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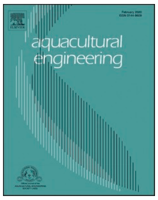

Nitrogen transformations across compartments of an aquaponic system

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ABSTRACT

The presence and transformations of nitrogen (N) in the environment depend on a variety of environmental factors but are also strongly influenced by anthropogenic activities such as modern agriculture. Understanding N transformations within the context of agricultural systems is crucial for efficient use thereof. The aim of this study was to investigate the changes in concentration of N forms (ammonium, nitrite, nitrate and organic N) within an aquaponic system, a modern agricultural system, in order to obtain insights into environmental pressures influencing N transformation processes. By measuring the concentrations of the individual N compounds, complemented by the determination of abiotic parameters and other relevant nutrients within the system water at 13 sampling points, significant differences between compartments that build up an aquaponic system could be demonstrated. These differences were attributed to individual microenvironments specific to the aerobic loop, anaerobic loop and radial flow settler as a connection between the two, shaping the microbial processes within the aquaponic system.

1. Introduction

Nitrogen (N) is an element occurring in all organisms, including humans. Being one of the most common elements on earth, nitrogen is continuously moved around the biosphere in what we know as the nitrogen cycle. The nitrogen cycle is strongly influenced by anthropogenic activities and is dependent on a variety of environmental factors (Widdison and Burt, 2008). For instance, modern agriculture systems are highly inefficient in their use of N, with between 50 and 70 % of applied N lost to the environment, instead of being converted into plant biomass. This can result in environmental toxicity and affects climate change (Coskun et al., 2017; Erisman et al., 2011; Fowler et al., 2013; Galloway et al., 2008; Schlesinger, 2009). Understanding N transformations and the microbial communities involved therein, as well as understanding the potential environmental impact of food production technologies that use N, is therefore crucial (Robertson and Groffman, 2007). While N transformations in soil and natural aquatic systems are often studied, there is still a noticeable lack of research regarding the N transformations in aquaponics, a newer food production technology combining recirculating aquaculture and hydroponic culture (Wongkiew et al., 2017).

In aquatic food production systems, four of nine N forms (Robertson and Groffman, 2007), organic N (N_{org}), ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-), require monitoring to avoid them reaching toxic concentrations for the organisms in the system (Dodds and Whiles, 2010; Timmons and Ebeling, 2010). Where an excess of N waste is present, its removal is necessary. This is particularly important for the more toxic forms NH_4^+ and NO_2^- and less critical for NO_3^- due to its lower relative toxicity (Timmons and Ebeling, 2010).

In aquaponic systems specifically, N is required to fulfill the nutritional requirements of fish and crops. The primary input of N into an aquaponic system is via proteins in fish feed as N_{org}. These are ingested, metabolized and transformed by the fish into ammonia (NH_3) and primarily released into the aqueous medium via passive gill diffusion (Randall and Wright, 1987). The remaining N_{org} present in the fish excreta, non-consumed feed and decaying biomass is mineralized to

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NH_4^+ (Cai et al., 2017). Some of these inorganic N (N_{inorg}) forms can be further transformed to NO_2^- and NO_3^- via nitrification, to nitrogen gas via denitrification and/or anammox, or assimilated into biomass by microbes and plants (Kułek, 2015; Robertson and Groffman, 2007; Widdison and Burt, 2008).

In aquaponic systems, N transformations mainly depend on the presence or absence of oxygen and organic carbon, which creates the correct environmental conditions for particular groups of microbes (Schmautz et al., 2020). The different compartments constituting the aquaponic systems are designed to steer the environmental conditions to achieve the desired microbial activity in order to ensure that concentrations of N_{org}, NH_4^+, NO_2^- and NO_3^- are kept below their tolerance range, in turn ensuring fish and plant welfare.

The aim of this study was to compare N concentrations in the different compartments of the aquaponic systems in order to obtain insights into the environmental conditions which could influence the N transformation processes in these systems and to what extent. By measuring the concentrations of the individual N compounds, complemented by the determination of other relevant nutrients in the system water, conclusions concerning the biochemical performance of the system can be drawn, and enable the metabolic processes in the aquaponic system to be steered in the right direction.

2. Materials and methods

Experiments were carried out at the Zurich University of Applied Sciences (Wädenswil, Switzerland) in the foliar greenhouse between June and September 2017. In this period, three parallel replicates of a 4.3 m^3 aquaponic system (Fig. 1) were freshly stocked with 20 ± 0.2 kg per system Nile tilapia (*Oreochromis niloticus*, stocked) and 63 lettuce plants (*Lactuca sativa* cultivar "YACHT" Salanova®) for three lettuce cycles each (Table S1). Experiments were conducted under the authorization of the Veterinary Office of the Canton of Zurich, no. ZH020/17.

