DEPARTMENT OF BIOLOGICAL AND
ENVIRONMENTAL SCIENCES

FRESHWATER AQUAPONICS: LOW-TECH ALTERNATIVE FOR NUTRIENT REMOVAL IN SWEDISH AQUACULTURE?

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Degree project for Master of Science (120 hec) with a major in Biology
Physiology and cell biology BIO727 (60 hec)
Second cycle

Semester/year:	Spring/Autumn 2024
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Abstract

The growing world population is putting conventional aquaculture to the test. Available land for food production is becoming scarcer, and excess nutrients can cause eutrophication of soils and waters. Aquaponics, the coupling of recirculating aquaculture systems (RAS) with the production of plants in growbeds without soil, so called hydroponics are a promising way to improve modern aquaculture. Due to a rising demand for seafood, it is therefore necessary to identify plant species suitable for nutrient removal from RAS and potential challenges. To achieve this, we cultivated green kale (*Brassica oleracea*), a leafy vegetable requiring relatively high amounts of nitrogen for good yields, in ebb- and flow hydroponic systems with process water from a RAS farming African catfish (*Clarias gariepinus*), an important upcoming species within Swedish aquaculture. We compared plant-growth and nutrient levels in the water against a control system with a standard fertilizer solution for hydroponics. The daily growth rate of the plants in the aquaponic treatment was significantly lower at 0.57 g/d than in the control at 0.97 g/d within the first 29 days of the experiment. The growth correlated with the nitrogen uptake of 49.81 mg/d for aquaponics and 87.37 mg/d for fertilised. Low leaf-pigment concentration in the aquaponic treatment suggested a lack of trace elements and/or suboptimal pH. The addition of trace element solution and pH adjustment with sulfuric acid led to a clear positive effect on the plant's growth and pigmentation. Focusing on researching sustainable and alternative ways to maintain optimal pH and a balanced nutritional profile for all organisms within aquaponic systems could improve growth and nutrient absorption, producing a valuable secondary product, while employing more circularity and sustainability.

1. Introduction

The rapidly growing global population presents significant challenges for humanity (United Nations, 2019). These challenges are particularly evident in the realm of nutrition. A larger population inherently leads to a higher demand for high-quality proteins that must be met. Especially in the production of terrestrial animal protein, a substantial amount of energy is required, significant amounts of greenhouse gases like carbon dioxide (CO_2) and methane (CH_3) are emitted and often, a large area of land is required to achieve sufficient yields. Aquaculture systems contribute significantly to meet this worldwide demand. Additionally, aquaculture is more efficient than the farming of terrestrial animals as terrestrial animals are endo- and aquatic are ectothermic, therefore using less energy through heat generation (Tlusty et al., 2018). Seafood is composed of high-quality proteins containing balanced important amino acids, essential omega 3-fatty acids and relevant vitamins (A, B and D)(Jayasekara et al., 2020). Common aquaculture systems like open cage salmon farms are however subject to several issues like rapid environmental changes (temperature), exposure to pathogens (fish lice) and in some contexts, damage to the environment through eutrophication and the risk of escaping animals. Recirculating aquaculture systems (RAS), have the potential to solve many of these common problems due to their circular and enclosed design, utilising several mechanical and biological water filtration processes (*Figure 1*).They can offer a more controlled environment, thus ensuring animals can be grown more efficiently while also improving their welfare (Martins et al., 2010). The amount of circularity in RAS varies, as some designs employ different filtration steps. Partial RAS commonly lack removal steps for nitrogen, which simplifies the design, but requires a higher rate of water exchanges. Full RAS, which employ other removal techniques for these compounds such as denitrification filters employ circulation degrees higher than 90%. This minimises the discharge of waste or nutrients into the environment and potentially the need for chemicals treating diseases, presenting a potential solution for developing production of high-quality protein with little environmental impact compared to traditional aquaculture (Ahmed & Turchini, 2021).

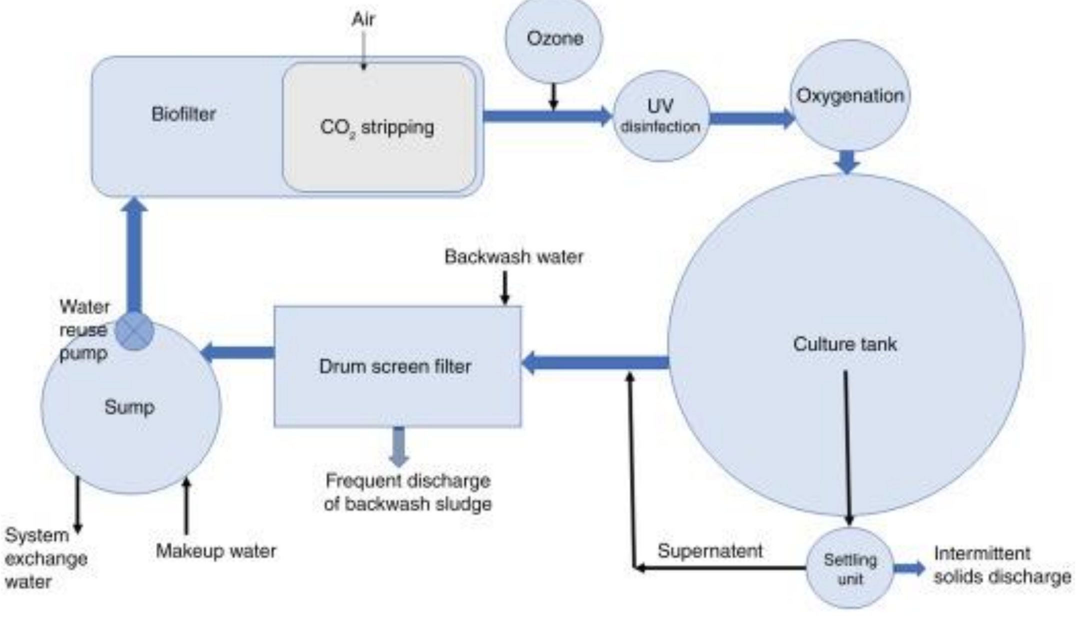


***Figure 1: Schematic overview of a typical RAS illustrating the flow of water (blue arrows), solids and gases through mechanical and biological filtration* stages** (Holan et al., 2020)

In all aquaculture systems, ammonia (NH_3)/ammonium (NH_4^+) is produced as a metabolic byproduct of the animals and decomposing feed residues, which can be neurotoxic for most fish species at concentrations above 0.5 mg/L (Roques, 2013), and presents a danger especially in closed systems where water is recirculated. Toxicity increases with rising pH values as the concentration of the more toxic, ionised form (NH_4^+) correlates with pH (Fösel, 2007). Through microbial nitrification (Figure 2), NH_3 is first oxidised into nitrite (NO_2^-) and then into nitrate (NO_3^-) (Tyson et al., 2008). In RAS, nitrification filters, such as fixed- and moving bed, are used for this purpose, providing a large surface area and oxygenation for nitrifying microbes.

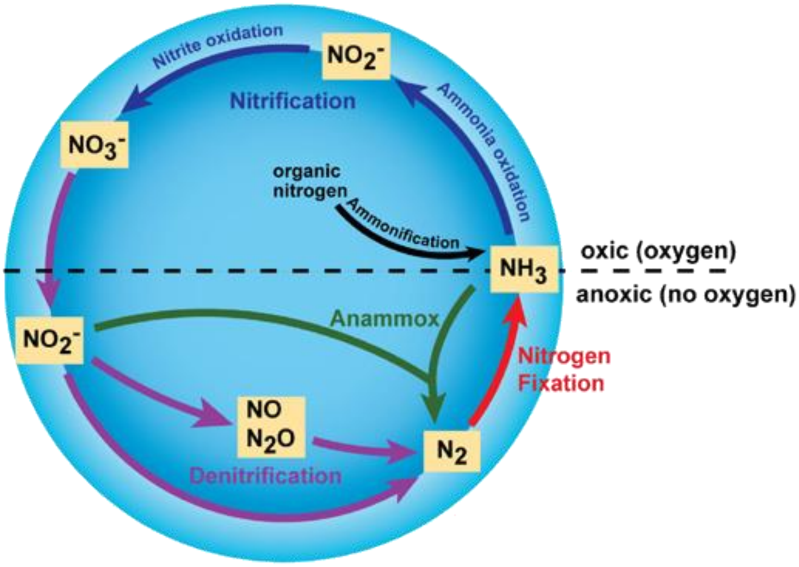


Figure 2: Microbial cycling of nitrogenous compounds in oxic- and anoxic conditions (Bernhard, 2010)

High concentrations of NO_2^- and NO_3^- affect the oxygen binding affinity of red blood cells by converting haemoglobin into methaemoglobin and will therefore compromise the animal's health. NO_3^- tolerance varies depending on species, larval stage and size, ion concentrations and other factors. A safe level for post-smolt Atlantic Salmon (*Salmo salar*) in RAS was identified at around or below 100 mg/L NO_3^- (Davidson et al., 2017). Before the critical level is reached, NO_3 needs to be removed from the system (Camargo et al., 2005). The removal is mostly performed by water exchange or with the use of denitrifying filters, in which anoxic bacteria reduce NO_3^- to harmless nitrogen-gas (N_2)(Hrubec et al., 1996). Disposing of nitrogenous waste is usually performed in traditional sewage treatment plants with denitrifying bacteria.

