Waltham, MA, USA) (II, III). Air humidity in the DWC units was measured by a humidity meter (Prego, Helsinki, Finland) and the light intensity in DWC was monitored by the digital light meter (Tasi TA6120, Suzhou, China) (I, II, III).

2.3 Methods for bacterial analyses

2.3.1 DNA extraction

DNA extraction was performed from samples of plants, water, feed, fish mucous, and gut. For the DNA extraction from the plant samples, the Nucleospin Plant II kit (Macherey-Nagel, Düren, Germany) was used while the DNA extraction from the samples of water, feed, and fish was carried out by using the Nucleospin Soil kit (Macherey-Nagel, Düren, Germany).

2.3.2 PCR amplification

The PCR amplification for the bacterial 16S ribosomal RNA (rRNA) gene was carried out for sequencing. The details of the primers used in PCR are given in Table 2.

TABLE 2 Details of PCR amplification for bacterial 16S ribosomal RNA (rRNA) gene performed in I and III.

Study	Gene	Target group	Nested	Region	Primer pairs	Reference
I	16S rRNA	Bacteria	Yes	V6-V8	799F and 1492R 1062F and 1390R	Chelius and Triplett 2001 Zheng et al. 1996
III	16S rRNA	Bacteria and archaea	No	V4	515F and 806R	Caporaso et al. 2011

In study I plant samples were analyzed while in III fish samples were analyzed. The different sample types in I and III required using different primer sets. In study I nested PCR was performed to limit the co-amplification of plant chloroplasts and mitochondria. The proportions of the expected bacterial amplicon (ca. 350 bp) in the product were evaluated by using image analysis of agarose gels with ImageJ software. The image analysis was performed to remove the mitochondrial amplification from plant sample amplicons (I) and unknown co-amplification of host DNA from fish sample amplicons (III). The mitochondrion and host DNA PCR product was removed from the samples by gel extraction. The products were pooled based on equimolar amounts of the bacterial amplicons.

2.3.3 Sequencing and sequence processing

Sequencing of bacterial amplicons was performed on Ion Torrent PGM. Ion PGM Hi-Q View OT2 Kit, PGM Hi-Q View Sequencing Kit, and Ion 316 Chip v2 were used for bacterial sequencing. The analysis of sequencing data was conducted with the Mothur software package v.1.43.0 (Schloss *et al*. 2009) following the relevant parts of the MiSeq SOP protocol (Kozich et al. 2013). Sequences were quality filtered and aligned against the Silva database v.1.38 (Quast *et al*. 2013). Chimeras and non-target sequences were removed, and operational taxonomic units (OTUs) were defined with a 97 % similarity cut-off. The Silva v. 1.38 database was used to get the taxonomic affiliation of the OTUs.

2.4 Methods for fatty acid analyses

In II when rainbow trout was grown with baby spinach in the aquaponic system, the total fat content of fish muscle (g kg^{-1} wet weight) was measured by the accredited in-house method JOK3008. The fat content measurement was performed by Natural Resources Institute, Finland (Luke).

In III, the lipid extraction was carried out by following the Folch protocol (Folch & Gerald 1957) with chloroform : methanol : water (2 : 1 : 0.75) from freeze-dried muscle samples (5 mg) of fish (III). Fatty acids were transmethylated with 1 % sulfuric acid in methanol. Fatty acid analysis was run with a gas chromatographer mass spectrometer (Shimadzu Ultra, Kyoto, Japan) containing a mass detector (GC-MS). Fatty acid methyl esters were classified and measured as previously published by Taipale *et al.* (2016).

2.5 Methods for off-flavor-causing compounds analyses

For measuring off-flavor-causing 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) the method reported in Lindholm-Lehto (2022) was followed (II). The analysis was performed by the Natural Resources Institute, Finland (Luke). The full method description and validation have been reported in Lindholm-Lehto (2022).

2.6 Calculations

The relative growth rate (RGR) of mint was measured as an increase in mass per unit of existing dry mass per day by using the formula

RGR = (Ln(final weight) - Ln(initial weight))/(T2 - T1),

where weight (g) and T2 - T1 = duration of the experiment (days).

The specific growth rate (SGR) of fish was measured by the formula

SGR = ((Ln(final weight) - Ln(initial weight))*100)/(T2 - T1),

where weight (g) and T2 - T1 = duration of the experiment (days). Feed conversion ratio (FCR) was calculated with the formula dry mass of food consumed (kg)/increase in fish wet weight (kg).

2.7 Statistical analyses

The data obtained from the experiments were analyzed using R or IBM SPSS 26 statistical packages and differences in values were considered significant when $p < 0.05$. Various statistical analyses that were used in I–III are listed in Table 3. Two-factor analysis of variance (ANOVA) followed by Tukey's post hoc test was conducted where time replicate was considered as a random factor and treatment (companion plant species) as a fixed factor (I). Differentially abundance analysis was performed (I, III) as described by Love *et al.* 2014, Mandal *et al.* 2015, Kaul *et al.* 2017, and Fernandes *et al.* 2014, and SourceTracker (I) as described by Knights *et al.* 2011.

TABLE 3 Statistical analyses (I, II, Ill).