2.1. Operation of the aquaponic system

Fish were fed ten times per day with a vegetarian feed, Tilapia Vegi, 3.0 mm and 4.5 mm containing 6% of N (Hokovit, Hofmann Nutrition AG, Bützberg, Switzerland), amounting to 2.5 % of their body weight as calculated at the beginning of each lettuce cycle. To maintain constant fish biomass during all three lettuce cycles, fish were weighed at the beginning and the end of each cycle and the biomass gained was removed (Table S2). To assure fish safety and prevent the accumulation of NH_4^+ and NO_2^-, two months prior to stocking the system with fish the biofilter was started using a biofilter starter (PURE + filter start gel, Evolution Aqua Ltd, Wigan, United Kingdom), ammonium dihydrogen phosphate as an NH_4^+ source and addition of fish feed as a carbon source.

Prior to the experiment, the lettuce was planted in rockwool cubes and irrigated only with tap water (until both cotyledons of the seedlings had completely opened) and thereafter with fertilizer solution (1800 μS cm^{-1}, Wuxal®, Maag, Dielsdorf, Switzerland). Once the roots were long enough (ca. 5 cm), the plants were transplanted to the aquaponic system into Styrofoam floating rafts (Dryhydroponics BV,' s-Gravenhage, The Netherlands). For the additional protection of the plants, beneficial organisms (*Ichneumonidae* as VerdaProtect and BasilProtect, *Phytoseiulus persimilis* as Phytoseiulus-SD-System and *Amblyseius cucumeris* obtained from Andermatt Biocontrol AG, Grossdietwil, Switzerland), were used as prescribed by a supplier. In addition, Agree® WP and Neem oil (Andermatt Biocontrol AG) were applied in the phyllosphere when the beneficial organisms proved insufficient. No fertilizer was supplemented into the system water, however, Iron Optifer (Ökohum GmbH, Herrenhof, CH) and KlinoSpray (Unipoint AG, Ossingen, CH) were applied biweekly as foliar fertilization.

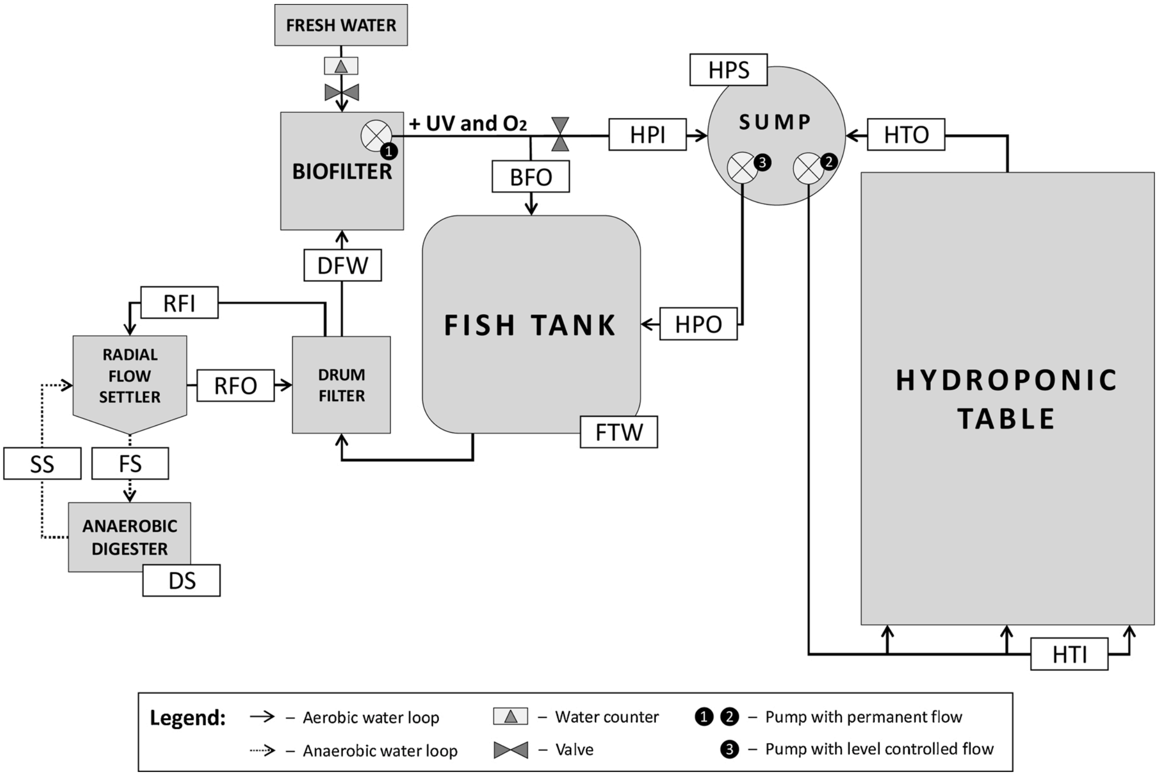

Fig. 1. Water flow with the sampling points (FTW, fish tank water; DFW, drum filter outflow water; BFO, biofilter outflow water; HPI, inflow into hydroponic part of the system; HPS, sump water; HTI, hydroponic table inflow; HTO, hydroponic table outflow; RFI, radial flow settler inflow; RFO, radial flow settler outflow; FS, settled fresh sludge; DS, digested sludge; SS, supernatant of digested sludge returned back to the system) in the aquaponic system as operated between 2017 and 2018.