NO_3^- and NH_3 are essential macronutrients for plants, which are usually added to agricultural fields or hydroponic systems in the form of fertiliser. Introducing a plant component into an aquaculture system is expected to utilise this resource, reduce waste, potentially minimising the risk for eutrophication, and improve water quality. As plants and animals might not share the same requirements on the culture conditions in regards of e.g. pH, temperature, or nutrition, it is important to identify compatible species and mechanisms to counteract the differing requirements. The coupling of aquaculture with hydroponic plant cultivation is called aquaponics. These systems utilise various irrigation techniques for the plants. Most common is deep water culture (DWC) in which the plants roots are submerged into the water permanently (*Figure 3*). This technique is easy to set up but is unsuitable for plants that develop complex root systems and do not grow well with permanently submerged roots, as these can rot when permanently submerged in water. Ebb and Flow-systems (E&F), that periodically flood a grow bed media and drain again, offer better aeration and media for healthy root systems to develop (*Figure 4*).

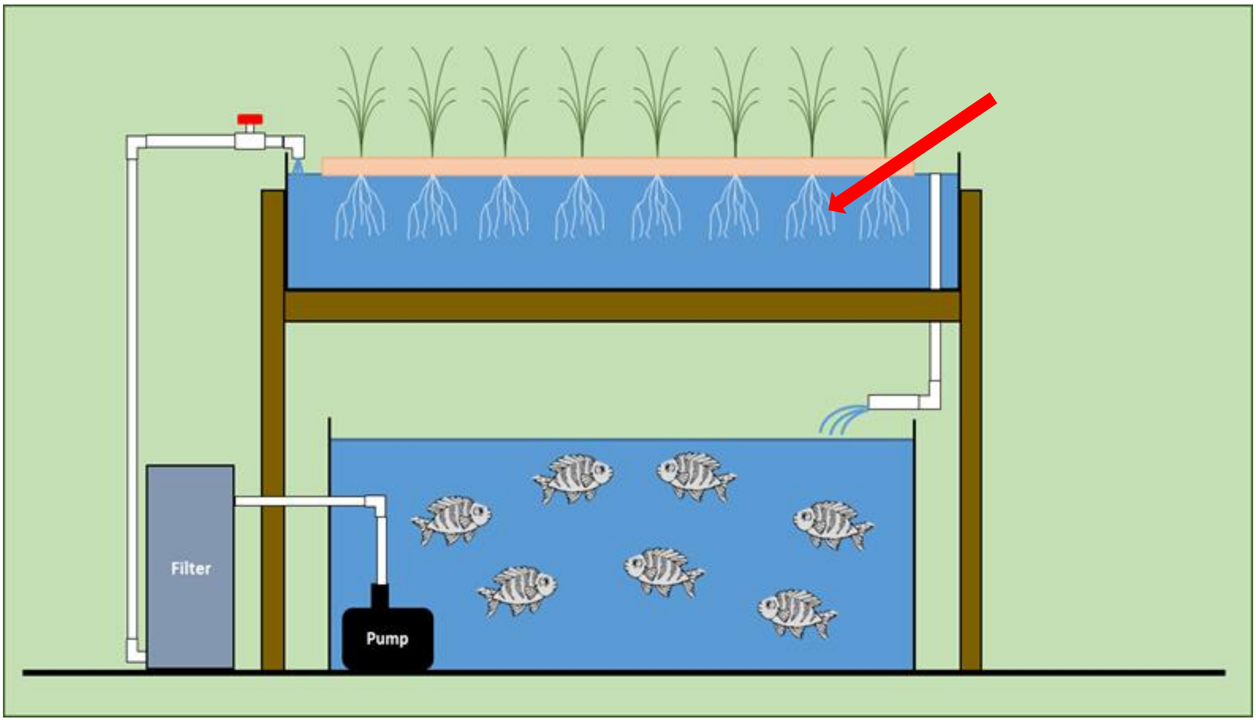


Figure 3:Plants fixed on the surface of a DWC-aquaponic system, with their roots freely submerged in water (red arrow) (Ampim et al., 2022)

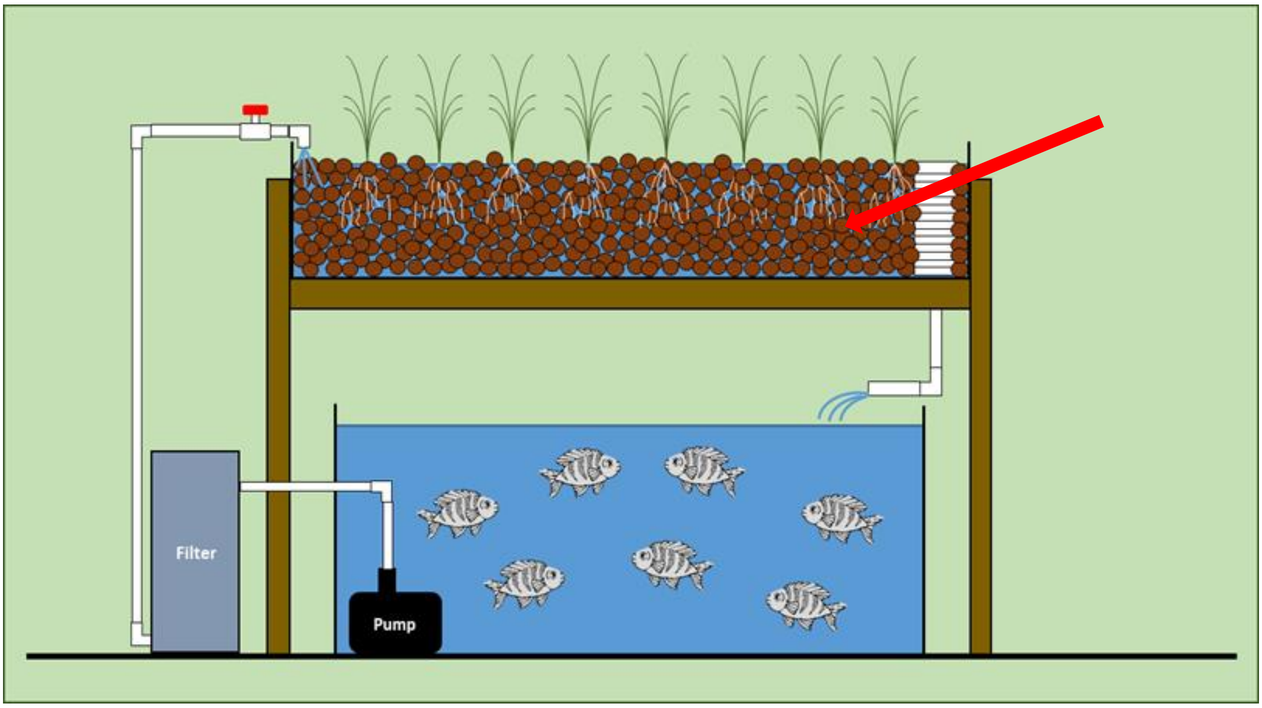


Figure 4: Plants growing in an E&F aquaponic systems, growing in light expanded clay aggregate (LECA) (red arrow) (Ampim et al., 2022)

Fish that are commonly farmed in aquaponic systems are cyprinids such as European carp (*Cyprinus carpio*), and cichlids like tilapia (genus *Oreochromis*), species that have currently little economic value in western countries (Prabu et al., 2019). A genus that has been gaining attention in RAS-research in Europe as well are African Catfish (*Clarias gariepinus*) (Figure 5). Due to its advantageous attributes for sustainable aquaculture, in sub-Saharan Africa the genus represents the most farmed fish, with Nigeria alone being responsible for 50.61% of the worldwide production of 316.727 MT in 2015 (Dauda et al., 2018). Catfish are adapted to rapid environmental fluctuations regarding water quality and availability of feed. They can breathe air and are able to consume a broad variety of feed, ranging from vegetable scraps to predation on other fish, which presents possibilities for developing feed with lower fishmeal contents (FAO, 2016). Kale (*Brassica oleracea*) (Figure 5) is a very well established plant for use in hydroponic systems that requires high nitrogen levels to ensure maximum growth and is therefore possibly well suited for nutrient removal in aquaponics (Chakwizira et al., 2013). Several varieties of the crop such as" Dwarf curled" are widely consumed all over the world and compared to other leafy vegetables like lettuces, spinach, and other *Brassica,* it has a relatively high protein content, ranging between 3.28-11.67% in fresh weight (Satheesh & Workneh Fanta, 2020).

Figure 5: B. oleracea (left) and C. gariepinus (right), image credit: Impecta Fröhandel (left), FAO (right)

1.1 Aim

The aim of this project was to discover new ways of nutrient removal in RAS by analysing the nutrient removal capacity of plants, in this case: kale in aquaponic systems. These offer in this case a lower degree of technicality and are supposedly a more easily employable method of removal, compared to denitrification filters. To achieve this, we cultivated kale in E&F hydroponic systems, comparing performance in freshwater with mineral-fertiliser against process water from a RAS with *C. gariepinus*. To develop aquaculture with more efficient use of nutrients and less waste, we analysed growth- and physiological parameters from *B. oleracea* of the "Dwarf curled" variety to identify compatibility with water properties, an optimal plant to animal ratio for sufficient nutrient removal, plant nutrition and to identify challenges and future research questions for the use of kale in aquaponic systems.