Studies	Analysis	Statistical program
I, III	Analysis of Variance (ANOVA)	SPSS
I	Two-factor analysis of variance (ANOVA)	SPSS
II, III	Repeated measure ANOVA	SPSS
II, III	Independent samples *t*-test	SPSS
I, III	Permutational multivariate analysis of variance	R
I, III	Non-metric multidimensional scaling	R
I	Source Tracker	R
I, III	Differential abundance analysis	R
I, III	Redundancy analysis	R

3 RESULTS AND DISCUSSION

3.1 Plant growth, companion planting, and microbes in lettuce

The overall growth of plants was similar or better in aquaponics compared to the growth of plants in hydroponics (II). The growth of baby spinach in aquaponics and hydroponics was similar in both systems (Fig. 6). The total plant weight in aquaponics was 43 % higher (40 % for shoot, 70 % for root) than in hydroponics. However, the baby spinach weight did not differ statistically significantly between treatments (II). Likewise, the shoot (aquaponics: 14.5 ± 1.7 cm, hydroponics: 12.2 ± 1.3 cm) and root (aquaponics: 37.8 ± 5.7 cm, hydroponics: 29.2 ± 4.6 cm) length ± SD of the spinach did not differ between hydroponics and aquaponics.

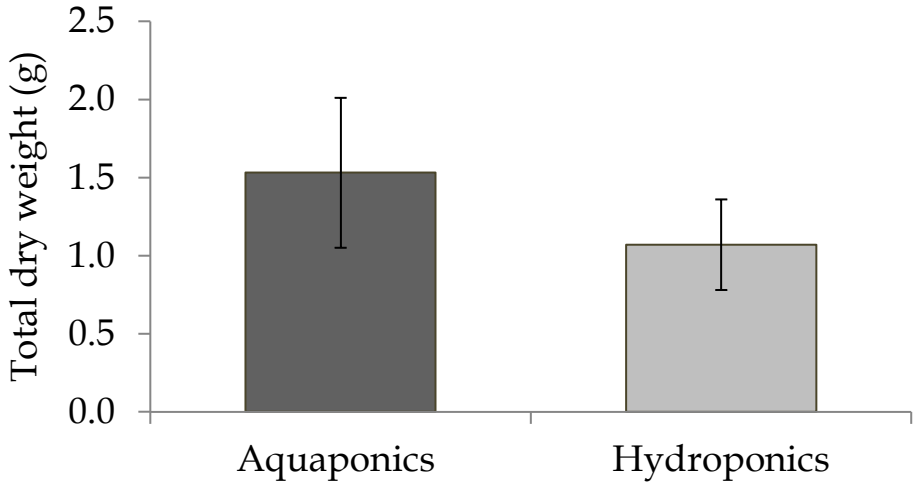

FIGURE 6 Dry weight (g) ± SD (n = 3) of baby spinach (*Spinacia oleracea*) grown in hydroponics or aquaponics for 42 days. For aquaponics, baby spinach was grown with rainbow trout (*Oncorhynchus mykiss*) in a coupled aquaponic system. There was no statistically significant difference between the treatments.

Other studies have also reported similar or higher growth of plants in

aquaponics compared to hydroponics (Pantanella *et al.* 2012, Delaide *et al.* 2016, Fischer *et al.* 2021, Xu *et al.* 2022) suggesting that the results of II are in line with other studies.

The growth of lettuce was enhanced when grown with companions especially when grown with mint and rucola (I) (Fig. 7). Other studies have also reported improved growth when plants are grown as companions (Geng *et al.* 2017) due to plant-plant interaction such as facilitating the growth of neighbour plants (Brooker 2006, Lugtenberg 2015). When plants are grown in proximity, they may increase the performance of companions through facilitative mechanisms (Marler and Callaway 2021). Through facilitative mechanisms, plants interrogate the identity of neighbour plants and respond negatively or positively to each other. Moreover, companion plants may suppress the harmful microbes and introduce beneficial ones thereby, improving plant productivity (Marler and Callaway 2021).

