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# Optimization of nitrogen use efficiency by means of fertigation management in an integrated aquaculture-agriculture system

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Recirculating aquaculture system (RAS) effluent has a high concentration of nitrogen, which can be a valuable asset as fertilizer for agricultural use in the vicinity of the RAS. A novel fully-automated experimental set-up of 24 lysimeter-plots was used to determine the optimal irrigation rate for cucumber plants fertigated with RAS effluents of three different nitrogen concentrations. Three RASs were stocked with 7, 14 and 21 kg of barramundi (*Lates Calcarifer*), which were fed 1.75% of their body mass daily. Then, 10% of the water in each RAS was exchanged daily, resulting in three nitrate–N concentrations of approximately 30, 60 and 90 mg/L. A synthetically fertilized control treatment was kept at 60 mg/L nitrate–N, and the other essential plant nutrients were kept at the same concentration for all treatments. The water from these four sources was then used to irrigate six plots per nitrate–N concentration treatment, at a rate of 1–6 times the amount transpired, which was measured in an automated fashion by the experimental set-up.

Two yield response to nitrogen fertigation models fit well to the measured data, and although they led to different conclusions in terms of optimal fertigation can be a useful tool for decision support. The nitrogen use efficiency dropped with increased fertigation, from about 80% to 55%, and more fertigation led to an exponential increase in drainage and gaseous emissions of nitrogen. The observed data can be used to optimize irrigation for each nitrogen concentration, but the values are highly dependent on climate, plant type, and root zone characteristics, demonstrating the need for more inclusive modelling. Determination of how to optimally make use of RAS effluent water and the comparison with synthetic fertilizer can lead to the development of simplified protocols concerning integrated aquaculture agriculture systems, making it accessible to a wider range of growers.



## 1. Introduction

Sustainably supplying the complete dietary need of each person on this globe is a concern that is growing adjacent to the world population. Major steps needed to meet this goal are to decrease food waste (Parfitt et al., 2010), to change our diets by consuming more plant and less animal protein (Di Paola et al., 2017), and to intensify production on existing cultivated areas (Lal, 2016), while reducing the environmental impact of this production. The many factors involved in sustainable food production form a complicated web spanning many disciplines (Motesharrei et al., 2017), the focus here will be limited to the last two steps, specifically in relation to the optimal use of nitrogen.

Nitrogen emissions from aquaculture and agriculture have received more attention as awareness of the effects of reactive nitrogen imbalances in the receiving atmosphere and ecosystems increases. Food production is responsible for more than 85% of the global anthropogenic reactive nitrogen emissions (Canfield et al., 2010), which lead to acidification ($NH_3$) (Guo et al., 2011), eutrophication ($NH_4^+$ and $NO_3^-$) (Aneja et al., 2008), change of species composition in ecosystems (Krupa, 2003), tropospheric ozone formation (NO) (Portmann et al., 2012), stratospheric ozone depletion

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**Abbreviations**

| | |
|---|---|
| DAT | days after transplant |
| DO | dissolved oxygen |
| DOM | dissolved organic matter |
| FCR | food conversion ratio |
| IAAS | integrated aquaculture agriculture system |
| I/T | Irrigation/Transpiration |
| MBBR | moving bed bioreactor |
| ME | Mitscherlich exponential |
| NUE | nitrogen use efficiency |
| QP | quadratic plateau |
| RAS | recirculating aquaculture system |
| TAN | total ammonia nitrogen |

($N_2O$) (Ravishankara et al., 2009), and significant greenhouse-gas (GHG) emission ($N_2O$) (Fowler et al., 2013). Historically the agricultural inputs and outputs of N had to be carefully balanced, but this ended with the availability of cheap synthetically sequestered N (Smil, 2001). The abovementioned impacts can be reduced by integrating agricultural systems, by meeting some of the need for N in plant cultivation with the N which is a by-product of raising animals.

The feed conversion ratio (FCR = food fed/biomass increase) of common farmed varieties of fish ranges from 1 to 1.8 (Boyd and McNevin, 2015), a level of efficiency among farmed animals matched only by insects (Oonincx et al., 2015). Fish feed in modern intensive aquaculture has a high protein (20–60%) content and is the major input of nitrogen into the system. The percentage of nitrogen retained in the body of the fish is between 15 and 30% (Boyd and McNevin, 2015; Neori et al., 2007) and the rest is released into the water, mostly (80–90%) as total ammonia nitrogen (TAN) and 10–20% as organic matter (Neori et al., 2007; Timmons and Ebeling, 2013). Most of the organic nitrogen is also biodegraded in the system to ammonia (Yogev et al., 2016), so that eventually this also ends up in mineral form. For example, a stock of 10 ton of fish would typically require a daily application of 200 kg of fish feed with a 40% protein content. This would result in a yearly by-product of more than 3700 kg of nitrogen dissolved in the water released from this fish farm, or approximately 75 kg nitrogen per ton of fish.