2. Material and methods

2.1 Experimental Setup

We cultivated *B. oleracea* in two different treatments using one hydroponic system as in Figure 6 each:

- Aquaponic: Process water from Swedish *C. gariepinus* partial RAS-farm (Pond Fish and Greens AB, Floda, Sweden), collected from the pump sump after passing through a nitrifying biofilter.
- Fertilised: Tap water mixed with Nova Max Grow® (Terra Aquatica, Fleurance, France) hydroponic fertiliser solution, concentration according to the manufacturer's recommendation (*Table 1*)

Each treatment consisted of a 200 L water reservoir, connected to a growbed by a FP-1500 submersible pump (BOYU, Huanggang Town, China), with a flow-rate of 1500 L/h through a flexible hose ending in an inlet in the bottom of a 90 L PP-bucket. To simulate an active biofiltration unit in the aquaponic treatment, we added a 30 L mesh box filled with plastic biofilter media, which was submerged in the reservoir, aerated and pre- conditioned with ammonia for 4 weeks prior to the experiment. The inlet for the growbeds also served as an outflow when draining. To flood the growbed up to 16 cm, an overflow outlet was installed. Both in- and outflows were fitted with plastic mesh-cages to prevent clogging. The round growbeds were filled with 60 L of light expanded clay aggregate (LECA) as soil and had an area of 0.283 m^2 each. We took note of the water level daily and refilled the reservoir with tap water occasionally based on the amount of evaporation.

We operated the systems under artificial lighting using LED Plant lamp E27 - 36W full spectrum LED (Sansi LED ltd., Union City, USA) for each growbed. The light intensity on the surface of the LECA ranged between 160-200 $\mu mol/cm^2/s$. The day/night rhythm was 12/12h and controlled with analogue power timers.

The ebb and flow cycle were controlled by an analogue power timer which switched on the pumps for a 15 min interval in each full hour while the lighting was turned on. During the nighttime, they switched on for 15 min every 2.5 h to prevent the roots from drying out.

We split the experiment into 2 phases, introducing 8 plants per treatment into the growbeds for the start of each phase, followed by a 29-day growing period, operating it as described above and removing them for sampling. For phase 2 we introduced new plants into the same systems on day 29, while initially adding 20.6 g of supplementary trace element fertiliser mix "Aquaponic Mix" (Terra Aquatica, Fleurance, France) *(Table 1)* into the aquaponic treatment. We added an additional amount of 214.5 g on day 45 after we observed severe deficiencies in the plant, caused by improper dosage of the fertiliser. On the same day, we adjusted pH in the aquaponic treatment as well by adding 13 mL of glacial sulphuric acid, harvesting them after the same number of days as in phase 1 on day 58.

Figure 6: Experimental setup, left: aquaponic treatment (red arrow=biofilter), right: fertilised treatment without biofilter

Table 1: chemical composition of fertilisers

Product	Compound	Content	Nitrogen-Phosphorus-Potassium-relation (NPK)	Recommended dosage	days used
Nova Max Grow®	K_2O	10%	7-4-10	1.5mL/l	0
	NO_3-N	6.1%			
	CaO	4%			
	P_2O_5	4%			
	SO_3	2%			
	MgO	1.5%			
	NH_3-N	0.9%			
	Fe-chelate-DPTA	0.1%			
Aquaponic Mix	K_2O	4%	0-0-4	0.5 mL/10L/day	29
	CaO	1%			
	Fe-chelate-EDDHA-11% DPTA0	0.09%			
	Zn-chelate-EDTA	0.01			
	Mn-chelate-EDTA	0.004%			
	Cu-chelate-EDTA	0.002%			
	B	0.0003%			

2.2 Plants

We acquired *B. oleracea* seeds of the "Dwarf Green Curled" variety from online retailer Impecta (Katrineholm, Sweden). We sew the seeds into cubes (Eazy Plug®, Goirle, Netherlands) made from organic, fibrous material on November 30th, 2023, for phase 1 and on January 18th, 2024, for phase 2. We soaked the cubes with tap water every 3rd day and placed them on a rack for draining to prevent overwatering. After sprouting, we applied low level LED lighting (measure intensity).

2.3 Plant growth

To monitor the growth of the plants, we weighed them before introducing them into the experimental setup. Using a SAUTER® RE 2021 (KERN & SOHN GmbH, Balingen-Frommern, Germany) digital scale, we acquired wet weights together with a fully soaked sowing-cube. We labelled and photographed each plant with an individual tag to identify them after removing them from the system after the growth period, repeating the previously mentioned weighing and imaging processes (Figure 7). We archived the photographs for potential additional analysis, such as root/shoot length or leaf area. After the weighing process we froze and stored the whole plants for potential carbon- and nitrogen content analysis. We used the weighing data to calculate specific growth rate (SGR) as seen below.

$$SGR = \frac{(Weight_{final} - Weight_{initial)}}{time_{growing\ period}}$$

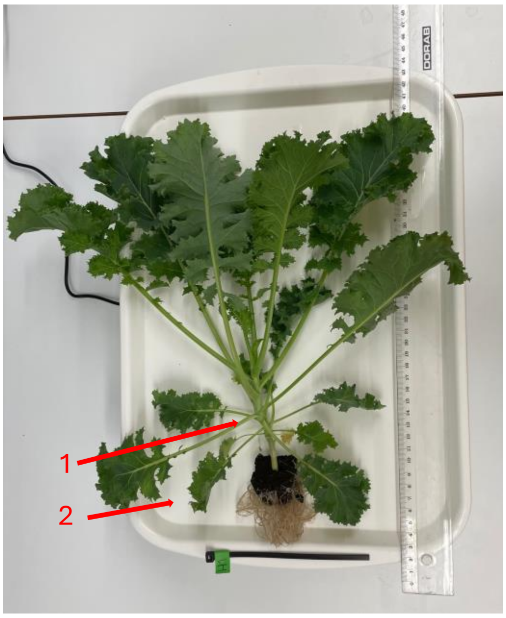


Figure 7: sampling picture of Brassica oleracea after 29 days of hydroponic growth, 1: sowing cube; 2: label

2.4 Nutrient uptake and water quality

We took samples of the water every day during the experimental phases, monitoring the water level in the process, to account for evaporation. Using a Hach Lange® DR 2800 (Hach Lange GmbH, Düsseldorf, Germany) spectrophotometer we analysed concentrations of dissolved Phosphate (PO_4) with powder pillow method 8048, NO_3^- with powder pillow method 8039, later (from April 4th, 2024) employing the LCK 339 cuvette test kit, due to unreliable results with the powder pillow method, NO_2^- with powder pillow method 8155 and NH_3 using powder pillow method 8155. Taking evaporation and removal of compounds through the sampling process into account, we calculated the total amounts of nutrients for each system as follows:

$$Total\ mass_{compound} = Volume_{reservoir} \times concentration_{compound} - Volume_{sample} \times concentration_{compound}$$

To identify nutrient removal rates, we calculated slopes of the daily amount of nutrients through all days in each respective phase. Using the FiveEasy Plus pH/mV meter (Mettler-Toledo GmbH, Gießen, Germany) we monitored the pH in each treatment daily.

2.5 Data analysis and visualisation

We compiled data in Microsoft Excel® to create figures and tables, utilising Excel´s data analysis add-on to analyse data for normality and perform statistical tests.

3. Results

3.1 Plant growth

Comparing the treatments with each other, we observed significant differences in growth rate in both phase 1 and 2 (phase 1: p=0.048; phase 2: p=0.012) using Student´s t-test for independent samples with differing variances. In phase 1, the fertilised system yielded an average plant weight of 49 g ±13.5 g, whereas the aquaponic treatment yielded 38.2 g ±5.43 g (Figure 8). The leaves of the plants in the aquaponic treatment were significantly lighter in colour and showed symptoms of iron deficiency. Before pH adjustment and increased use of micronutrient fertiliser, leaves bleached and died off. New non- discoloured leaves started to grow after the adjustments made on day 45 (Figure 9). We did not observe any significant differences within treatments when comparing both phases with each other after employing the same test method as mentioned above.