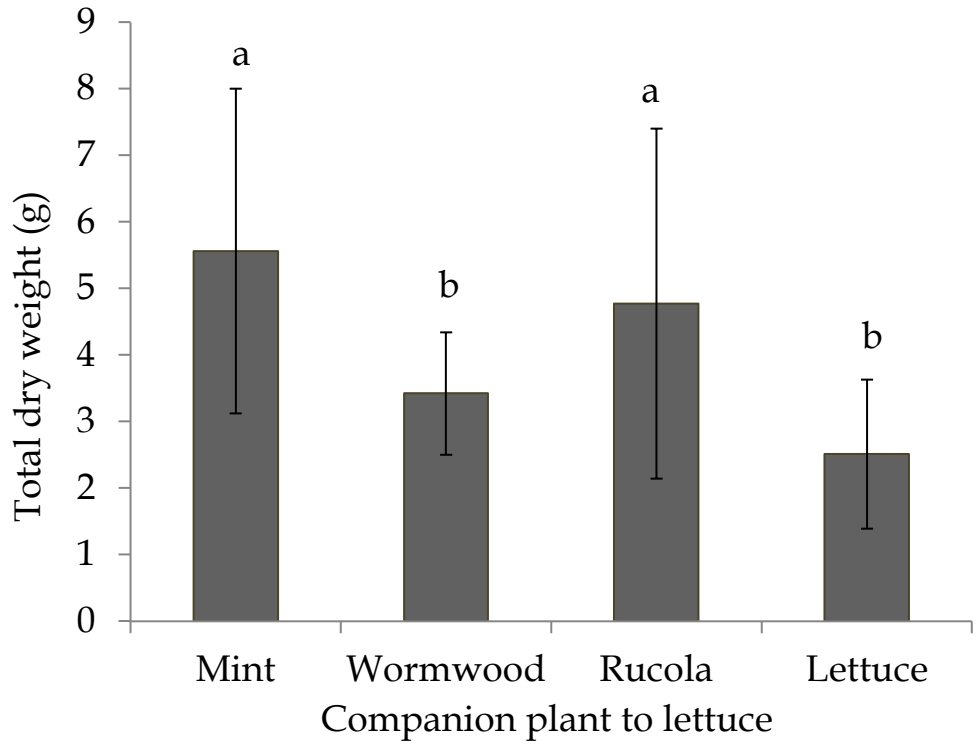

FIGURE 7 Total dry weight (g) ± SD of lettuce (*Lactuca sativa*) when grown with companion plant mint (*Mentha spicata*), wormwood (*Artemisia absinthium*), rucola (*Diplotaxis tenuifolia*) and alone in aquaponics for 30 days in a decoupled aquaponics system. In RAS rainbow trout (*Oncorhynchus mykiss* initial weight 228 ± 15.4 g) were grown for 30 days. Two-factor ANOVA. The two factors in the model were companion plant species (4) and time replicates (3). Bars denoted with different letters differ statistically significantly ($p < 0.05$).

Companion planting influences the microbial diversity in the companion plants (Navrátilová *et al.* 2019, Liu *et al.* 2020) which may affect the growth of plants positively (Pii *et al.* 2015, Geng *et al.* 2017). On the other hand, microbes associated with companions may affect the plant's growth negatively (Farrar *et al.* 2014). In study I, the bacterial community composition in lettuce grown with

three companion plant species was explored. The bacterial community composition in lettuce was affected by the presence of a companion plant. When lettuce was grown with mint and wormwood almost 50 % of the bacterial community in lettuce potentially originated from the mint or wormwood (I). Further, it was investigated if the lettuce growth was affected due to certain groups of bacteria or bacterial diversity, and the results revealed that the presence of specific bacterial genera enhanced the growth of lettuce (Table 4, I). However, bacterial diversity did not influence the productivity of the system. Instead, the increase in lettuce growth was positively correlated with a specific subset of bacterial taxa when grown with mint (I). This latter part of the finding is in contrast to the traditional view that system diversity is important in determining a system's productivity (Weidner *et al.* 2015). However, this finding highlights the possibility of improving the productivity of aquaponic systems by introducing specific microbes into the system. The bacterial genera of differentially abundant OTUs that were associated with increased lettuce biomass (I) have been reported to alleviate abiotic stress such as nutrient imbalance and improve nutrient acquisition in plants (Table 4, I).

TABLE 4 Operational taxonomic units (OTUs) found in the analyses of I and III in aquaponics and RAS and their potential function in plants and fish.

Genus of differentially abundant OTUs	Enrichment	Potential function	Reference
		Study I	
Rhodobacter	Positively associated with lettuce biomass	Alleviate abiotic stress and disease symptoms	Gravel *et al.* 2007
Pseudomonas	Positively associated with lettuce biomass	Alleviate abiotic stress and disease symptoms	Sammar *et al.* 2021
Hypomicrobium	Positively associated with lettuce biomass	Increase nitrogen acquisition from water	Wang *et al.* 2021
Arcicella	Positively associated with lettuce biomass	Increase phosphorus acquisition	Chai *et al.* 2017
		Study III	
Azospirillaceae	Enriched in aquaponics but not in RAS	Promote plant growth, organic matter decomposition in aquatic ecosystem	Fukami *et al.* 2018 Ferreira *et al.* 2020
Bacteroidia	Enriched in aquaponics but not in RAS	Related to fish metabolic activity and abundant in fish gut fed on plant source oil	Desai *et al.* 2012 Wu *et al.* 2012
Mycoplasma	Lower relative abundance in aquaponics fish gut than in RAS	Adaptive advantage to salmonids by mutulism. Linked to feed utilization and improved growth	Rasmussen *et al.* 2021

Another interesting finding was that lettuce did not acquire a significant proportion of microbial community from the RAS effluents (I). This finding is important from a practical and consumer point of view. The microbes in RAS water could facilitate the transmission of pathogens in plants grown on RAS effluents and make them unmarketable. Thus, it is important to examine if the microbes are transmitted from the RAS effluents to the plants grown in aquaponics. The study I revealed that the bacterial community in lettuce prominently developed from the neighbour plants, and it was not acquired noticeably from the RAS effluents. However, further research is required to understand the transmission of microbes from RAS effluents to plants grown in aquaponics.

In study I, the experiment was repeated three times to serve as replicates in time. The time replicates influenced the lettuce growth and bacterial

community composition significantly which highlights the importance of considering factors such as daily fluctuations in weather, tap water quality, and probably random drift during the pre-experimental and experimental conditions (I) while planning these kinds of systems.