2.2. Sampling procedure

Water samples for chemical analysis were taken six times during the experiment (Table S1) at 13 different locations within the system (Fig. 1) together with fresh tap water samples. Temperature, pH, electrical conductivity and dissolved oxygen were measured on the spot, while samples for nutrient analysis were stored in falcon tubes and placed into a polystyrene box containing cooling elements until the end of the sampling process and then stored at - 20 °C until further analysis. Parameters analyzed, sample preparation and analytical methods used in this study are described in Table 1.

2.3. Water flow in the aquaponic system and sampling points

Fish tank water (FTW) was sampled directly in the tank 20 cm under the water surface. Water from the fish tank flowed continuously through the bottom drainage to the solids removal unit, where fish feces and feed residues were mechanically separated from the system water by a drum filter with a 40 μm mesh. Using gravity, solids-free water (DFW) flowed to the biofilter. To maintain a constant water level and to control water consumption in the system, fresh tap water was added to the biofilter via a mechanically controlled water valve and analogous water counter. A circulation pump installed in the biofilter continuously (5 m^3 h^{-1}) pumped water through the UV treatment system (77 W radiation flux at 254 nm with a 35 % efficiency) and the oxygenation zone back to the fish tank, where the biofilter outflow water (BFO) was sampled before entering the fish tank. The computer-controlled valve, installed between the oxygenation zone and the fish tank, opened every 5 min for 2 min, resulting in a water flow rate of 0.5 m^3 h^{-1} into the sump (HPI). Water in the sump (HPS) was sampled 20 cm under the water surface. A separate pump continuously transferred the water to the hydroponic raft table (HTI) at a flow rate of 0.36 m^3 h^{-1} and, from there, back to the sump over the drainage point at the end of the hydroponic table (HTO). A level sensor-controlled pump then pumped the water back to the fish tank (HPO), maintaining a constant water level in the sump.

Backwash water from the drum filter was discharged to the solids thickening unit, i.e. radial flow settler (RFS), where the inflow water was sampled (RFI). The solids-free water from the RFS overflowed (RFO) back to the drum filter inlet pipe. About 7 L of settled, thickened sludge, i.e. fresh sludge (FS) in the RFS, was manually removed three times per week. The thickened sludge was added to the anaerobic digester (DS). At the same time, 7 L of the supernatant from the anaerobic digester (SS) was added back to the RFS, which returned water full of nutrients to the main water loop of the system. Thus, the aquaponic system consisted of an aerobic loop (FTW, DFW, BFO, HPI, HPS, HTI, HTO and HPO) and an anaerobic loop (FS, DS and SS), while the RFS (RFI and RFO) served as a connection between the two (Schmautz et al., 2020).

2.4. Data analyses

All statistical analyses and graphics were carried out with R statistical software version 3.6.1 (R Core Team, 2018) and packages 'agricolae' (de Mendiburu, 2019), 'devtools' (Wickham et al., 2019b), 'dplyr' (Wickham et al., 2019a), 'ggbiplot' (Vu, 2011), 'ggplot2' (Wickham, 2016), 'ggpubr' (Kassambara, 2019) and 'moments' (Komsta and Novomestky, 2015). To test for differences, a Kruskal-Wallis test was performed, followed by a Wilcoxon rank-sum test, with a significance level of $\alpha = 5\%$. Principal component analysis (PCA) was used to test the influence of different parameters between compartments.

3. Results and discussion

During the 12-week experimentation and analysis period, total nitrogen (TN) was primarily present in the form of NO_3 (85 %) and averaged at 64.5 mg L^{-1} in the fish tank water (Table 2, Table S3). Nitrate concentrations slowly increased over time, from 36 mg L^{-1} in week 27–74 mg L^{-1} in week 39 (Fig. S1), suggesting NO_3- accumulation and imbalance between plant requirements and N generation (Wongkiew et al., 2017).

As discussed before (Shin et al., 2004), in aquatic plant-based treatment systems, the removal efficiency of different nutrients is related to various physical, chemical, and biological interactions. Accumulation of N could be the result of lower N plant uptake, due to plants being limited by other nutrients (iron, manganese, copper, molybdenum and zinc) which were not introduced in sufficient amounts by the fish feed (Table S3). With the addition of micronutrients to the system water, this ratio could be changed since these are usually the most limiting nutrients in the aquaponic system (Delaide et al., 2017). While commonly limiting, P and K (Bittsánszky et al., 2016) were present in sufficient concentrations during the experiment (Table S3).