Intensification of plant-based agricultural production requires large inputs of nitrogen, which can result in increasing fluxes of $NO_3^-$–N to the ground and surface water bodies (Dahan et al., 2014). Gaseous emissions of nitrogen can constitute a substantial loss of the nitrogen applied, the amount of which depends on the form of the nitrogen and the method of application (Aneja et al., 2008), as well as root zone properties such as temperature (Oikawa et al., 2015), water content (Bergsma et al., 2002), pH and possibly the presence of bio-char (Obia et al., 2015). From an environmental point of view, the form of the N emitted is more important, whether $NH_3$ (Krupa, 2003), $N_2O$ (Butterbach-Bahl et al., 2013), NO (Akiyama et al., 2004) or inert $N_2$. To illustrate the order of magnitude of the anthropogenic influence on the global N cycle, in 2010, 133 M tons of $NH_3$–N was sequestered by means of the Haber-Bosch process. Together with 30 M tons of N from fossil fuel combustion and 60 M tons from N-fixing cultivations, the sum of reactive N of anthropogenic origin (210 M ton) has surpassed all natural N fixation combined (203 M ton) on a yearly basis (Fowler et al., 2013). Typical N use efficiency in agriculture is around 30% (Erisman et al., 2008), forming a huge potential flux of N to surrounding environments by means of leaching or gaseous emissions.

An integrated aquaculture-agriculture system (IAAS) has the potential to remove nitrogen from aquaculture effluent and to proportionally reduce the need for fertilizer (Goddek et al., 2016). The benefits of integrating fish and plant cultivation have been appreciated for more than 1500 years (Coche, 1967), but in practice, this technique has remained extremely limited. The main reasons for this are the high start-up cost and technical skills required (Konig et al., 2018; Love et al., 2015) and discrepancies in the complementarity of fish and plant requirements (Seawright et al., 1998; Tyson et al., 2008).

As fish can be grown at high $NO_3^-$–N concentrations, nitrogen need not be a limiting factor in an IAAS. The $NO_3^-$–N concentration of the effluent water from a recirculating aquaculture system (RAS) is a function of fish density and water exchange, which is determined by requirements for fish health, water use and treatment, and heat retention in the RAS. The amount of irrigation water needed to supply the plant with the optimal amount of water and nitrogen depends on the irrigation water's nitrogen concentration. Although several studies have been done on the nitrogen balance in aquaponic systems (Endut et al., 2014; Hu et al., 2015; Wongkiew et al., 2017; Zou et al., 2016), as of yet, no study has been published concerning optimization of the fertigation in an IAAS. A better understanding of how to apply RAS effluent water in terms of fertigation can pave the way to integrating nitrogen management of fertigation and aquaculture, and potentially other sources of waste water that contain nitrogen and organic matter.

The objective of this study was to determine the optimal irrigation amount for three different RAS effluent $NO_3^-$–N concentrations. The motivating hypothesis is that each nitrogen concentration has an optimal irrigation regime at which the highest yield can be achieved with the lowest amount of nitrogen fluxes out of the root zone to the groundwater or atmosphere. Determination of how to optimally make use of RAS effluent water and the comparison with synthetic fertilizer can lead to the development of simplified protocols concerning the IAAS, making it accessible to a wider range of growers.

## 2. Materials and methods

### 2.1. RAS and its effluent

The experiment was carried out in three IAA systems and one synthetically fertilized control system at the "Yair" Agricultural R&D Station (30°46′45.3″N 35°14′27.1″E), situated in Israel's Central Arava Valley. A substantial amount of Israel's export crops are produced in this desert (annual precipitation 25–50 mm), which is characterized by a mild winter and extremely hot summer (Goldreich and Karni, 2001). The high temperatures make this region ideal for growing warm-water fish, and the mild winters enable the cultivation of crops all year.

The fish and plant compartments were located in two adjacent climate-controlled greenhouses. In the RAS component (Fig. 1), the water circulated at a flow rate of 1 $m^3\ h^{-1}$ from the fish tank (800 L) to a clarifier (300 L) to a moving bed bioreactor (MBBR) (400 L) and from there back to the fish tank. Sludge that settled to the bottom of the clarifier was flushed out every 2 h (resulting in about 30 L of sludge removed per kg feed) to a sludge collection tank (500 L) where it underwent anaerobic digestion. The MBBR contained plastic bio-beads (Aridal Ltd, Israel) at a density of 5 L per kg of fish and was used to oxidize ammonia to nitrate. Oxygen in the filter was maintained at 90% saturation. A heating coil in the reactor kept the water in the fish tank between 28 and 30 °C. Once a day, 150 L of water was transferred from the MBBR through a 0.13-mm screen filter (with continual backwash to the bio-filter) to an intermediary irrigation tank, and was replaced after removal of the irrigation water.