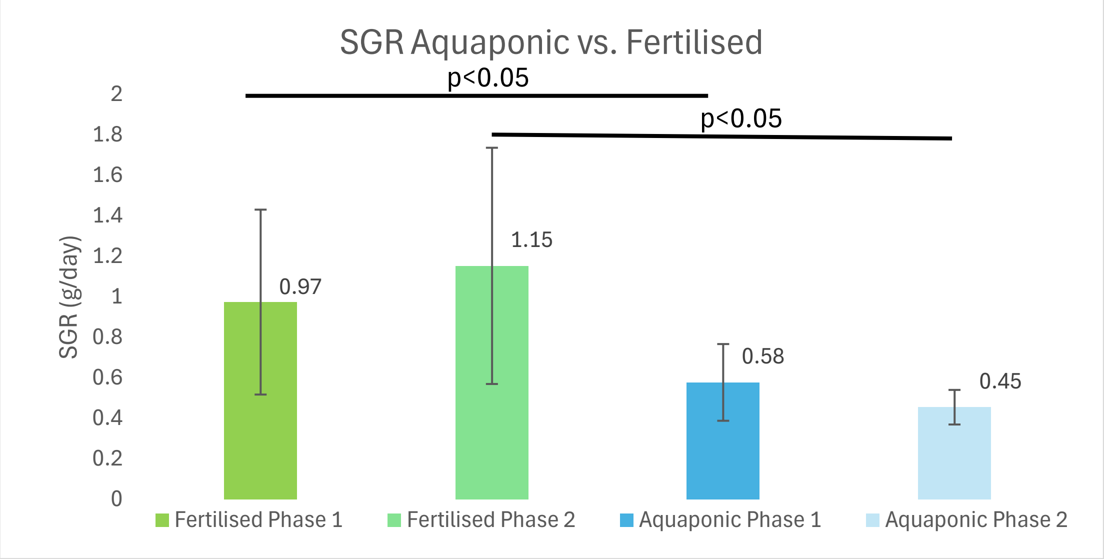


Figure 8: Specific growth rate of B. oleracea through 29 days of hydroponic cultivation in fertilised and aquaponic treatments grown in 2 phases, average± standard deviation, Student´s t-test, n=8

Figure 9: bleaching leaves of B. oleracea on day 45 (left) and new, healthy leaves on day 50 in the aquaponic treatment (right)

3.2 Water parameters

3.2.1 pH

We observed declining pH values for the fertilised system in both phases, ranging between 6.56 and 4.94. In the aquaponic system, we identified rising pH values ranging between 7.38 and 8.08 in phase 1. After the addition of sulphuric acid to the aquaponic system on day 45, pH decreased down to 5.29 and started to increase and stabilise up to 7.25.

3.2.2 NH_3

The presence of NH_3 declined rapidly in the aquaponic treatment, levelling out after the first day at very low concentrations between 0.01 and 0.06 mg/l (Figure 10). After adding sulphuric acid to adjust pH on day 45, the level increased rapidly, reaching a maximum at 105.3 mg on day 49 and then started to decrease, reaching pre-pH adjustment values of 8 mg on day 59. In the fertilised system, we observed a rapid decline in NH_3 throughout the entire experiment starting from levels as high as 2756 mg (Figure 11), compared to 19.5 in the aquaponic treatment.

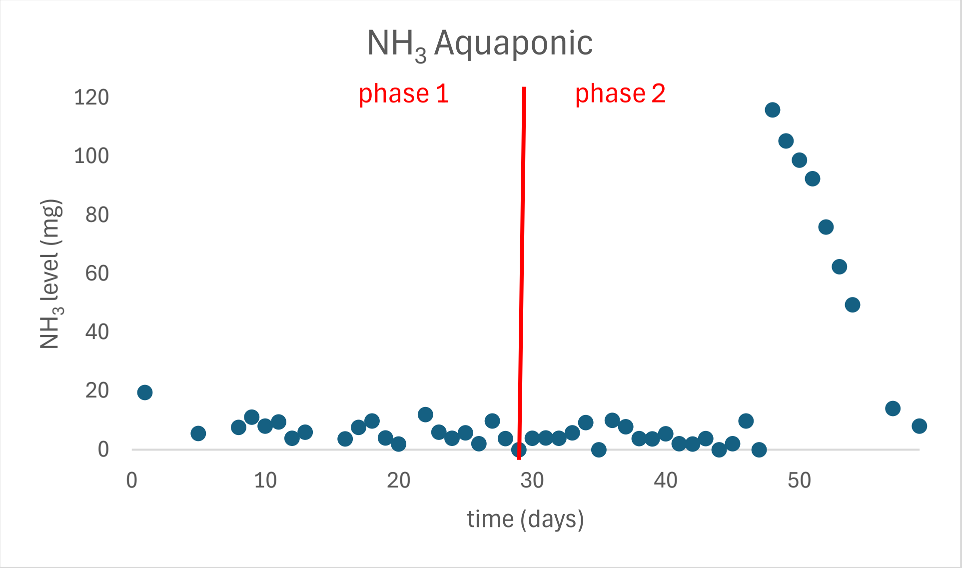


Figure 10: NH$_3$ levels in the aquaponic treatment in phase 1 and 2, n=1

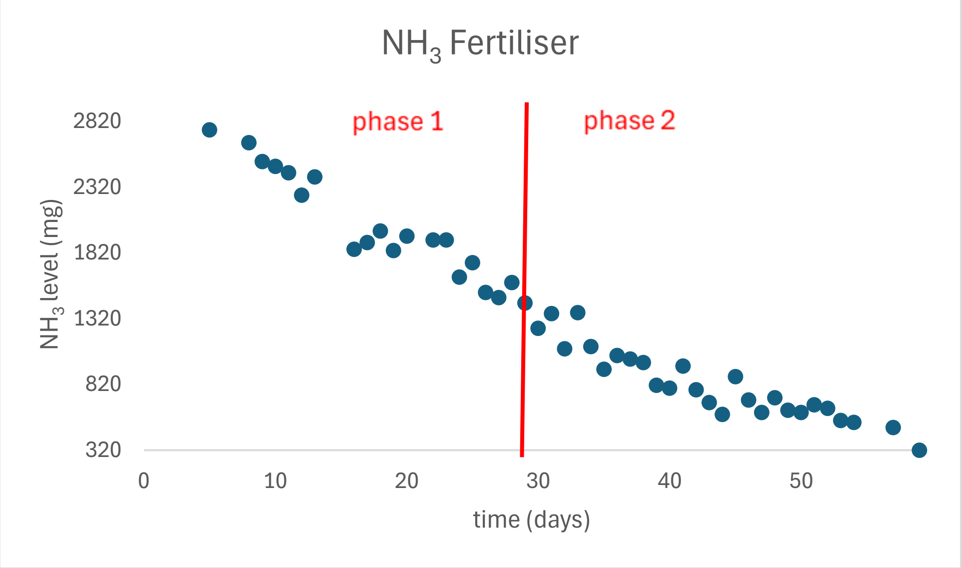


Figure 11: NH$_3$ levels in the fertilised treatment in phase 1 and 2, n=1

3.2.3 NO$_2^-$

The NO$_2^-$ level in the aquaponic treatment (Figure 12) remained constantly low (2-0 mg) after day 7 until pH adjustment with sulphuric acid, then displaying an increase until reaching its maximum at 14.06 mg on day 54. From then, the level decreased down to 7 mg on day 59. Whereas in the fertilised treatment a high starting level of 538 mg kept increasing rapidly, peaking at 1391 mg on day 8 and then rapidly declined until day 17, balancing out on low levels down to 0.8 mg up until the end of the experiment (Figure 13).