3.1. Nitrogen transformations between compartments of the aquaponic system

INPUT As the most prominent input of N to the aquaponic system, fish feed provided essential nutrients for the growth and development of the fish, including 30 g N day^{-1}. Alongside feed, small amounts of N (0.05 g N day^{-1}) were added via fresh tap water used to compensate for evapotranspiration.

FISH Freshwater fish excrete NH_3 via their gills, urine and feces, which is in equilibrium with NH_4^+, depending on the ambient pH, temperature and salinity (Timmons and Ebeling, 2010). During the experiment, pH in the fish tank was ≈ 7.3 and temperature was ≈ 26.0 °C. Thus, more than 98.5 % of N was present in the NH_4^+ form (Emerson et al., 1975). Along with the total ammonia nitrogen (TAN), the sum of NH_3 and NH_4^+, fish also excrete between 6–15% of N as N_{org} via feces (Timmons and

Table 1

Measured parameters (pH, temperature, electrical conductivity, dissolved oxygen, dissolved nutrients and organic nitrogen), sample preparation and further analysis.

Parameter	Where?	Sample preparation	Lab equipment	Company
pH [-], **T** [°C]	Direct, on the sampling spot	–	Probe PHC10103 & HQ40d portable multimeter	Hach Lange, Loveland, CO, USA
El. conductivity [μS cm^{-1}]	Direct, on the sampling spot		Probe CDC40103 & HQ40d portable multimeter	Hach Lange, Loveland, CO, USA
Dissolved oxygen [mg L^{-1}]	Direct, on the sampling spot	–	Probe LDO10101 & HQ40d portable multimeter	Hach Lange, Loveland, CO, USA
Na, NH_4^+, K^+ Ca^{2+}, Mg^{2+} [mg L^{-1}]	Stored at -20 °C in 15 mL falcon tube, laboratory	Filtered, 0.22 μm, 1 μL 2 M HNO_3 per 1 mL sample	930 Compact IC flex	Metrohm Schweiz AG, Zofingen, CH
Cl^-, NO_2^-, NO_3^-, PO_4^{2-}, SO_4^{2-} [mg L^{-1}]	Stored in 15 mL falcon tube, laboratory	Filtered, 0.22 μm	930 Compact IC flex	Metrohm Schweiz AG, Zofingen, CH
B, **Mo**, **Cu**, **Fe**, **Mn**, **Zn** [mg L^{-1}]	Stored at -20 °C in 15 mL falcon tube, laboratory	Filtered, 0.22 μm	ICP-AES, Varian Vista AX CCD Simultaneous	Agilent Technologies, Santa Clara, CA, USA
N_{org}[a] [mg L^{-1}]	Stored at -20 °C in 50 mL falcon tube, laboratory	–	KjelMaster K-375, SpeedDigester K-439, Scrubber K-415	BÜCHI Labortechnik AG, Flawil CH

[a] Calculated by subtracting NH_4^+-N from the measured total Kjeldahl nitrogen.

Table 2

Mean ± SEM of different nitrogen forms presented as a percentage of total nitrogen in the water samples from different compartments of an aquaponic system (FTW, fish tank water; DFW, drum filter outflow water; BFO, biofilter outflow water; HPI, inflow into hydroponic part of the system; HPS, sump water; HTI, hydroponic table inflow; HTO, hydroponic table outflow; HPO, outflow from hydroponic part of the system; RFI, radial flow settler inflow; RFO, radial flow settler outflow; FS, settled fresh sludge; DS, digested sludge; SS, supernatant of digested sludge returned back to the system) of an aquaponic system, measured six times during the experiment. Letters represent the significant differences between the compartments of the system based on a Kruskal-Wallis test followed by a Wilcoxon rank-sum test (α = 5%, n > 14).