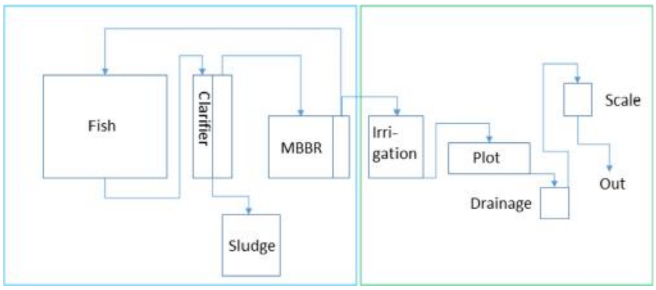


**Fig. 1.** Set-up of the fish compartment on the left, and the plant compartment on the right.

The barramundi (*Lates Calcarifer*) grown in the three systems were weighed before the start of the experiment, after a two-week interval when some fish were removed to maintain the same total fish biomass, and at the end of the experiment, and were fed about 1.75% of their target body mass (7, 14 and 21 kg) daily with a commercial fish feed with 45% protein (Raanan Fish Feed, Israel). This feeding and water change regime resulted in irrigation water of three distinct $NO_3^-$–N and nitrogen as dissolved organic matter (DOM–N) concentrations, with the target $NO_3^-$–N concentrations set at 30, 60 and 90 mg/L (excluding the DOM–N).

The only source of nitrogen for the 3 IAAS's was the feed which contained 7.2% nitrogen (feed with 45% protein of which 16% is nitrogen). The amount of nitrogen taken up by the fish was calculated from the increase in fish biomass multiplied by the biomass nitrogen concentration measured by the Kjeldhal method (AOAC, 2012). The sludge was assumed to undergo complete digestion and mineralization of the DOM-N to $NH_4^+$–N (Yogev et al., 2017) and the fraction of nitrogen removed with the sludge at the clarifier was estimated from the $NH_4^+$–N concentration measured weekly in the supernatant in the anaerobic digester multiplied by the amount of sludge removed that week. The fraction of $NO_3^-$–N removed from the system was the sum of the daily amounts of water removed multiplied by their $NO_3^-$–N concentrations. The fraction of DOM–N removed was the average of nine samples measured for each fertigation treatment multiplied by the total amount of water removed (see section 2.3). The change in nitrogen dissolved in the system was the difference in the system's $NO_3^-$–N concentration at the beginning and end of the experiment multiplied by the system's volume.

### 2.2. Plant cultivation

The plant component of the system was located in a 6-m-by-17-m plastic-covered greenhouse orientated in an east-west direction. Cooling fans located on the western side and an evaporative cooling pad on the eastern side were both automatically turned on when the air temperature was above 30 °C. A 30% shade net (Aluminet, Ginegar, Israel) was placed over the greenhouse to assist with temperature control. Cucumber was used as a model plant, chosen for its short growing season with a continuous and high fruit yield. A fruiting crop was chosen as generally they are of high value and have a longer shelf-life than the lettuce typically used in aquaponic experiments, which is important in a region focussed on export; furthermore their cultivation is more complex due to the oxygen requirements for the root zone.

Twenty-four experimental plots were laid out in a random-block design in eight rows of three plots in a north-south direction. The plots consisted of white expanded polystyrene boxes that were 1-m-long, 0.5-m-wide, and 0.2-m-deep and filled with 100 L of perlite each (Perlite 206, Agrikal, Israel). Automatic valves (Aquative Plus, Netafim, Israel) on the irrigation mains allowed water to flow through a lateral to the designated plot at specified times, and amounts were measured by means of a flow meter (SF: 0.1-L pulse, Arad, Israel). The water in excess of the perlite-holding capacity seeped out and was collected in a small channel below the polystyrene box, and from there flowed out to a container located in a trench adjacent to the greenhouse (Fig. 1). From these containers, the drainage water was vacuum-pumped (Boxer, Debem, Italy) through a manifold of electronic valves (5281A-GP, Bermad, Israel) to a container located on a scale (SH 600, BeKet, Israel) and weighed in order to determine the quantity for each of the 24 plots. The daily transpiration was calculated by subtracting the amount of drainage from the amount of irrigation water.