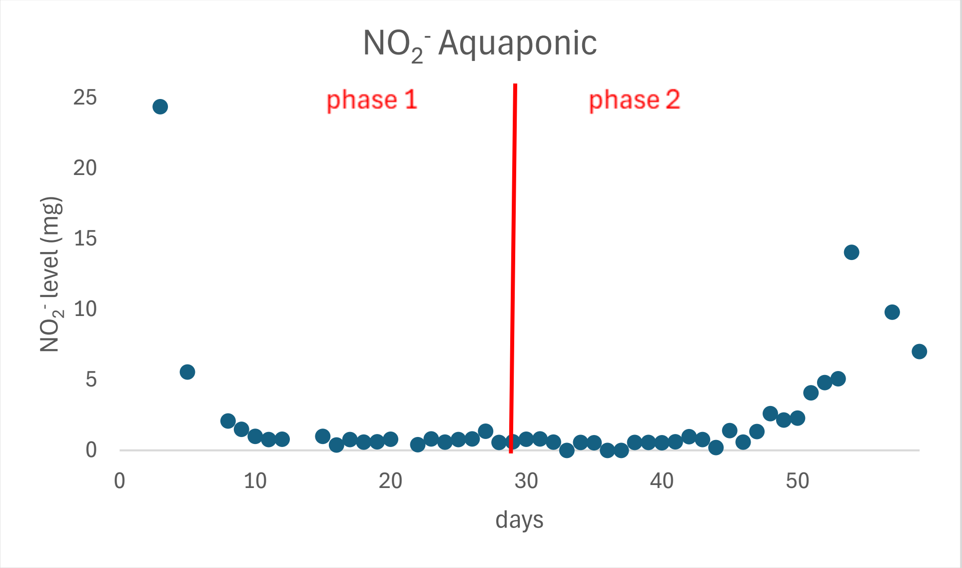


Figure 12: NO_2^- levels in the aquaponic treatment in phase 1 and 2, n=1

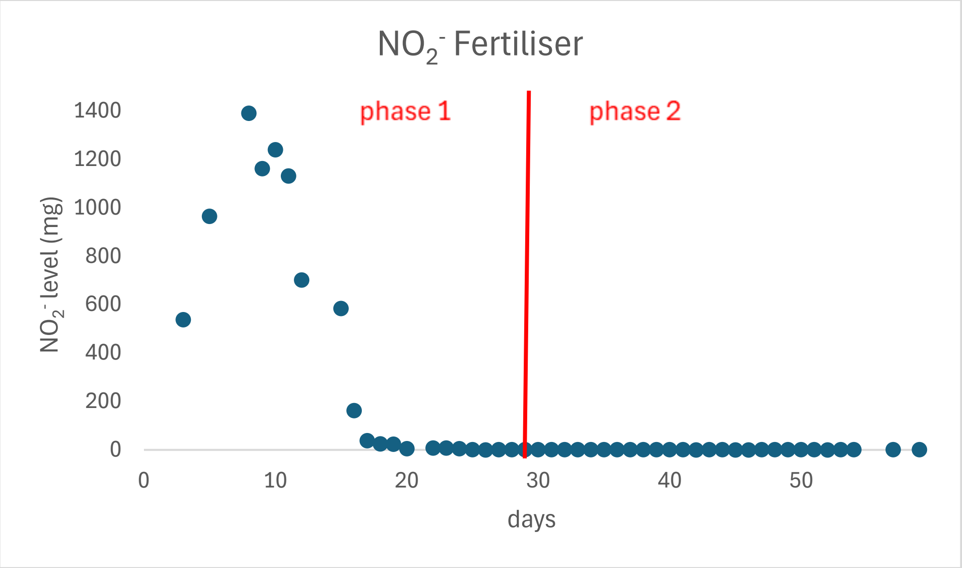


Figure 13: NO_2^- levels in the fertilised treatment in phase 1 and 2, n=1

3.2.4 NO_3^-

NO_3^- concentrations exhibited relative stability within the aquaponic treatment starting from day 18 onwards, demonstrating a slight decline until the conclusion of the experiment, punctuated by periodic spikes (Figure 14). Conversely, the fertilised system exhibited an increase in NO_3^- levels between day 18 and 22, subsequently transitioning into a gradual decline similar to that observed in the aquaponic treatment (Figure 15). NO_3^- concentrations were consistently approximately twice as high in the fertilised system compared to the aquaponic system, constituting the predominant fraction of dissolved nitrogenous compounds in both treatments.

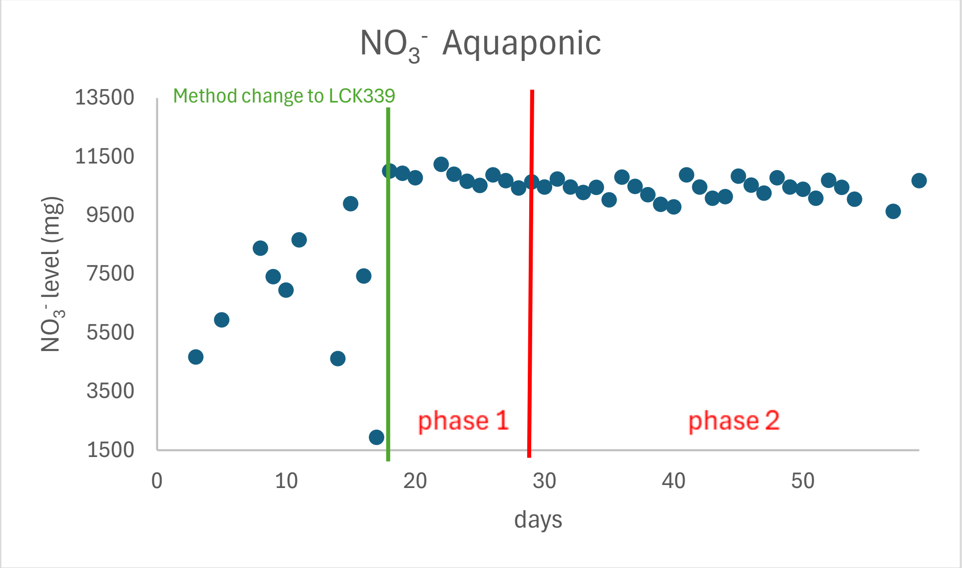


Figure 14: NO_3^- levels in the aquaponic treatment in phase 1 and 2, n=1

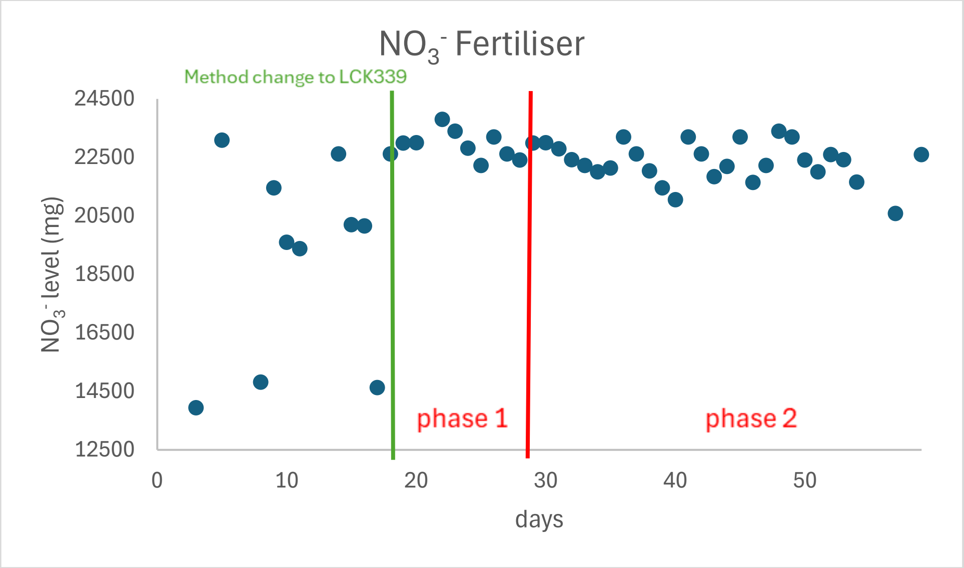


Figure 15: NO_3^- levels in the fertilised treatment in phase 1 and 2, n=1

3.2.5 total -N

Both treatments displayed a consistent reduction of nitrogen over the course of the experiment, showing similar fluctuations between the sampling days as described for NO_3^- (Figure 16, Figure 17). The data presented starts at day 19 of the experiment, due to previously mentioned complications with the powder-pillow methodology. In the final 10 days of phase 1, the average daily removal was 1.75-fold higher in the fertilised- than in the aquaponic treatment. For the whole duration of phase 2, this proportion was higher with a factor of 25.5. However, when comparing only the final 10 days of each treatment, the difference decreased (Table 2). N-levels were generally around twice as high in the fertilised- as in the aquaponic treatment.

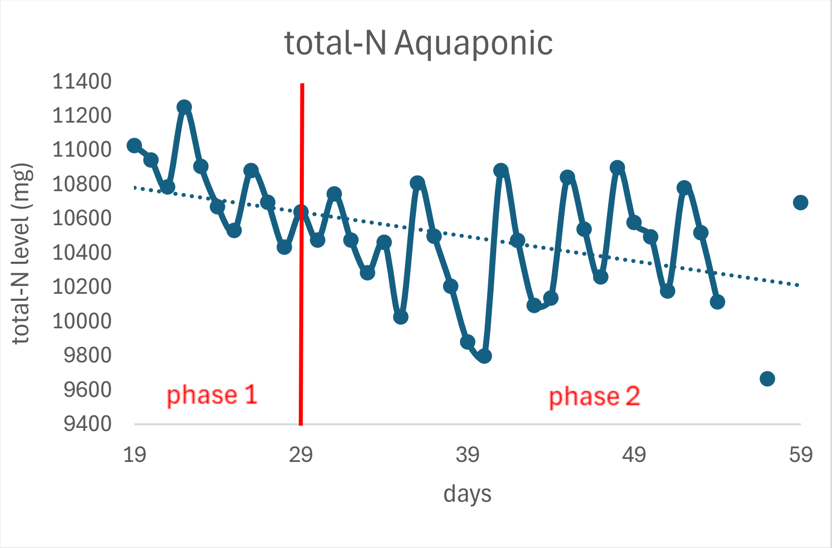


Figure 16: total-N level in the aquaponic treatment in phase 1 and 2, n=1

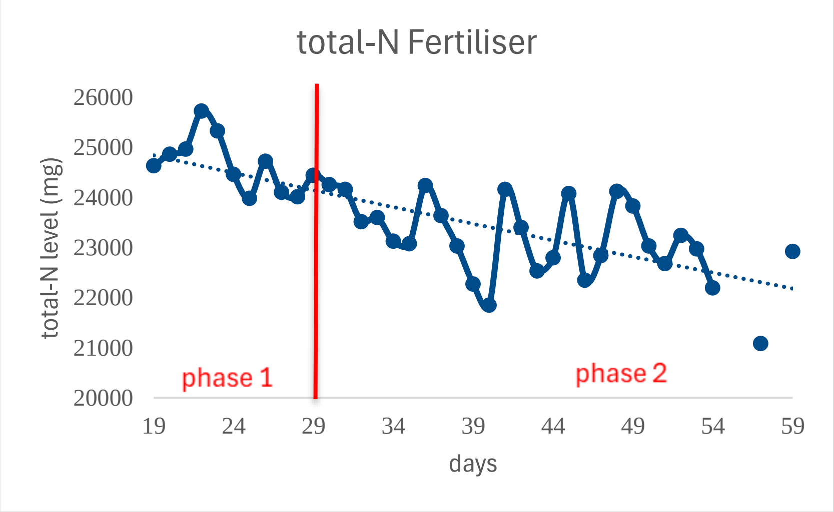


Figure 17: total-N level in the fertilised treatment in phase 1 and 2, n=1

Table 2: Nitrogen removal in aquaponic and fertilised treatments

Phase	**removal aquaponic (mg/day)**	**removal fertilised (mg/day)**	**Proportion fertilised/aquaponic**
1 (day 19-29)	-49.81	-87.37	1.75
2 (day 30-59)	-1.78	-45.60	25.55
2 (day 49-59)	-27.13	-88.26	3.25

3.2.6 ortho-PO_4

Ortho-PO_4 levels declined in all treatments and phases, whereas the average daily removal rate was spread widely with a removal of ~14.65 mg/day for the aquaponic- (Figure 18), and 132.29 mg/day for the fertilised systems (Figure 19) in phase 1. The initial amount for the aquaponic treatment was lower than in the fertilised treatment, with 3356 mg compared to 24216 mg.