	System water (aerobic loop)								Radial flow settler (loop connection)		Sludge (anaerobic loop)		
	FTW	DFW	BFO	HPI	HPS	HTI	HTO	HPO	RFI	RFO	FS	DS	SS
NH_4^+-N [%]	0.21± 0.02 a	0.27± 0.05 a	0.20± 0.02 ab	0.19± 0.02 ab	0.14± 0.01 b	0.16± 0.01 ab	0.13± 0.01b	0.17± 0.02 ab	4.07± 1.23 c	7.07± 2.11 c	3.43± 0.80 c	41.65± 1.54 d	51.46± 2.77 d
NO_2^--N [%]	0.18± 0.02 a	0.20± 0.01 a	0.22± 0.02 ab	0.25± 0.02 ab	0.18± 0.02 a	0.19± 0.01 a	0.17± 0.01 a	0.17± 0.02 a	1.17± 0.19 bc	1.80± 0.27 c	0.03± 0.01 d	0.02± 0.01 d	0.01± 0.00 d
NO_3^--N [%]	85.04 ± 1.44 bc	87.23 ± 1.27 abc	88.67 ± 1.15 abc	92.47 ± 0.69 a	89.95 ± 0.87 ab	87.58 ± 0.45 bc	83.79 ± 1.04 c	86.87 ± 1.34 bc	61.70 ± 3.40 d	64.96 ± 3.16 d	0.15± 0.10 e	0.07± 0.02 e	0.09± 0.02 e
N_{org} [%]	14.58 ± 1.45 bcf	12.29 ± 1.29 abc	10.85 ± 1.17 abc	7.09 ± 0.68 a	9.73 ± 0.86 ab	12.07 ± 0.45 bc	15.91 ± 1.05 c	12.79 ± 1.35 bc	33.07 ± 3.75 de	26.17 ± 2.87 df	96.39± 0.80 g	58.26 ± 1.54 h	48.44 ± 2.76 eh
Total N [mg L^{-1}]	70.96 ± 2.10 a	70.61 ± 2.76 a	66.49 ± 3.28 a	60.71 ± 1.76 a	67.12 ± 2.13 a	66.54 ± 2.11 a	72.00 ± 2.47 a	66.14 ± 1.35 a	80.93 ± 5.49 a	62.97 ± 6.17 a	528.33 ± 68.08 b	427.10 ± 36.31 b	327.38 ± 27.16 b

Ebeling, 2010), depending on the N content of the fish feed and the metabolism of the specific fish species (Lupatsch and Kissil, 1998; Schneider et al., 2004; Wongkiew et al., 2017).

FISH TANK Higher percentages of NH_4^+ were detected in the aquaculture compartments (FTW and DFW) when compared to the hydroponic compartments (HPS, HTI and HTO) in the experimental aquaponic system (Fig. 2, Table 2, Table S3) due to excretion of NH_3 by the fish (Timmons and Ebeling, 2010). Besides NH_4^+, there was also an