Before each irrigation event, a valve (24VAC NC 25 mm, Bermad, Israel) at the end of each of the four irrigation main lines was opened to allow all the water in the main line, that had warmed up in the greenhouse, to return through another pipe to the irrigation tank, where the water temperature was continually monitored and cooled to 24 °C. Eight times a day, a measured amount of irrigation water from each of the four systems was pumped into the weighed container in order to calibrate the water meters, so that the water balance of all 24 plots was carried out by the same scale.

Three cucumber seedlings (Sanyal, Soli, Israel) were transplanted to each plot on 7/8/2017 (dates will subsequently be referred to as days after transplant, DAT). Each plant had its own irrigation water emitter, and an oxygen sensor (KE-50, Figaro, Japan) was buried at 5-cm depth next to the central plant. The plots were covered with white plastic mulching in order to minimize evaporation. As the water used in this experiment was saline (Electrical conductivity of 2.5 $dSm^{-1}$), the drainage water from the plants was not returned to the fish systems in order to eliminate salinity build-up which would limit plant growth.

Transpiration was calculated daily for each of the 24 plots for the duration of the experiment. The number of leaf nodes was counted daily until the plants reached the trellising wire at 2-m height. From 20 DAT the cucumbers were harvested daily and weighed individually for each of the three plants in each plot. At the end of the season (40 DAT), total fresh biomass yield was weighed, and the plant was split into fruit, stems and leaves and weighed again. Five leaves and three stem samples were taken from each plant at equally spaced distances from the bottom to the top of the plant, and three ripe cucumbers of the last harvest were taken as representative samples for each plant. These samples were weighed and dried to determine dry matter content, and then tested for N and carbon content (OEA-CHNS Flash, 2000, Thermo Fisher Scientific, MA, USA).

The root zone was cut into three blocks of equal size, and the perlite was washed off the roots through a 3-mm sieve, after which the roots were dried and weighed. The root N content was not measured, but an estimated value of 5% was used for all treatments in subsequent calculations.

### 2.3. Fertigation and water quality

The experimental treatments consisted of the four irrigation water $NO_3^-$–N concentrations from three RASs (referred to as treatments “A” for aquaculture followed by a number indicating the $NO_3^-$–N concentration) and one synthetically fertilized system of 60 mg/L $NO_3^-$–N referred to as S60, each in combination with six irrigation treatments without replicates. The nitrogen source of the S60 treatment was a synthetic fertilizer (MOR+, ICL, Israel), and the prepared mixture was continually mixed and aerated in the same way as the fish systems. $K_2SO_4$ ($K_2SO_4$, Solucros, Belgium), PK (MKP, Haifa Chemicals, Israel), and a cocktail of micronutrients (Super Koratin, ICL, Israel) were added to all irrigation water to keep their levels similar in all treatments (Appendix 1).

The pH in the two higher density RASs dropped due to the hydrogen released during nitrification, and was kept at pH 6.5 by an automated KOH dispenser (Profilux, GHL, Germany). The lower density RAS did not lower the water used (pH 8.2) much, so a 10% $H_2SO_4$ solution was dispensed automatically to the irrigation water removed from this system and to the synthetically fertilized irrigation water in order to lower their pH to 6.5.

Irrigation water was sampled daily and drainage water of all 24 plots was sampled three times a week to measure EC (DDS 120W, Bante Instruments, China) and $NO_3^-$−N (Reflectoquant Nitrate Test 116971, Merck KGaA, Germany and Orion Star A214, Thermo Fisher Scientific, MA, USA). Water samples from the MBBR were tested daily for pH (HI 9126, Hanna Instruments, RI USA), $NO_2^-$−N (Nitrite test 14658, Merck KGaA, Germany), and $NH_4^+$−N (Ammonium Test 11117, Merck KGaA, Germany), and the dissolved oxygen (DO) levels were measured in the MBBR daily (Handy Polaris, OxyGuard, Denmark). Irrigation water was sampled daily and tested for $NO_3^-$−N (Reflectoquant Nitrate Test 116971, Merck KGaA, Germany), and weekly for P (Reflectoquant Phosphate Test 116978, Merck KGaA, Germany), and about every five days for nitrogen in the dissolved organic matter (DOM). DOM−N was calculated as the difference between the $NO_3^-$−N concentrations determined by spectrophotometry (Spark 10M, Tecan, Switzerland) after digestion of the organic matter to $NO_3^-$−N and $NO_3^-$−N measured in the same sample that was filtered (Glass fiber filter 66078, Pall Corporation, USA), but did not undergo digestion (Eaton et al., 1995). Supernatant from the sludge digestion tanks was tested weekly for TAN (Reflectoquant Ammonium Test 116977, Merck KGaA, Germany). Samples from all irrigation water were tested once (K, Ca, Mg, Na, Mn, Fe, Zn, Cu and B by means of atomic absorption, AA240FF, Varian, USA; P and K by means of spectrophotometry, Lambda 25, Perkin Elmer, USA; Cl by means of titration, 665 Dosimat, Metrohm, Switzerland) in order to confirm that these nutrients were being added in the right proportion (Appendix 1).