3.2 Microbial communities in fish mucous and gut

The microbes in aquaponics could influence the fish positively or negatively (Joyce *et al.* 2019). Fishes are in direct contact with the circulating water and hence in continuous contact with dynamic microbiota. This microbiota may have implications for fish health. The microbial communities in the mucous prevent the fish from the attack of pathogens and provide a defence against diseases (Turnbaugh *et al.* 2006, Krajmalnik-Brown *et al.* 2012, Rowland *et al.* 2018). Mucosal microbiota interact with environmental antigens and intestinal microbiota interact with the host. The lymphoid tissues in the gut must develop mechanisms to distinguish between pathogenic and commensal microorganisms. The colonization of normal beneficial microbiota in the gut improves the immune regulatory functions of the gut but these regulatory functions can be disturbed by an imbalanced microbiota and cause diseases (Pérez *et al.* 2010).

In III, the microbial communities differed in the mucous and anterior gut of the rainbow trout when rainbow trout was grown with mint compared to RAS. The only difference between the two systems was the presence of mint and all other parameters were kept the same in both treatments. Thus, it could be speculated that the difference in microbial communities in mucous and gut of rainbow trout in aquaponics was possibly due to the mint. Mint is a promising source of root exudates and secondary metabolites (Mimica-Dukic and Bozin 2008, Surendran *et al.* 2017). The root exudates may contain several biologically active molecules such as sugars, amino acids, organic acids, vitamins, enzymes, fatty acids, carbohydrates, aromatics, and flavonoids. Root exudates are a source of carbon for microbes (Singh and Mukerji 2006, Pantigoso *et al.* 2023).

The results from differential abundance analysis revealed that two Azospirillaceae OTUs were enriched in fish mucous in aquaponics compared to fish in RAS. Fifteen fish mucous OTUs showed lower relative abundance while one OTU of the genus *Mycoplasma* showed lower relative abundance in the posterior gut of the fish reared in aquaponics compared to RAS. The bacterial OTUs that were enriched in aquaponics have been reported to promote plant growth, provide protection against pathogens, and to improve fish growth (Table 4, III). *Mycoplasma* has been reported to be the dominant genus in the gut of farmed fish (Rasmussen *et al.* 2021). They can adapt to the gut environment of salmonids and form a mutualistic relationship with the gut resulting in improved fish growth possibly due to increased feed utilization of the fish (Rasmussen *et al.* 2021). However, lower abundance of *Mycoplasma* is reported in fish gut fed on high ω-3 PUFA with lower feed consumption. (Jin *et al.* 2019). In III, fish consumed less feed in aquaponics compared to RAS. Therefore, it

could be speculated that the difference in the relative abundance of *Mycoplasma* in the fish gut in RAS and aquaponics could be due to the difference in feed consumption. Further, it was tested if bacterial community variation in the fish mucous, anterior gut, and posterior gut was associated with fish weight, but no significant relationship was discovered in either of the treatment.

3.3 Fish growth and fatty acids in fish muscle

The weight gain (aquaponics: 137.1 ± 11.3 g, RAS: 109.3 ± 3 g) and specific growth rates (aquaponics: 2.0 ± 0.1 g, RAS: 1.7 ± 0.08 g) of fish were higher in aquaponics when grown with baby spinach compared to RAS (II). When rainbow trout was grown with mint the weight gain (aquaponics: 269.6 ± 31.8, RAS: 240 ± 11) and specific growth rate (aquaponics: 2.1 ± 0.1, RAS: 2.0 ± 0.06) did not differ significantly between treatments (III). SGR in III was higher than in II possibly due to different temperatures and initial size of the fish (Jobling 1993, Akbulut *et al.* 2002). Pulkkinen *et al.* (2019) reported a SGR of 1.6 ± 0.03 for RAS grown rainbow with similar rearing conditions and initial size as in II. Thus, the higher SGR in II shows good fish growth indicating also that the conditions for growing rainbow trout both in RAS and aquaponics were good in II and III.

FCR is the ratio of the dry mass of feed to fish wet weight gain in a certain period. Lower FCR values indicate higher feed conversion efficiency into fish weight gain. The lower FCR reduces the impact of feed on the environment (Turcios *et al.* 2014) and decreases the cost of fish production (Martínez-Llorens *et al.* 2007). The FCR for juvenile rainbow trout fed on dry pellets in RAS is generally one or below one (Pulkkinen *et al.* 2019, Salgado-Ismodes *et al.* 2020, Tunçelli and Pirhonen 2021). The feed conversion ratio (FCR) was lower in aquaponics (II: 0.9 ± 0.08, III: 0.8 ± 0.10) than in RAS (II: 1.06 ± 0.03, III : 1.0 ± 0.07) in both studies when rainbow trout was grown with spinach (II) and mint (III). The feed consumption (aquaponics: 110 ± 0.01 g, RAS: 112 ± 0.03 g) did not differ significantly when rainbow trout was grown with baby spinach (II), but the fish feed consumption (aquaponics: 198.9 ± 8 g, RAS: 233.2 ± 10.3 g) was lower in aquaponics than in RAS when rainbow trout was grown with mint (III). In II and III, the plausible reasons for the improved fish growth were better water quality in the aquaponic system due to lower dissolved inorganic nitrogen (DIN) (II, III). In III, the amount of feed consumed by fish in aquaponics was significantly lower than in RAS. However, the fish maintained the same weight and growth rates as in RAS. Consequently, FCR, SGR, and feed consumption in aquaponics can be affected by the choice of plant species. Moreover, fish feed consumption was reduced in aquaponics (III). Thus, mint could be used in aquaponics to lower feed consumption.