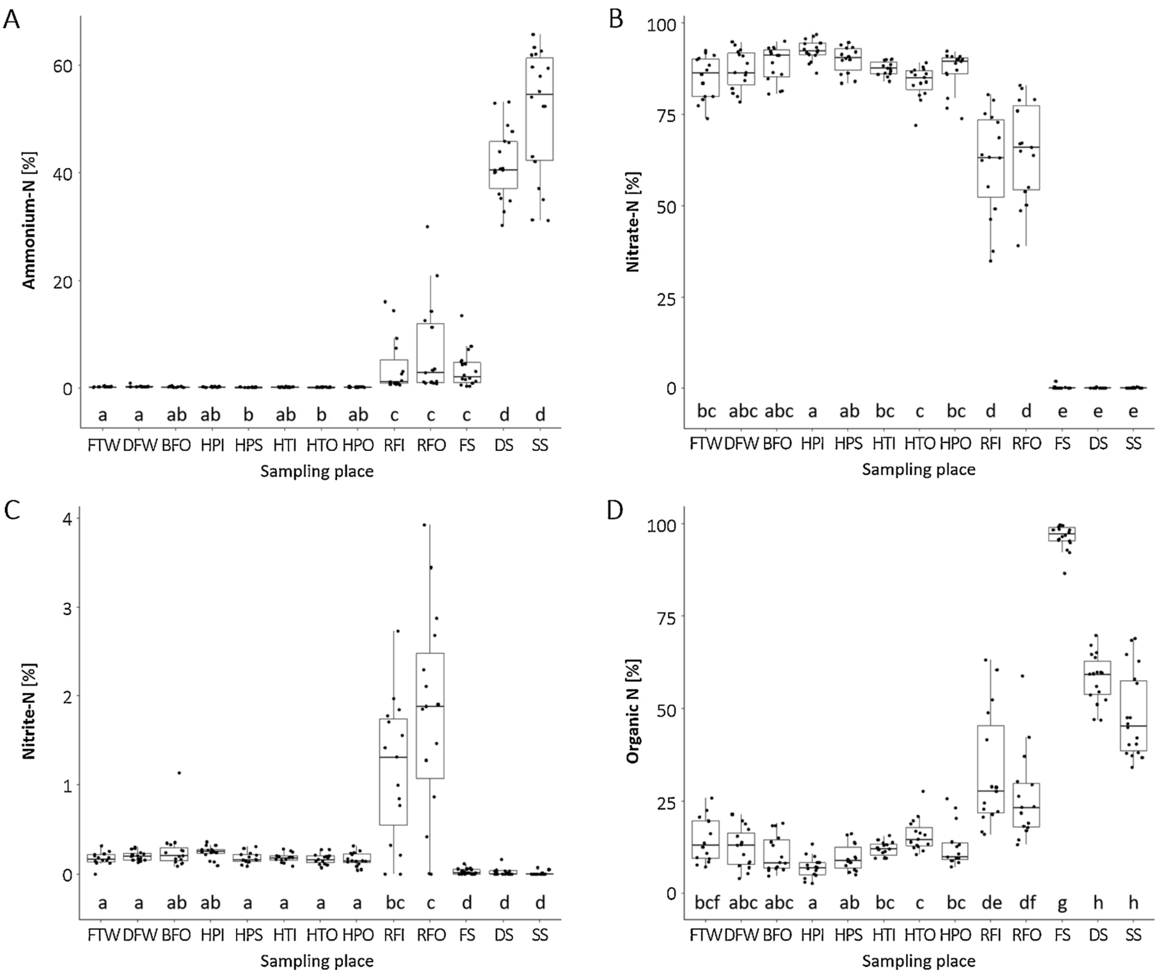

Fig. 2. Different nitrogen forms in different compartments (FTW, fish tank water; DFW, drum filter outflow water; BFO, biofilter outflow water; HPI, inflow into hydroponic part of the system; HPS, sump water; HTI, hydroponic table inflow; HTO, hydroponic table outflow; HPO, outflow from hydroponic part of the system; RFI, radial flow settler inflow; RFO, radial flow settler outflow; FS, settled fresh sludge; DS, digested sludge; SS, supernatant of digested sludge returned back to the system) of the aquaponic system, measured six times during the experiment. Letters represent the significant differences between the compartments of the system based on a Kruskal-Wallis test followed by a Wilcoxon rank-sum test (α = 5%, n > 14).

increase of N_{org} in the fish tank due to the presence of N_{org} in the fish feces (Lupatsch and Kissil, 1998; Schneider et al., 2004; Timmons and Ebeling, 2010; Wongkiew et al., 2017).

DRUM FILTER The solids removal unit, i.e. the drum filter, provided a continuous removal of the N-rich waste (Dolan et al., 2013). This can be observed by a slight decrease of N_{org} from FTW via DFW to BFO. High concentrations of organic particles can compromise gill function and provide a habitat that enables the proliferation of pathogens, but can also influence the efficiency of all other water treatment systems, increase the biological oxygen demand, mineralization and TAN production, and provide a substrate for the growth of heterotrophic microorganisms in the biofilter that displaces the nitrifying bacteria (Dolan et al., 2013; Johnson and Chen, 2006; Summerfelt and Penne, 2005).

BIOFILTER As the primary function of the biofilter, nitrification is responsible for the transformation of TAN to NO_3^-. In the presence of oxygen, NH_3 is oxidized by ammonia-oxidizing bacteria and ammonia-oxidizing archaea, followed by the oxidation of the resulting NO_2^- to NO_3^- by nitrite-oxidizing bacteria (Kowalchuk and Stephen, 2001). Additionally to the two-step nitrification process involving different microorganisms, members of the genus *Nitrospira* are able to perform complete nitrification from NH_4^+ to NO_3^- (Daims et al., 2015). During the experiment, sufficient oxygen levels (≈ 9.8 mg L^{-1}), water temperature (≈ 26 °C) and low organic carbon concentrations facilitated by the mechanical filter (Schmautz et al., 2020) allowed effective nitrification in the biofilter.

UV TREATMENT Along with solids removal, UV treatment also plays a vital role in the system by causing microbial inactivation. Thus, UV treatment decreases the likelihood of viral, bacterial and fungal diseases and acts as a form of microbial control (Kasai et al., 2002; Timmons and Ebeling, 2010). While UV treatment has an indirect effect on the N transformations by damaging or killing organisms involved in N-cycling, no literature was found that UV light can directly influence N transformations.

HYDROPONIC SYSTEM In the aquaponic system, the sump served as a connection between aquaculture and hydroponic sub-systems, constantly mixing water from both parts. Comparing the results, an increase of N_{org} from HPS to HTO was observed due to small particles in the water potentially originating from dead plant material and biofilm remnants. The surface area of the hydroponic table can provide suitable conditions for the attachment of microbial community and microbial processes such as nitrification and denitrification (Schmautz et al., 2020). These processes can be influenced by the release of organic compounds and oxygen from plant roots (Landi et al., 2006; Wu et al., 2016; Yin et al., 2013). Alongside the microbial N transformations, plants play an important role by performing NH_4^+ and NO_3^- uptake from the system water. Previous studies have demonstrated that the NH_4^+ to NO_3- ratio can affect the rate of plant growth and biomass allocation. Most species can grow better and accumulate more N when grown in a mixture of NH_4^+ and NO_3- (Ali et al., 2001; Guo et al., 2002; Wu et al., 2016). Results of this study showed a slight decrease of NH_4^+ concentration, while NO_3- and N_{org} concentrations slightly increased between HTI and HTO, however, the differences were not significant. It was also reported that NH_4^+ application together with NO_3- is effective in reducing NO_3- accumulation in leafy vegetables (Gunes et al., 1994; Ikeda and Tan, 1998; Zhu et al., 1997). High NO_3- concentrations in edible plant parts constitute a potential threat for human health, and therefore many countries have set maximum permissible values through legislation (Savvas et al., 2006). Regulation (EC) No 1881/2006 states, that the lettuce cultivated in greenhouses and harvested between April and September should not exceed 4 g NO_3 kg $^{-1}$ (Commission Regulation (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs, 2010). Using a similar aquaponic system, measured concentrations were found to be below this threshold value (Nozzi et al., 2018).