The irrigation treatments were determined as a fraction of the transpiration: Irrigation/Transpiration (I/T). The target I/T treatments were 1, 2, 3, 4, 5 and 6 and started after two weeks of irrigating all the plants equally to ensure good seedling establishment. The irrigation amounts were adjusted on a daily basis according to the I/T treatments when the transpiration rose rapidly, and were left unchanged when the transpiration levelled off. The target I/T treatments were changed upon limitation in the available irrigation water, but the ratios between the I/T treatments within each nitrogen concentration treatment remained constant.

### 2.4. Data analysis

Data was collected by means of five data loggers (Mega-Arduino-Italy, CR800-Campbell Scientific-UT-USA, Profilux-GHL-Germany) and stored in a Microsoft SQL server. The data was continuously displayed online by means of Microsoft's reporting service in order to track the many parameters being monitored, and notifications of possible errors (temperature of the water in the RAS, irrigation water or the greenhouse, irrigation or drainage amounts or flow rates, etc.) were sent automatically so that the problem could be dealt with immediately. Further data analyses, a statistical analysis and graph-making were carried out in Microsoft Excel and Python. Statistical significance of the difference between the $NO_3^-$−N treatments was determined by means of a single factor ANOVA with Tukey HSD as a post hoc analysis ($p < 0.01$).

The yield response to $NO_3^-$−N fertigated was fit to the measured data by minimizing the sum of square errors by means of the Solver packet of Microsoft Excel. Two models were used to analyze the yield response, the Quadratic Plateau (QM) and Mitscherlich Exponential (ME) model (Cerrato and Blackmer, 1990). The QP model is defined by:

$$Y = a + bX + cX^2 \text{ if } X < C \quad (1)$$

$$Y = Y_{max} \text{ if } X \geq C \quad (2)$$

Where $Y$ is the yield of cucumbers (kg m$^{-2}$), $X$ is the amount of $NO_3^-$−N fertigated (g m$^{-2}$), $a$ is the intercept, $b$ is the slope, $c$ is the quadratic coefficient, $C$ is the critical rate of fertigation, which is at the intersection of the quadratic response and the plateau and $Y_{max}$ is the plateau yield.

The ME model is defined by:

$$Y = Y_{max}(1\text{-}exp^{-K1(X+K2)}) \quad (3)$$

Where $Y$ is the yield of cucumbers (kg m$^{-2}$), $X$ is the amount of $NO_3^-$−N fertigated (g m$^{-2}$), $Y_{max}$ is the maximum yield possible, and $K1$ and $K2$ are fitted constants corresponding to the Mitscherlich effect factor and the estimate of the soil derived available N respectively (Cerrato and Blackmer, 1990).

## 3. Results and discussion

### 3.1. RAS performance and N balance

During the 40 days of the experiment, the fish in systems A30, A60 and A90 were fed 4.96, 9.8 and 14.7 kg of feed, respectively. The mass gains of the fish over the 40-day period for systems A30, A60 and A90 were 3.7 kg, 7.7 kg, and 7.8 kg respectively, implying FCRs of 1.35, 1.27, and 1.88, respectively. A technical problem stopped the air supply for several hours two weeks into the experiment leading to the death of some fish, which were immediately replaced by other fish of the same total mass. As the A90 system had the highest fish density it was most affected by this event; the subsequent drop in $NO_3^-$−N concentrations of the water, and the reduction in fish growth may be due to this event. The water exchange rate was fixed at 10% of the system volume per day, which maintained a good water quality sufficient for good fish performance. An overview of the nitrogen source and sinks is given in Table 1. The $NH_4^+$−N and $NO_2^-$−N concentrations were kept <1 and <0.25 mg/L respectively by means of the MBBR as concentrations of few mg/L are toxic to fish, and were thus neglected from the table. The nitrogen concentration of the fish was about 3%, which is a normal value for barramundi grown in aquaculture (Glencross et al., 2008; Katersky and Carter, 2007; Raso and Anderson, 2003) and corresponded to 20–30% of the nitrogen in the food. Generally, about 70% of the applied nitrogen was released into the water. The DOM−N concentration in system A30 was below detection, whereas systems A60 and A90 had similar average concentrations that accounted for 8.6 and 5.6% of the nitrogen put into the system respectively. The amount of nitrogen estimated to have left the system as sludge was 5–9%. There was little change in dissolved nitrogen for systems A30