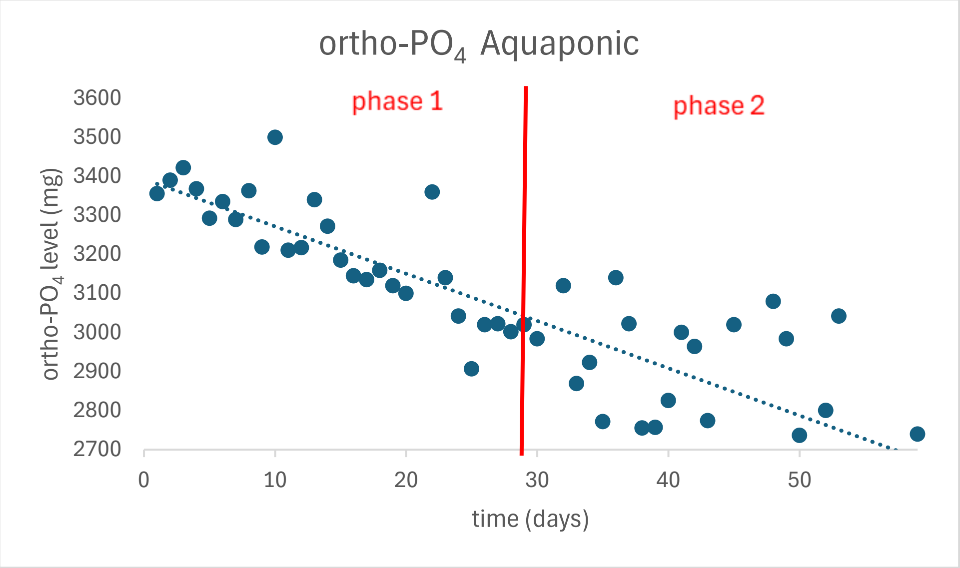


Figure 18: total amount of ortho-PO_4 in the aquaponic treatment in phase 1 and 2, n=1

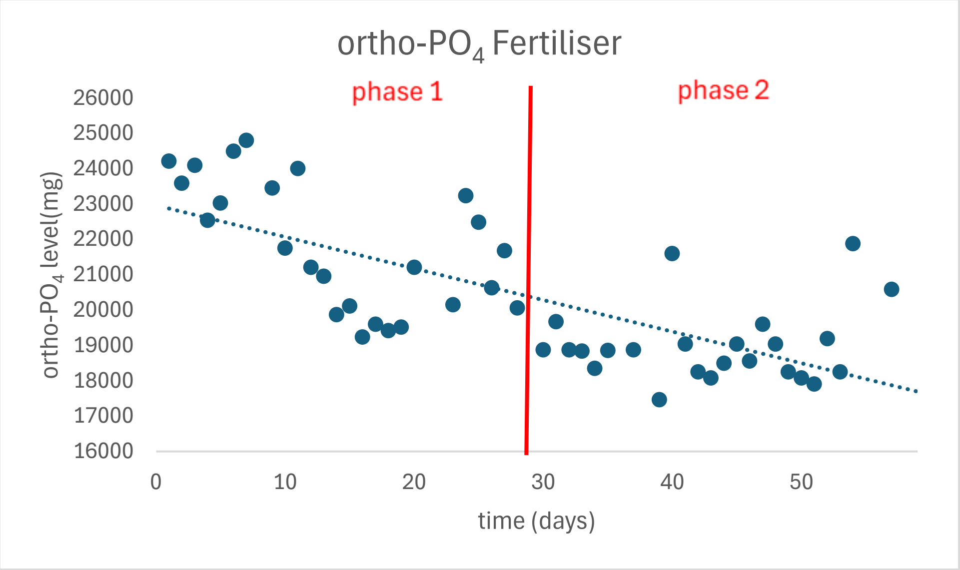


Figure 19: total amount of ortho-PO_4 in the fertilised treatment in phase 1 and 2, n=1

4. Discussion

4.1 Plant growth

Based on our growth results, it is evident that the aquaponic treatment performed very poorly in phase 1. A possible explanation for this might be deficiency in mineral micro-nutrients, as the addition of trace element fertiliser increased performance in the later stages of phase 2. Aquaponic systems often lack sufficient concentrations of plant- available iron (Kasozi et al., 2019). The observed change in colouration of the leaves to a more pale- green between the leaf-veins (interveinal chlorosis), is a clear indicator for iron deficiency as iron is a crucial component of chlorophyll synthesis (Kosegarten et al., 1998). To counteract these deficiencies, it is widespread practice in commercial- and hobbyist aquaponics to either introduce the minerals through special feed or add them directly to the system in form of special fertilisers, which is what we did for phase 2 in the experiment. Regrettably, there has been a miscommunication between us and the fertiliser reseller, leading to inadequate application of the fertiliser. Initially, it was applied only once, despite the necessity for daily supplementation according to the manufacturer's recommendations. We counteracted this later by adding 14 times the daily amount 14 days before the end of the phase, but since the plants showed severe symptoms of deficiency within the first two weeks of phase 2, the growth performance cannot be compared to the fertilised treatment. However, after adjusting the fertiliser concentration and the pH (see below), the plants showed immediate recovery, suggesting that proper growth can be assured by the supplementation of micronutrients and that nitrogenous compounds and ortho-PO_4 were no limiting factors. The experimental setup limited the amount of added nutrients in the aquaponic treatment to only one initial addition, which is not entirely representative of a true coupled aquaponic system. In these, nutrients are consistently added though the feeding of the fish and will also change in concentration as the fish grow and consume more feed. In such a system, the need for the supplementation of micronutrients might be lower and the overall growth of the plants higher.

The pH had to be adjusted to ensure good growth. pH in most freshwater RAS-effluent commonly ranges around 7.2, which is outside of the ideal range for most plants grown in hydroponics, including kale, as it prefers slightly acidic pH in hydroponics and displayed up to 76% higher growth when cultivated in systems with a pH between 5.5-6 compared to 7.2 in experiments conducted by (Wortman, 2015). This problem can be counteracted by either employing decoupled aquaponics, growing the plants in separate hydroponic units that are not directly connected to the RAS, this however reduces the amount of recirculation and is not often feasible to set up at large scale farms, whereas this might be more easily performed in smaller setups (Goddek et al., 2019). Alternatively, the hydroponic unit's pH in a fully coupled system could be adjusted by buffering and adjusting within the individual components of the RAS, but it will still compromise the optimal pH for each respective component. Identifying plant species that can handle higher pH and respective higher salinities as well, could be another solution. Salt water tolerant plants (halophytes), like *Crambe maritima* tolerate and grow well in very variable environmental conditions regarding pH and salinity. *C. maritima* used to be a crop along coastal regions in northern- and western Europe, which has the potential to be used as a filter in aquaponics, containing high protein-levels and is demanding considerable amounts of nitrogen. Its compatibility with hydroponics is unknown and must be studied. There are however other halophytes that are being used in aquaponics today, *Salicornia europea* is another edible halophyte which is experiencing high demand from restaurants and hotels, being implemented in fine dining, and achieving high market prices. It has been subject to research in hydro- and aquaponics and is being commercially produced in conjunction with RAS. Both species do not require high salinities to grow well being facultative halophytes and are also suitable for freshwater aquaculture.

4.2 Water parameters

The dynamics of the different forms of nitrogenous compounds were developing as expected in both systems. The fertilised system had an initial exponential increase in NO_2^- which resembled the growth and establishment of nitrifying bacteria, which oxidise NH_3, converting it to NO_2^-. The following decrease of NO_2^- and rising levels of NO_3^- showed that nitrite-oxidising bacteria were growing and oxidising the NO_2^- into NO_3^-. During the oxidising process, the concentration of H^+ increases and pH is reduced, which is an effect that we were also able to observe in our systems. This development is common for establishing nitrifying biofilters in RAS, suggesting that the growbeds filled with LECA and tank surface provided a suitable environment with adequate surface area to establish possibly adequate nitrification for RAS wastewater. For low-tech systems this could potentially mean that they could be designed around utilising the growbeds as biofilters, not including a separate compartment and therefore reducing complexity and investment costs. The active biofilter in the aquaponic treatment kept NO_2^- and NH_3 levels at low ranges from the start of the experiment. However, its function was impaired when pH was adjusted mid- phase 2, as the bacteria could not handle the abrupt change, which resulted in a surge of NH_3 and later NO_2^- and consequently returning to pre- pH- adjustment levels.