RADIAL FLOW SETTLER Rinsed waste from the drum filter requires further thickening to remove the excess of liquid still present. Sedimentation is one of the most suitable methods to accomplish this (Cripps and Bergheim, 2000). It has been estimated that 97 % of solids could be captured in the settling unit if re-suspension is not a problem (Henderson and Bromage, 1988; Johnson and Chen, 2006) and a clear supernatant returned via the drum filter to the aerobic loop of the system. Sampling showed high variation between the samples taken in the RFS (Fig. 2), as a result of the fluctuation in N concentrations in the RFS depending on the time of previous drum filter rinsing and the amount of removed waste at that time. There was a significant increase of NH_4^+ at both RFS sampling points compared to other aerobic loop compartments. The percentage of NH_4^+ increased from RFI to RFO due to the reduction in N_{org} concentration, suggesting sedimentation of N_{org} rich particles and degradation of organic material (Table 2, Table S3). The presence of a higher percentage of NO_2^- suggests an incomplete transformation of N_{inorg}, either via nitrification or denitrification pathways. There was a decrease of TN between RFI and RFO, suggesting denitrification and the removal of the larger particles via sedimentation. Denitrification can account for up to 60 % of N losses due to anoxic conditions in sedimentation tanks, where high amounts of suspended solids accumulate (Hu et al., 2015).

FRESH SLUDGE Approximately 5% of the total daily TN input was discharged via the RFS drainage into the anaerobic digester. Of this RFS drainage discharge, more than 95 % of the TN was present in the N_{org} form. Total N was significantly higher in the FS compared to the aerobic loop of the system. At the same time, the percentage of N_{inorg} was significantly lower compared to any other compartment.

ANAEROBIC DIGESTER Mesophilic (25–45 °C) anaerobic digestion was used to break down the organic matter originating from the fish waste and uneaten feed into bioavailable nutrients for subsequent use as plant nutrition (Delaide et al., 2018; Goddek et al., 2018; Marchaim, 1992; Monsees et al., 2017). In the anaerobic digestion process, carried out by facultative and obligate anaerobic microorganisms, organic sludge underwent changes in its chemical, biological and physical properties during the various processes such as fermentation, methanogenesis and denitrification (Appels et al., 2008; Mirzoyan et al., 2010; Mshandete et al., 2005; Schmautz et al., 2020). Denitrification in oxygen-depleted zones may account for as much as 21 % of the N loss (van Rijn, 2013; van Rijn et al., 2006). Results of this study showed a slight decrease of TN from the FS to the DS. The loss of N could be explained by the processes described above. The percentage of N_{org} decreased compared to the sludge originating from RFS compartments, suggesting the degradation of N_{org} into N_{inorg} forms. Compared to the aerobic loop, the anaerobic loop samples had the highest percentage of NH_4^+ (176.1 mg NH_4^+-N L^{-1}) and the lowest percentages of NO_2- and NO_3-, possibly as the result of anaerobiosis. Furthermore, under anaerobic conditions, both carbon limitation and excess can affect the activity of denitrifying bacteria as reported by van Rijn et al. (2006), the former causes the accumulation of intermediate products, such as NO and N_2O, and the latter results in NO_3- reduction to NH_4^+. Inhibition of NH_3 starts at 2500 mg L^{-1} for mesophilic reactors (Yenigün and Demirel, 2013), confirming that, in our experiments, NH_3 inhibition was not present. No significant differences could be shown between anaerobic DS and SS sampling points.