**Table 1**
The source and sinks of nitrogen in the three RAS's reported in grams of nitrogen.

| Source/Sink | A30 | A60 | A90 |
|---|---|---|---|
| Food | 357 | 706 | 1058 |
| Fish | 110 | 231 | 234 |
| Irrigation $NO_3^-$ | 150 | 289 | 377 |
| Irrigation DOM | 0 | 61 | 60 |
| Sludge $NH_4^+$ | 20 | 36 | 90 |
| Change $NO_3^-$ | 6 | 11 | 31 |
| Missing | 72 | 78 | 265 |

and A60, but system A90 did change due to a reduction in the $NO_3^-$−N concentration. The missing nitrogen was assumed to have left the system in a gaseous form. The nitrogen distributions presented here are comparable to others carried out on RAS (Neori et al., 2007; Yogev et al., 2017), and differences in N distribution can be attributed to fish type and the RAS set-up.

### 3.2. Fertigation and transpiration

The climate control in the greenhouse kept the average greenhouse air temperature at 29.8 ± 4.0 °C, the average relative humidity was 71 ± 9%, and the average photosynthetic photon flux density was 620 ± 178 μmol $m^{-2}$ $s^{-1}$.

The irrigation water $NO_3^-$−N concentrations of the four treatments varied slightly over time (Fig. 2 a). The season-average $NO_3^-$−N concentrations for treatments A30, A60, A90 and S60 were 32, 61, 86 and 58 mg/L respectively and except for A60 and S60 were significantly different from each other ($p < 0.01$). The total amount of $NO_3^-$−N applied to each treatment over the 40-day season was the product of the concentration and amount of water applied (Fig. 2 b). The transpiration was calculated on a daily basis and was similar for all plots until the I/T treatments were started at 14 DAT (Fig. 3). As the transpiration increased the target I/T treatments required more water than was available from the RAS, so at 20 DAT the maximal I/T treatments for systems A90 and A60 were lowered to 4.5; the maximal I/T for A30 was increased to 7 that same day, and at 24 DAT, the maximal I/T for A90 was decreased to 3.5, after which all I/T treatments remained constant.

As the change in media water content due to water uptake was not measured, the I/T treatment with a target of 1 was actually less than 1, so that the irrigation for the lowest I/T treatments of each $NO_3^-$−N treatment did not increase for the duration of the experiment (14–40 DAT). This method resulted in deficit irrigation for the lowest I/T treatment of all $NO_3^-$−N treatments.

For the lowest $NO_3^-$−N treatment (A30), the increase in I/T resulted in a steady increase in transpiration. Except the lowest I/T treatments all plots had drainage water daily, so the increase in transpiration is assumed to be mostly due to the additional nitrogen associated with the higher I/T treatments, which enabled increased growth. The transpiration of the highest four I/T treatments for the three higher $NO_3^-$−N concentration treatments were clustered together, indicating that they approached saturation in terms of $NO_3^-$−N requirement.

### 3.3. Plant growth and N uptake

At the time the I/T treatments were started (14 DAT), there was already an influence of the $NO_3^-$−N concentration treatments on the average leaf numbers per plant, which were 13.3, 15.0, 15.8 and 13.8 for A30, A60, A90 and S60, respectively, but this was the only visible difference, and all plants appeared healthy. However, Farneselli et al. (2015) observed in their nitrogen fertigation studies

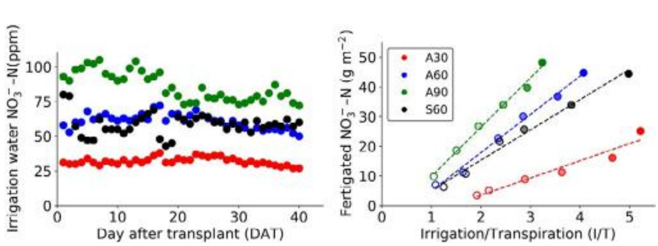


**Fig. 2.** (a) Irrigation water $NO_3^-$−N concentrations over time and (b) amount of fertigated N−$NO_3^-$ for the each treatment, the darker hue indicates the higher I/T treatment.