To assess the nutritional quality of the rainbow trout % lipid content (II) total ω-3 PUFA, and 22 fatty acids (III) were measured from rainbow trout muscle. The analysis revealed that the % lipid content of rainbow trout when grown with baby spinach was the same in both aquaponics and RAS (II). In III

the total ω-3 PUFA and docosahexaenoic acid (DHA) contents were similar when rainbow trout were grown with mint in aquaponics compared to RAS (III). A study on mint and tilapia in aquaponics has reported higher weights and fatty acids contents when fish was grown with mint and chickpeas compared to fish grown without mint and chickpeas in tanks (Alameen *et al.* 2023) suggesting that mint may improve the fatty acid contents of the fish in aquaponics. The fish fatty acids composition is related to feed consumption and feeding source (Gomes *et al.* 2016, Turchini *et al.* 2018, Taipale *et al.* 2022). Fish in aquaponics were expected to have altered fatty acid content compared to fish grown in RAS because of less feed intake in aquaponics (III). However, fish had a similar fatty acid content both in RAS and aquaponics (III). Rainbow trout maintained its growth in aquaponics the same as in RAS despite less feed consumption in aquaponics (III).

3.4 Nitrification

In RAS the concentrations of ammonia, nitrite, nitrate, and pH level should be managed to keep the water parameters within the recommended limits for fish growth and survival. The results of II and III showed that the water quality was better in aquaponics in terms of lower DIN and due to faster disappearance of TAN, nitrite, and nitrate in circulating water as compared to RAS. It suggests that plants in aquaponics absorbed nitrogenous compounds (II), (III) and affected the DIN concentration in aquaponics.

In II and III, the DIN was compared between aquaponics and RAS when rainbow trout was grown with baby spinach (II) and mint (III). The results from both studies were similar in terms of the rapid disappearance of TAN, nitrite, and nitrate during nitrification start-up in aquaponics compared to RAS. In both studies, the concentration of TAN and nitrite decreased to zero earlier in aquaponics compared to RAS and the concentration of nitrate was lower in aquaponics compared to RAS. Results indicate that plants or hydroponic part, in general, can act as biofilter when the RAS biofilter is not mature yet. Plants may provide nitrifying microbes that can establish into the biofilter to facilitate the maturation of the biofilter. Nitrification start-up has been studied in biofilter of RAS (Pulkkinen *et al.* 2019) but so far there is no information on if plants affect the biofilter maturation and facilitate nitrification start-up in the aquaponic system. Results from II and III suggest that in aquaponics plants speeded up the biofilter maturation. Moreover, the rapid disappearance of DIN in aquaponics can reduce the stress on the fish by reducing the exposure time of fish to elevating TAN, nitrite, and nitrate (Baßmann *et al.* 2017) during the start-up of nitrification.

The nitrification process causes the production of hydrogen ions which results in a pH fall in the RAS water. The pH management in RAS is crucial for the survival of fish. In aquaponics, the buffering can be done by adding chelated nutrients to circulating water directly, with fish feed or by foliar sprays (Roosta and Hamidpour 2011). The fish feed does not contain sufficient calcium and potassium needed for plant growth (Lennard 2021). Adding basic calcium

and potassium salts paired with carbonate, bicarbonate or hydroxyl ions help to maintain the pH of circulating water within the acceptable limits of fish and plants along with providing additional calcium and potassium that plants require (Lennard 2021). In II and III the pH in aquaponics and RAS decreased during the experiment but in aquaponics remained neutral (approximately 7) compared to RAS suggesting that the plants in aquaponics may play a role to maintain the pH in aquaponics. The plants may buffer the pH of the circulating water (Makhdom *et al.* 2017). Plants release an anion when absorbing a cation and make the root medium alkaline (Touraine *et al.* 1988, Jackson *et al.* 1989). Alternatively, denitrification and recovery of alkalinity in biofilter could be related to the stability of the pH in aquaponics (Timmons and Ebeling 2013).