The removal of nitrogen was clearly limited by the growth of the plants, as the removal was proportional to it for both experimental phases. Since uptake of nitrogen was still only marginal and the concentration in both systems stayed relatively high, it is unlikely for nitrogen to be the limiting factor for the plants growth. Whereas the concentration of plants/area was adequate in the beginning of the phases, the kale plants appeared to compete for light in the later stages, with some plants shading others in the fertilised treatment. This might be an explanation for the high standard deviation in this treatment and could have been prevented by employing lower density planting. Overall, this suggest, that the systems were not operating close to their limits, and we would have been able to increase nitrogen removal by adding more growbed area and plants. The removal data was however imprecise, the fluctuations mentioned in the results were most likely due to improper accounting for evaporation. The ventilation for the room that the systems were situated in was subject to change and it therefore varied from our suspected evaporation rates. For future experiments it might be desirable to reduce evaporation or utilise a method for more precise monitoring, while also adding more plants per reservoir. Our data does not give precise indications of how much nitrogen was taken up by the plants, as adsorption to surfaces an uptake by bacteria cannot be excluded. Analysing the plants nitrogen concentration could have aided in acquiring that information but was not possible due to lack of time and unavailability of the laboratory facilities. However, the removal suggests suitability for use as complementary or sole removal tool in RAS. NH_3 is the preferred form for uptake of nitrogen in plants, so in a RAS, placing the hydroponic unit before the nitrification filter, unlike our simulated system, might be preferable. This might also explain why the fertilised system displayed higher growth rates, as the NH_3 concentration was higher than in the aquaponic unit. Effluent water from E&F systems like the one in this experimental setup has an increased aeration level than the influent and it might therefore more efficient to apply E&F´s before biofiltration or degassing steps.

Ortho-PO_4 levels decreased in both treatments and phases, but we were not able to identify the amount of ortho-PO_4 taken up by the plants as the reduction was generally much higher than what the plants could accumulate. The expected accumulation of phosphorus for kale in the harvested biomass from the fertilised system in phase 1 according to (U.S. Department of Agriculture, 2019) resembled only ~8% of the reduction in phosphorus in the form of ortho-PO_4. In the aquaponic treatment, this was not as pronounced, but the removal was also higher than expected. Although we expected that the bacterial biomass in both biofilter and growbed media would also marginally extract and accumulate phosphorus within the system, the removal was too high just to be explained by that. Most likely, the available ortho-PO_4 was primarily bound by cations and therefore made unavailable for the plants (Cerozi & Fitzsimmons, 2016). The fluctuation in pH affects the binding affinity of these cations and could therefore be a possible explanation to the fluctuating ortho-PO_4 levels, as available ortho-PO_4 such as $H_2PO_4^-$ and HPO_4^{2-} decreases with decreasing pH-values (Cerozi & Fitzsimmons, 2016). The sludge that was present in the aquaponic treatment might have had an influence on the availability of PO_2 too, as sludge from RAS contains organic phosphorus, which might become mineralised through microbial activity. Generally, ortho-PO_4 levels were sufficient to not represent a limiting factor to the plant's growth, but it is also not possible to draw conclusions about the plants effect on ortho-PO_4 level reduction and water quality improvements.

Including a control for the fertilised- and aquaponic system, utilising the same system design without planting kale within the growbeds could give us further insights. The effects of ortho-PO_4 on fish health and growth are not very well researched for many species, however recent research suggests that it might be of low concern for some species like *Psetta maxima*, even at concentrations as high as 75 mg/l (van Bussel et al., 2013). However, phosphorus accumulating in sludge and potentially contaminating the environment presents risks through eutrophication. Unlike nitrogenous fertilisers, which can be easily synthesised from atmospheric (N_2), using the Haber-Bosch method, phosphorus is a limited, mined resource, which has been subject to rapid price fluctuation. Between 2020 and 2023, the average market price increased by 400% (Brownlie et al., 2023). This instability underlines the importance and need for a more sustainable and circular use of phosphorus through methods like aquaponics or biochar, which is a method to adsorb nutrients to a substrate which can then be employed as fertiliser in agricultural fields (Strawn et al., 2023).

4.3 Conclusion

In conclusion, our study sheds light on the intricate dynamics of growth and water parameters within aquaponic systems. Despite initial setbacks in phase 1, characterized by poor growth likely due to micronutrient deficiencies, our experiment revealed the critical role of supplementing essential minerals for optimal plant development. The incorrect methodology, regarding fertilizer application in phase 2 hindered our ability to compare growth accurately, but subsequent adjustments indicated an immediate positive impact on plant health, underscoring the importance of meticulous system management. The behaviour of nitrogenous compounds exhibited expected patterns, with nitrification processes supporting water quality in both systems. However, challenges such as pH fluctuations and bacterial adaptation underscore the need for careful system monitoring and management. Despite uncertainties in our data regarding nitrogen removal quantification, our results suggest the viability of aquaponics as a primary or complementary nitrogen removal tool in recirculating aquaculture systems. Moreover, our examination of water parameters highlighted the complexities of nutrient cycling, regarding ortho-PO_4^- dynamics. While ortho-PO_4^- levels remained non-limiting for plant growth, the disproportionate reduction observed suggests the potential for further investigation into nutrient uptake mechanisms. Additionally, our findings suggest potential for more efficient ortho-PO_4^- use through aquaponics, considering its limited availability and fluctuating market value. Overall, our study underscores the importance of fine-tuning system parameters, addressing nutrient deficiencies, and implementing sustainable practices to optimize growth and water quality in aquaponic systems. Further research into nutrient dynamics, plant species selection, and system design will advance our understanding and facilitate the widespread adoption of aquaponics as a viable and sustainable agricultural practice.

5. Acknowledgements

I would like to thank my supervisor Jonathan Roques for making this project possible, helping me to plan around all the complications that occurred and introducing me to other projects, helping me to expand my knowledge and academic network. I would also like to thank the Fish-Endocrinology-Lab group at Gothenburg University for being such an inclusive, friendly and helpful group of people. I extend my gratitude to my parents for their ongoing support throughout my academic journey and my whole life. Their encouragement has been a constant source of motivation. I would like to thank my friends, both old and new, at Gothenburg University for their companionship, inspiration and help.

6. References

Ahmed, N., & Turchini, G. M. (2021). Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. *Journal of Cleaner Production*, *297*, 126604. https://doi.org/https://doi.org/10.1016/j.jclepro.2021.126604

Ampim, P. A. Y., Obeng, E., & Olvera-Gonzalez, E. (2022). Indoor Vegetable Production: An Alternative Approach to Increasing Cultivation. *Plants*, *11*(21), 2843. https://doi.org/10.3390/plants11212843

Bernhard, A. (2010). The Nitrogen Cycle: Processes, Players, and Human Impact. *Nature Education Knowledge*, *3*(10), 25–25.

Brownlie, W. J., Sutton, M. A., Cordell, D., Reay, D. S., Heal, K. V., Withers, P. J. A., Vanderbeck, I., & Spears, B. M. (2023). Phosphorus price spikes: A wake-up call for phosphorus resilience. *Frontiers in Sustainable Food Systems*, *7*. https://doi.org/10.3389/fsufs.2023.1088776

Camargo, J. A., Alonso, A., & Salamanca, A. (2005). Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere*, *58*(9), 1255–1267. https://doi.org/10.1016/j.chemosphere.2004.10.044

Cerozi, B. da S., & Fitzsimmons, K. (2016). The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. *Bioresource Technology*, *219*, 778–781. https://doi.org/10.1016/j.biortech.2016.08.079

Chakwizira, E., Richard, G., Maley, S., George, M., & Michel, A. (2013). *Water and nitrogen use efficiency of forage kale crops*. *43*, 1–16.

Dauda, A. B., Natrah, I., Karim, M., Kamarudin, M. S., & Bichi, A. u H. (2018). African Catfish Aquaculture in Malaysia and Nigeria: Status, Trends and Prospects. *Fisheries and Aquaculture Journal*, *09*(01). https://doi.org/10.4172/2150-3508.1000237

Davidson, J., Good, C., Williams, C., & Summerfelt, S. T. (2017). Evaluating the chronic effects of nitrate on the health and performance of post-smolt Atlantic salmon Salmo salar in freshwater recirculation aquaculture systems. *Aquacultural Engineering*, *79*, 1–8. https://doi.org/https://doi.org/10.1016/j.aquaeng.2017.08.003

FAO. (2016). *Cultured Aquatic Species Information Programe Clarias gariepinus*.

Fösel, B. (2007). *Mikrobiologie der Stickstoffentfernung in den Biofiltern einer marinen Aquakultur mit geschlossenem Wasserkreislauf*. University Bayreuth.

Goddek, S., Joyce, A., Kotzen, B., & Burnell, G. M. (2019). *Aquaponics Food Production Systems* (S. Goddek, A. Joyce, B. Kotzen, & G. M. Burnell, Eds.). Springer International Publishing. https://doi.org/10.1007/978-3-030-15943-6

Holan, A. B., Good, C., & Powell, M. D. (2020). Health management in recirculating aquaculture systems (RAS). In *Aquaculture Health Management* (pp. 281–318). Elsevier. https://doi.org/10.1016/B978-0-12-813359-0.00009-9

Hrubec, T., Smith, S., & Robertson, J. (1996). Nitrate Toxicity: A Potential Problem of Recirculating Systems. *Successes and Failures in Commercial Recirculating Aquaculture*, *1*.