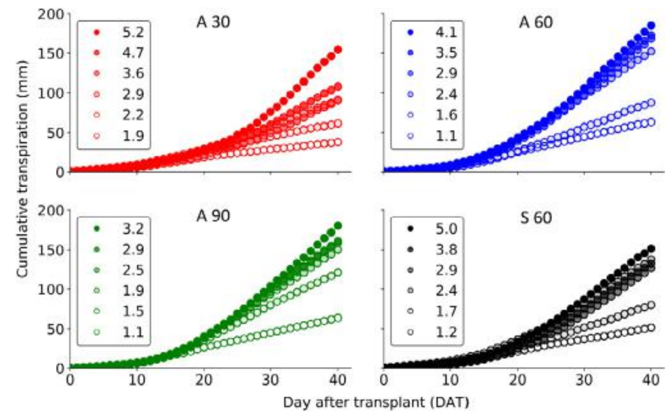


**Fig. 3.** Cumulative transpiration graphed for each of the I/T treatments (whose season-average values are noted in the legends).

on tomatoes that the low fertigation frequency was not able to supply the critical nitrogen concentration in the early growth stages, which resulted in lower biomass accumulation, demonstrating the importance of sufficient nitrogen in the early growth stages. Cucumbers were harvested from 20 to 40 DAT, and the total cucumber yields for each $NO_3^-$−N concentration treatment as a function of total fertigated $NO_3^-$N depicted in Fig. 4. The lowest $NO_3^-$−N treatment (A30) showed a continual increase in yield with increasing I/T, similar to the transpiration of the same treatment in Fig. 3; this correlation of relative transpiration to relative yield was pointed out by De Wit (1958).

Two models of yield response to applied nitrogen fertilizer, 'quadratic with plateau' (QP) and 'Mitscherlich-exponential' (ME), have been plotted in Fig. 4, and statistically both appear to fit the data well (respective $R^2$ values of 0.953 and 0.945). According to the ME model the maximal yield has not been achieved and is estimated to be 17.5 kg $m^{-2}$, requiring 82 g $m^{-2}$ to achieve 90% of the maximal yield. According to the QP model the maximal yield is at 11.5 kg $m^{-2}$, and this threshold is reached with less fertigated nitrogen, 30 g $m^{-2}$ instead of 39 g $m^{-2}$ as predicted by ME model. Cerrato and Blackmer (1990) found the QP model best described the corn yield data they were modelling, but pointed out that the abrupt discontinuity at the point identified as economic optimum is difficult to accept from a biological point of view, and the yield in the response curve can be overestimated there.

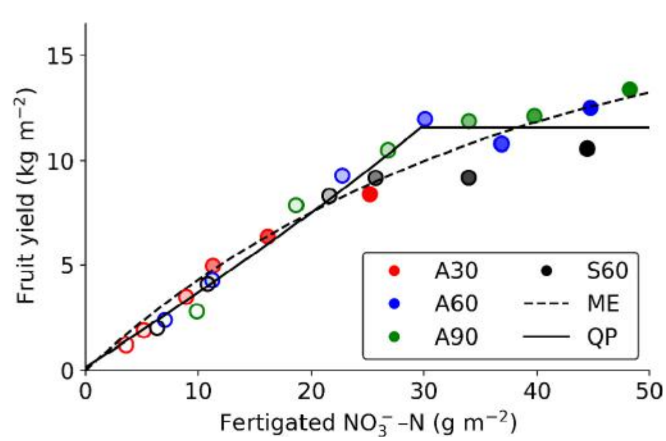


**Fig. 4.** Total cucumber yield for each of the $NO_3^-$−N treatments as a function fertigated $NO_3^-$−N. The yield response to $NO_3^-$−N fertigation has been modelled with the Mitscherlich Exponential (ME) and the Quadratic Plateau (QP) functions, with $R^2$ values of 0.945 and 0.953 respectively. The darker hue of the data points indicates the higher I/T treatment.

In terms of decision support the amount of nitrogen required to achieve a specific yield can be read from Fig. 4 (from either modelled line), and the I/T required to achieve that application for each of the irrigation water $NO_3^-$−N concentrations can then be read from Fig. 2 b, for example $NO_3^-$−N 30 g m$^{-2}$ would require an I/T of 2.1 for A90, and 3.0 for A60. The yield of the synthetically fertilized treatment (S60) was consistently below the threshold of the QP model, besides $NO_3^-$−N, these aquaculture treatments contained DOM−N, and it is likely that some $NH_4^+$−N become available for uptake due to the mineralization of this organic matter in the root zone, making the $NO_3^-$:$NH_4^+$ ratio more favorable to the plants as it is easier for plants to regulate intracellular pH when both forms of nitrogen are supplied (Hawkesford et al., 2012). An alternative explanation could be a beneficial microbial community that established in the root zone of the IAAS plots but not in the synthetically fertilized plots (Zhao et al., 2018).