3.5 Off-flavor-causing compounds

In II, the concentration of GSM and MIB in fish muscle, water, and baby spinach was investigated. The concentration of GSM was significantly higher in the roots and MIB in shoots of baby spinach grown in aquaponics compared to baby spinach grown in hydroponics. The concentrations of the GSM and MIB in baby spinach shoots were below the sensory detection (700–900 ng kg^{-1}) (Persson 1980, Young *et al.* 1996). In conclusion, baby spinach was edible from both hydroponic and aquaponic system because GSM and MIB remained below the sensory detection. As to fish GSM was found in low concentrations (400–500 ng kg^{-1}) and remained below the limit of sensory detection (700–900 ng kg^{-1}). However, the concentration of MIB in fish muscles was above the sensory detection limit (700–900 ng kg^{-1}) (Persson 1980, Young *et al.* 1996) and did not differ between RAS (1474 ± 240 ng kg^{-1}) and aquaponics (1612 ± 2 99 ng kg^{-1}). In II, the concentrations of GSM (2–8 ng l^{-1}) were at low and MIB at moderate levels (15–35 ng l^{-1}) in the water of RAS and aquaponics compared to other studies on RAS water (Burr *et al.* 2012, Suurnäkki *et al.* 2020). The concentrations of GSM and MIB did not differ between RAS and aquaponics water which suggests that baby spinach cannot be used for the significant removal of off-flavor-causing compounds from RAS water. However, I suggest that plants have the potential to remove off-flavor-causing compounds from RAS water and the selection of plant species may play a role in the removal of off-flavor-causing compounds. In II, baby spinach had a high concentration of MIB in roots (1261 ng kg^{-1}) and shoots (1079 ng kg^{-1}) of seedlings at the time of transplanting which may have affected the removal of MIB from circulating water and hence from fish. Plants that do not contain off-flavor-causing compounds naturally may be a good choice to investigate the removal of these compounds from RAS water. The removal of off-flavor compounds is necessary because it reduces the consumer acceptability of the fish. Further research is suggested to investigate the potential of other plants than baby spinach to remove off-flavor-causing compounds from RAS water.

4 CONCLUDING REMARKS

Due to the recycling of waste, minimum water use, and minimum addition of nutrients aquaponics provides solutions for several sustainability issues in agriculture and aquaculture. Most importantly aquaponics contributes to the mitigation of nutrient discharge from RAS. Therefore, there is growing interest in aquaponics but there are challenges in the establishment of successful commercial aquaponics farming, and further research is required.

Companion planting and microbes associated with plants are well-known research areas in traditional agriculture but are overlooked in aquaponics. Companion plants and microbes associated with plants could be useful in enhancing the growth of plants in aquaponics. The results of the study I showed that lettuce growth was facilitated in aquaponics by companion planting. Companion plants mint and rucola improved the growth of lettuce when grown with lettuce (I). The bacterial community composition of lettuce was affected by the presence of companion plants. When mint was grown as a companion the growth of lettuce was enhanced due to the presence of specific bacterial taxa in lettuce (I). The bacterial genera *Rhodobacter*, *Pseudomonas*, *Hypomicrobium,* and *Arcicella* were detected in lettuce when grown with mint and were associated with the increase of lettuce biomass (I). The bacterial diversity in lettuce did not vary due to the effect of the companion plant and it did not play a role in enhancing the growth of lettuce (I). The results of this study emphasize the importance of the bacterial components in optimizing the productivity of aquaponics (I).

Nitrification is the most important process for the functioning of an aquaponic system. High concentrations of TAN and nitrite during the start-up of nitrification result in high mortality of fishes that not only causes financial losses but also requires management of the water quality within the survival limits of fishes. The results of II and III indicated that plants have the potential to affect the nitrification start-up along with removing TAN, nitrite, and nitrate from the circulating water. In II and III nitrification started earlier and the conversion process of TAN to nitrite and nitrite to nitrate took place earlier in aquaponics compared to RAS. Consequently, the plants in aquaponics can affect the start-up of nitrification by acting as a biofilter. Plant or hydroponic component of aquaponics help to buffer the sharp rise of TAN, nitrite, and

nitrate when the biofilter is not fully mature and assist in faster maturation of the biofilter.

The accumulation of off-flavor-causing compounds GSM and MIB in RAS water is a common problem, and the removal of these compounds is essential to make the fish marketable. The results from II suggested that plants in aquaponics have the potential to decrease the accumulation of these substances in fish flesh. Baby spinach absorbed the off-flavor-causing compounds from circulating water and contained higher concentrations of off-flavor-causing compounds in aquaponics compared to the plants grown in hydroponics (II). Baby spinach also reduced the concentration of GSM in fish flesh (II). However, the concentration of the GSM and MIB remained the same in RAS and aquaponics water. Consequently, baby spinach is not a very feasible plant to remove GSM and MIB from RAS water. Further research is required by using several plant species to understand the removal of off-flavor-causing compounds in aquaponics.

The fish growth was enhanced due to improved FCR (II, III) and improved SGR (II) in aquaponics compared to fish reared in RAS. The feed consumed by fish when grown with mint was significantly lower compared to feed consumed by fish in RAS (III) but it was similar in RAS and aquaponics when grown with baby spinach (II). Fish consumed less feed in aquaponics with mint but maintained similar weights as in RAS suggesting that the choice of plant species in aquaponics could affect the FCR, SGR, and feed consumption of fish. Moreover, mint affected the microbial communities of fish when grown in aquaponics (III).

The result of this dissertation showed that in both coupled (II, III) and decoupled (I) aquaponic systems plants grew well. The plants can grow equally well in aquaponics as in hydroponics (II).