3.2. Nitrogen interactions with other abiotic parameters

Nitrogen transformations in the aquatic production systems involve a wide range of interactions between physical, chemical and biological parameters. The knowledge of potential interactions amongst parameters is crucial in understanding and predicting changes in water quality and system performance (Espinal and Matulić, 2019; Timmons and Ebeling, 2010) while simultaneously assuring the optimal conditions for organisms. Based on a PCA data analysis of the additionally measured water parameters (Fig. 3), the first axis explained 50.7 % of the variation while the second axis explained 17.9 %, together explaining more than

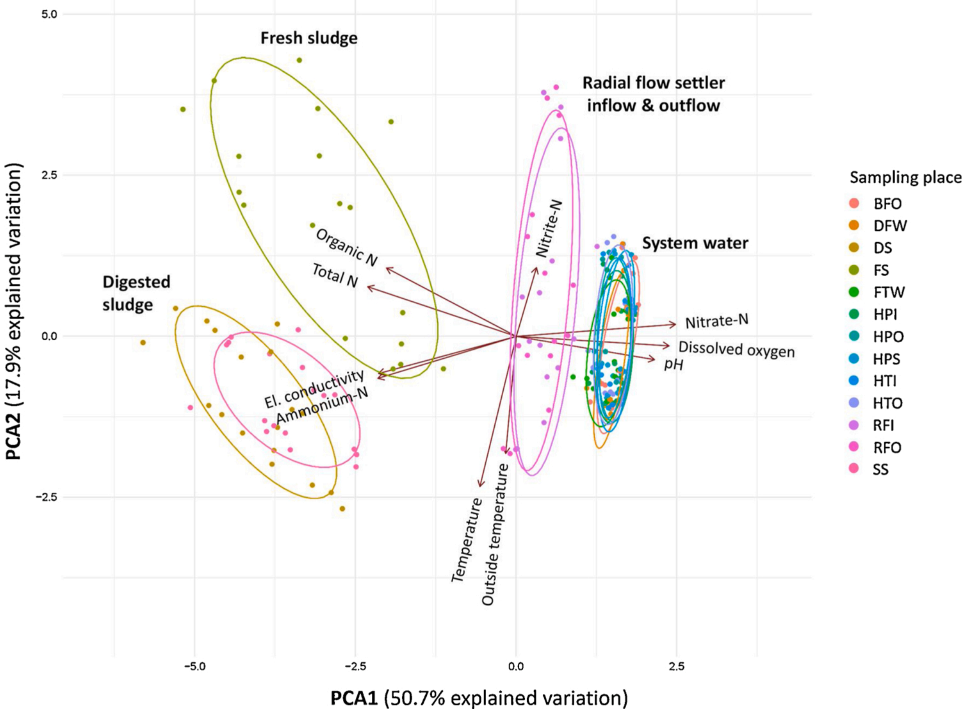

Fig. 3. Principal component analysis (PCA) with 95 % confidence eclipses of measured abiotic parameters and different nitrogen forms in the different compartments (FTW, fish tank water; DFW, drum filter outflow water; BFO, biofilter outflow water; HPI, inflow into hydroponic part of the system; HPS, sump water; HTI, hydroponic table inflow; HTO, hydroponic table outflow; HPO, outflow from hydroponic part of the system; RFI, radial flow settler inflow; RFO, radial flow settler outflow; FS, settled fresh sludge; DS, digested sludge; SS, supernatant of digested sludge returned back to the system) of the aquaponic system, measured six times during the experiment, explaining 68.6 % of data variance (n > 14).

68 % of the variation between selected parameters.

The dataset showed a clear distinction between aerobic loop, FS and digested sludge (DS and SS), with the RFS as a connection between the aerobic and anaerobic loop, confirming the results of Schmautz et al. (2020) looking into the microbial diversity in different compartments of the aquaponic system. Aerobic loop samples had high NO_3^- and oxygen levels, while the RFS had higher NO_2^- levels with increased influence from the ambient temperature, causing variation in the temperature of the measured samples, and causing FS to have high levels of TN and N_{org}. In contrast, digested sludge (DS and SS) had high electrical conductivity and NH_4^+ content.

Measuring the concentrations of individual N compounds within the aquaponic system, in addition to other relevant abiotic parameters, assists in drawing conclusions concerning the performance of the organisms present in the system, that is, that they are able to support in steering the metabolic processes involved. While large differences in the water parameters between compartments were not to be expected due to the high water circulation rate and low water volume of the system, it could be shown that N concentrations, ratios and abiotic parameter values varied significantly amongst the compartments. Thus, each compartment represented a different microenvironment responsible for specific microbial processes within the aquaponic system (Schmautz et al., 2020). While this is the first paper to describe detailed N transformations within such a system, further research using nutrient-mass-flow analyses and metagenomics to support these findings is necessary in order to better understand the role of microbial communities in these processes and allow the translation of these processes to other managed systems. In doing so, the long-term operation of such systems could be secured by assuring N conservation through its removal from wastewater, overcoming existing environmental challenges.

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CRediT authorship contribution statement

Zala Schmautz: Conceptualization, Data curation, Investigation, Software, Visualization, Writing - original draft. **Carlos A. Espinal:** Investigation, Methodology, Writing - review & editing. **Theo H.M. Smits:** Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Supervision, Writing - original draft, Writing - review & editing. **Emmanuel Frossard:** Conceptualization, Supervision, Writing - review & editing. **Ranka Junge:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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