Jayasekara, C., Mendis, E., & Kim, S. (2020). Seafood in the Human Diet for Better Nutrition and Health. In *Encyclopedia of Marine Biotechnology* (pp. 2939–2959). Wiley. https://doi.org/10.1002/9781119143802.ch131

Kasozi, N., Tandlich, R., Fick, M., Kaiser, H., & Wilhelmi, B. (2019). Iron supplementation and management in aquaponic systems: A review. *Aquaculture Reports*, *15*, 100221. https://doi.org/10.1016/j.aqrep.2019.100221

Kosegarten, H., Wilson, G. H., & Esch, A. (1998). The effect of nitrate nutrition on iron chlorosis and leaf growth in sunflower (Helianthus annuus L.). *European Journal of Agronomy*, *8*(3–4), 283–292. https://doi.org/10.1016/S1161-0301(98)00021-5

Prabu, E., Rajagopalsamy, C. B. T., Ahilan, B., Jeevagan, I. J. M. A., & Renuhadevi, M. (2019). Tilapia – An Excellent Candidate Species for World Aquaculture: A Review. *Annual Research & Review in Biology*, 1–14. https://doi.org/10.9734/arrb/2019/v31i330052

Roques, J. A. C. (2013). *Aspects of fish welfare in aquaculture practices*.

Satheesh, N., & Workneh Fanta, S. (2020). Kale: Review on nutritional composition, bio-active compounds, anti-nutritional factors, health beneficial properties and value-added products. *Cogent Food & Agriculture*, *6*(1), 1811048. https://doi.org/10.1080/23311932.2020.1811048

Strawn, D. G., Crump, A. R., Peak, D., Garcia-Perez, M., & Möller, G. (2023). Reactivity of Fe-amended biochar for phosphorus removal and recycling from wastewater. *PLOS Water*, *2*(4), e0000092. https://doi.org/10.1371/journal.pwat.0000092

Tlusty, M., Tyedmers, P., Ziegler, F., Jonell, M., Henriksson, P., Newton, R., Little, D., Fry, J., Love, D., & Cao, L. (2018). Commentary: Comparing efficiency in aquatic and terrestrial animal production systems. *Environmental Research Letters*, *13*. https://doi.org/10.1088/1748-9326/aae945

Tyson, R. V., Simonne, E. H., Treadwell, D. D., White, J. M., & Simonne, A. (2008). Reconciling pH for Ammonia Biofiltration and Cucumber Yield in a Recirculating Aquaponic System with Perlite Biofilters. *HortScience*, *43*(3), 719–724. https://doi.org/10.21273/HORTSCI.43.3.719

United Nations Department of Economic and Social Affairs. (2019). *World Population Prospects 2019*.

U.S. Department of Agriculture. (2019, January 4). *FoodData Central, KALE, RAW*.

van Bussel, C. G. J., Mahlmann, L., Kroeckel, S., Schroeder, J. P., & Schulz, C. (2013). The effect of high ortho-phosphate water levels on growth, feed intake, nutrient utilization and health status of juvenile turbot (Psetta maxima) reared in intensive recirculating aquaculture systems (RAS). *Aquacultural Engineering*, *57*, 63–70. https://doi.org/10.1016/j.aquaeng.2013.08.003

Wortman, S. E. (2015). Crop physiological response to nutrient solution electrical conductivity and pH in an ebb-and-flow hydroponic system. *Scientia Horticulturae*, *194*, 34–42. https://doi.org/10.1016/j.scienta.2015.07.045

Appendix 1: Popular science summary

Aquaponics: A key for aquaculture with a smaller environmental impact?

The "price" of seafood production

Seafood consumption and production have been increasing in recent times. As wild fisheries have already exceeded their maximum capacity, the aquaculture industry has to supply the demand. Generally, aquaculture is more efficient than the farming of terrestrial animals, specifically in feed conversion. To produce a kg of pork, 1.8-10 kg of feed are required, whereas Atlantic salmon converts only 1.1 kg of feed into 1 kg of body mass. As with animal farming, waste products need to be disposed of and can leach into the environment. In fish-farms, specifically open cages at sea and lakes, this waste is directly transferred into the environment, increasing the concentration of nutrients like phosphorus and nitrate. Extreme algal blooms are caused by elevated concentrations of these nutrients and can lead to oxygen deprived zones and death of animals in these so-called dead zones, presenting an ever-increasing problem in coastal areas and especially in the Baltic Sea.

What are Aquaponics?

The recent development in aquaculture shows a shift towards the use of recirculating aquaculture systems (RAS) that are mostly situated on land and mostly isolated from the environment. In these systems, the water is recirculated and has to be treated to remove the nutrients, as these will accumulate and harm the animals. Parts of the water will still be exchanged, which transfers the nutrients to other facilities like sewage treatment plants. Another alternative to reduce the nutrient concentration is to use them as plant fertiliser. Phosphorus and Nitrate are important nutrients for plants and often have to be supplemented to ensure proper growth. Introducing plants into a RAS increases water quality while enabling farmers to grow a second marketable crop. Common crops are mostly leafy greens like lettuce and microgreens like cress, but also plants like strawberries and tomatoes. Aquaculture systems coupled together with the growth of plants are called aquaponics.

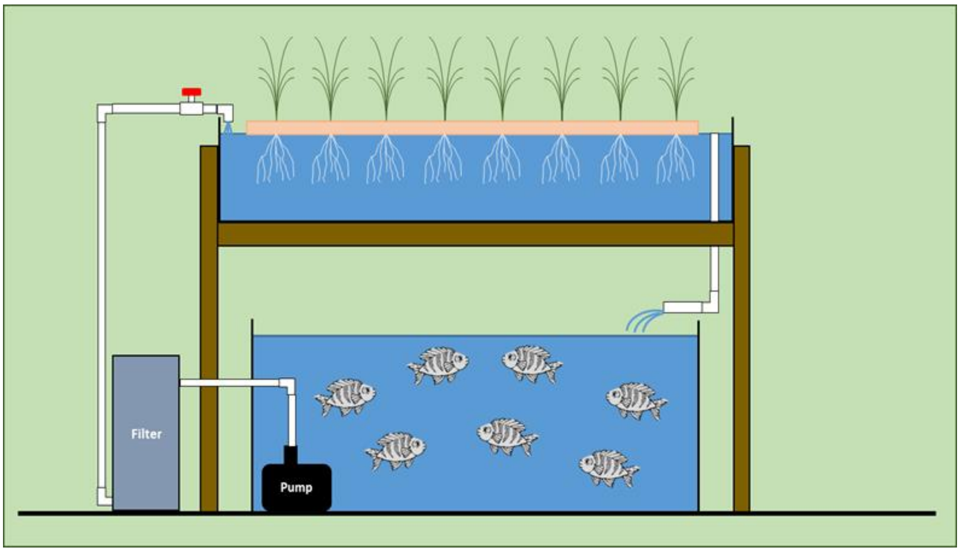
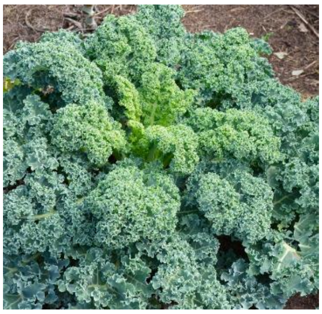


Figure 20: Aquaponic system (Lance Beecher(2021)

Our research

African catfish stands out as a promising species within aquaculture, contributing to the success of aquaponic systems. This species offers adaptability to various aquaculture setups, robust growth rates, and efficient feed conversion, reducing production costs and environmental impact. In our recent study, we investigated the potential of aquaponics by growing green kale, a leafy vegetable known for its nutritional benefits. Kale is rich in vitamins A, C, and K, as well as minerals like calcium and manganese. It is also high in antioxidants, fibre, and protein, making it a valuable addition to the diet and well marketable product.



from left to right: African catfish (FAO,2016), green kale(ndr.de, 2023)

We compared the growth of the kale plants and nutrient levels of the water in aquaponic systems utilizing wastewater from an African catfish farm as nutrients with those grown using conventional fertilizer. The results showed that while the daily growth rate of plants in the aquaponic system was lower, they still effectively absorbed nitrogen from the fish waste. Removing excess nitrogen from the system is advantageous as it helps prevent water pollution and maintains a balanced nutrient environment, contributing to the overall health and sustainability of the aquaponic ecosystem. However, we noted challenges such as suboptimal pH levels and deficiencies in trace elements like iron, which affected plant growth and pigmentation. By adjusting pH levels and supplementing with trace elements, we were able to improve plant growth and health.

Our study highlights the importance of further research into sustainable methods for maintaining optimal conditions in aquaponic systems. By addressing these challenges, aquaponic systems have the potential to not only support meeting the growing demand for seafood but also produce valuable secondary products in a more environmentally friendly and sustainable way, leveraging the benefits of species like African catfish and nutrient-rich crops like kale.