It is challenging to manage aquaponic systems due to the different growth requirements of the plants, fish, and microorganisms but with proper knowledge and management skills, these systems can be managed. During this research, several questions remained unanswered particularly the effect of mint metabolites on fish growth. However, there are some promising results from this research that can be used to improve the working of RAS technology. I suggest using plants to assist the biofilter maturation during the start-up of nitrification and improving the water quality of RAS particularly, in terms of the lower DIN. I recommend further research on companion planting in aquaponics using several combinations of plants to investigate the transfer of microbes between plants and their role in improving plant productivity. Companion planting is a good tool to improve the growth and introduce plant-associated bacteria into the system, but further research is required on how the plant's associated bacteria affect the growth of fish in aquaponics. Moreover, research on several plant species is recommended to study the removal of GSM and MIB from RAS water. Different plants have different capacities for the removal of nutrients. Therefore, different plants may affect the removal of off-flavor-causing compounds differently. Furthermore, it should be investigated which plant species can help to induce nitrifying bacterial communities in aquaponics and how they influence the nitrification process in aquaponics.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Kasvien vaikutus mikrobeihin, veden laatuun ja kalojen kasvuun aquaponic -systeemissä

Kalanviljelyssä kaloja kasvatetaan nykyisin yhä enemmän ns. kiertovesiviljelyssä, jossa kala-altaista poistuva vesi suodatetaan ja uutta vettä altaisiin tulee vain murto-osa verrattuna perinteiseen läpivirtausviljelyyn. Suodatuksessa vedestä poistetaan mekaanisilla suodattimilla kiintoainesta ja biologisilla suodattimilla kalojen proteiinihajotuksen lopputuotteena syntyvä ammoniakki/ammoniumtyppi hapetetaan bakteerien avulla kaloille suhteellisen haitattomaksi nitraatiksi nitrifikaatioprosessissa. Suodatuksen ja veden ilmastuksen jälkeen vesi voidaan pumpata takaisin kala-altaille. Veden kierrätys nostaa sen typpi- ja fosforipitoisuutta Kun tämä runsasravinteinen vesi poistuu kiertovesilaitokselta ympäristöön, se aiheuttaa alapuolisissa vesistöissä rehevöitymistä.

Kasvihuoneissa kasveja voidaan kasvattaa ilman multaa ns. hydroponic-menetelmällä. Tällöin kasvien juuristolle johdetaan vettä, johon on lisätty tarvittavat ravinteet. Aquaponic-kasvatuksessa yhdistetään kalojen kiertovesikasvatus ja kasvien hydroponic-kasvatus. Tällöin kalanviljelyn ravinteikasta poistovettä hyödynnetään kasvien kasvatuksessa, mikä vähentää ympäristöön päätyvää ravinnekuormitusta. Tässä väitöskirjassa olen tutkinut, miten aquaponic-kasvatus vaikuttaa kasvien ja kalojen kasvuun, niiden mikrobistoon, nitrifikaatioprosessin käynnistymiseen ja makuvirheitä aiheuttaviin haitta-aineisiin. Kaikissa osakokeissa kalana oli kirjolohi (*Oncorhynchus mykiss*), joka on ylivoimaisesti yleisin ruokakalaksi kasvatettava kala Suomessa. Aquaponic-systeemeissä kasvatetaan useimmiten trooppisia kalalajeja ja kirjolohi on selvästi harvemmin käytettävä kalalaji. Se sopii kuitenkin Suomen olosuhteisiin paremmin kuin trooppiset lajit, ja kirjolohelle on valmiit markkinat Suomessa.

Ensimmäisessä osakokeessa tutkittiin kasvien mikrobistoja, kun kasvit kasvatettiin kirjolohen kiertovesiviljelyn poistovedessä. Kokeessa kasvatettiin lehtisalaattia (*Lactuca sativa*) joko yksinään tai yhdessä mintun (*Mentha spicata*), isohietasinapin eli rukolan (*Diplotaxis tenuifolia*) tai malin (*Artemisia absinthium*) kanssa. Lehtisalaatin kasvu parani, kun se kasvatettiin yhdessä mintun tai rukolan kanssa. Lehtisalaatissa oleva mikrobisto vaikutti positiivisesti sen kasvuun, kun minttu oli sen seuralaiskasvina.

Toisessa kokeessa tutkittiin pinaatin (*Spinacia oleracea*) kasvua aquaponic- ja hydroponic-menetelmillä ja kirjolohien kasvua aquaponic- ja kiertovesisysteemeissä. Uudessa biosuodattimessa nitrifikaation käynnistyminen kunnolla voi kestää jopa kaksi kuukautta. Siksi tässä kokeessa tutkittiin myös erityisesti, nopeuttavatko kasvit nitrifikaation käynnistymistä. Kiertovesikasvatuksen yksi suurimmista ongelmista on kaloihin kertyvät makuvirheitä aiheuttavat yhdisteet, joita tuottavat suodattimien bakteerit. Tässä kokeessa halusin selvittää, onko kasveilla vaikutusta makuvirheitä aiheuttavien geosmiinin (GSM) ja 2-metyyli-isoborneolin (MIB) pitoisuuksiin vedessä, kaloissa ja kasveissa. Pinaatin kasvu ei eronnut aquaponic- ja hydroponic-menetelmissä. Aquaponic-kasvatuksessa pinaatin GSM- ja MIB-pitoisuudet nousivat, mutta aquaponic-kasvatuksessa kirjolohen GSM-pitoisuus oli alhaisempi kuin kiertovesikasva-

tuksessa. Molempien järjestelmien kasvatusvedessä GSM- ja MIB-pitoisuudet olivat yhtä suuria, joten kokonaisuutena kasvien vaikutus makuvirheaineiden vähentämisessä oli vain hyvin vähäinen. Aquaponic-systeemissä kirjolohet kasvoivat nopeammin ja niiden rehun käyttö oli tehokkaampaa kuin kiertovesikasvatuksessa. Nitrifikaatio alkoi nopeammin aquaponic-systeemissä kuin kiertovesikasvatuksessa ja aquaponic-systeemissä veden nitraattipitoisuus oli myös selvästi pienempi.

Kolmannessa osakokeessa verrattiin kiertovesikasvatusta ja aquaponic-systeemiä, jossa kasvatettiin minttua. Mittareina olivat kalan ihon ja suoliston mikrobisto, kalojen kasvu, rehukerroin, veden laatu ja kalan omega-3-rasvahapot. Minttu paransi veden laatua verrattuna kiertovesisysteemiin, ja näissä kahdessa eri systeemissä myös kalojen mikrobistot poikkesivat toisistaan. Sen sijaan kalojen kasvussa tai rasvahappojen määrässä ei ollut merkittäviä eroa, mutta kalat käyttivät rehua tehokkaammin aquaponic-systeemissä kuin kiertovesikasvatuksessa.

Kokeiden perusteella näyttää siltä, että kun kasveja kasvatetaan yhdessä kalojen kanssa, niillä on positiivisia vaikutuksia kaloihin ja rehun hyväksikäyttöön, mikä johtuu ilmeisimmin parantuneesta veden laadusta. Myös kalojen mikrobisto muuttuu kasvien vaikutuksesta. Kasvit kasvoivat yhtä hyvin kalojen tuottamalla ravinteikkaalla vedellä kuin keinotekoisella kasviravinteella. Kokeissa käytettyjen kasvilajien avulla ei kuitenkaan voida poistaa kovin merkittävästi vedessä olevia makuvirheitä aiheuttavia yhdisteitä.

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ORIGINAL PAPERS

I

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II

IS AQUAPONICS BENEFICIAL IN TERMS OF FISH AND PLANT GROWTH AND WATER QUALITY IN COMPARISON TO SEPARATE RECIRCULATING AQUACULTURE AND HYDROPONIC SYSTEMS?

by

Faiqa Atique, Petra Lindholm-Lehto, & Juhani Pirhonen 2022

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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?

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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).

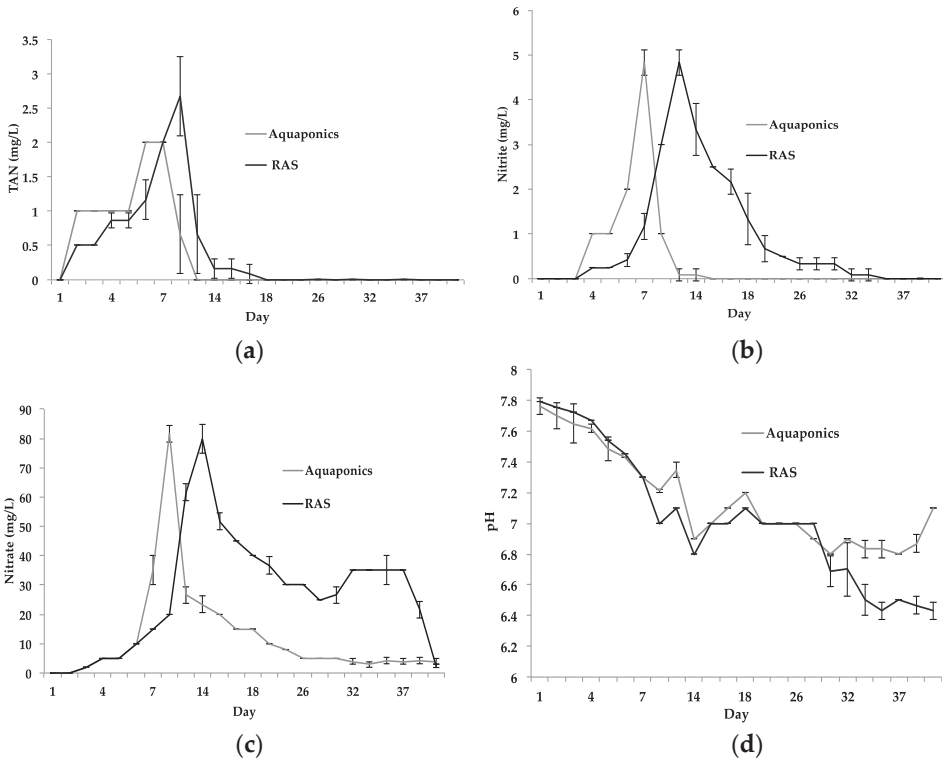

Figure 1. The mean concentration (mg/L) ± SD (n = 3) of (**a**) total ammonium nitrogen (TAN) (**b**) nitrite, (**c**) nitrate, and (**d**) pH in RAS and aquaponics treatments during the 42-day experiment. For the aquaponics treatment spinach was grown in a coupled aquaponics system together with rainbow 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 ± 49.74 (aquaponics) to 85.32 ± 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 ± 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 ± 142.9) and hydroponics (206.6 mg/kg ± 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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III

IMPROVED WATER QUALITY, FEED CONVERSION, AND MODIFIED MICROBIOME OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) IN AQUAPONICS AS COMPARED TO RECIRCULATING AQUACULTURE SYSTEM

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