### 3.4. Plant N allocation

The allocation of nitrogen to the different plant parts as a function of the amount of fertigated $NO_3^-$−N is shown in Fig. 5. The relative amount of nitrogen allocated tothe fruit decreased with reduction in fertigated $NO_3^-$−N but remained similar across the range of fertigated $NO_3^-$−N for the stems. The fraction of nitrogen allocated to the leaves and roots increased with reduction in fertigated $NO_3^-$−N. Polynomial trend lines were plotted in order to clarify the effect of the of total fertigated $NO_3^-$−N on the nitrogen allocation to the different plant parts, as the $NO_3^-$−N treatment alone did not appear to have much effect. The reduction in the shoot to root ratio, seen with the dramatic increase in the root biomass in the low fertigated $NO_3^-$−N range is a known response to nitrogen deficiency (Kudoyarova et al., 2015). Though the N concentration of leaf dry matter ranged from about 1 to 4% (data not shown), the fraction of nitrogen allocated to the leaves changed much less across the N availability range. In cucumber plants a large portion of the nitrogen taken up is allocated to the leaves first, and from there is transported to the fruits (Tanemura et al., 2008), implying a potential control mechanism for optimizing total plant growth under nitrogen limitation. The increase in the leaf to fruit nitrogen ratio may be due to this mechanism, as the leaf nitrogen concentration of the nitrogen starved plants was lower (data not shown) and so less nitrogen would be available to translocate to the fruit.

### 3.5. N balance on plant compartment

The three sinks of the fertigated nitrogen were the plant uptake, the drainage water, and the gaseous emission of nitrogen (Fig. 6). The nitrogen remaining in the perlite at the end of the season was not measured, but as the root zone nitrogen concentration at planting was the same as the irrigation water nitrogen concentration, the difference in the root zone nitrogen between the beginning and end of the season would be a relatively small fraction of the balance. The DOM−N forms a significant part of the nitrogen balance that has been left out, as the amount of DOM−N in the root zone and in the drainage water was not measured. The DOM−N that was mineralized would increase the gaseous nitrogen emissions for treatments A60 and A90, as the mineral nitrogen in the drainage and plant biomass was quantified. This omission is important to rectify in future experiments.

The same QP and ME models also fit the nitrogen uptake data well ($R^2$ of 0.913 and 0.895 respectively), but polynomial trend lines were fit to the graphs displaying nitrogen distribution to the plant, drainage and missing nitrogen, as the sum of these trend lines should equal the amount of $NO_3^-$−N fertigated and the other models are designed for yield. When the total amount of fertigated nitrogen during the 40-day experiment exceeded 30 g m$^{-2}$, the slope of the trend line fit to the nitrogen uptake data tapered off, while the nitrogen lost to drainage increased exponentially. Increasing the $NO_3^-$−N application from 30 to 50 g m$^{-2}$increased the $NO_3^-$−N uptake from 22 to 27 g, but as a consequence increased the $NO_3^-$−N lost to drainage from 4 to 14 g m$^{-2}$, $NO_3^-$−N missing from 4 to 8 g m$^{-2}$, and reduced the NUE from 75 to 50% (Fig. 6). The values are of course highly dependent on the climatic and root zone conditions, length of the experiment, as well as plant type and variety demonstrating the need for monitoring of the water status in the soil-plant-atmosphere continuum during the growing season, and more inclusive modelling for decision support (Dabach et al., 2015).

The missing nitrogen was assumed to be due to gaseous emissions, and had a slight exponential increase with increasing amounts of applied nitrogen. As the plants were grown in perlite media, the root zone oxygen concentration did not vary much between the I/T treatments (all between 15 and 20%), the increased availability of carbon in the aquaculture water could lead to an increase in denitrification (Weier et al., 1993), but this was not seen in the results, when comparing the S60 and A60 treatments for example. The only observed parameter that correlated with the

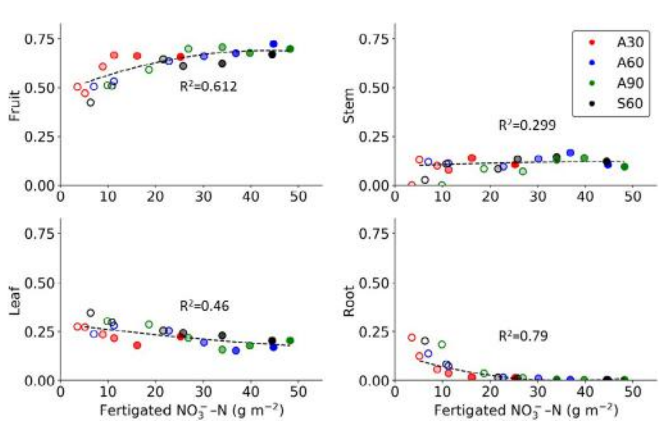


**Fig. 5.** Distribution of N uptake per treatment over the plant parts. The darker hue indicates the higher I/T treatment. All trend lines are second order polynomials with $R^2$ values reported on the graph.

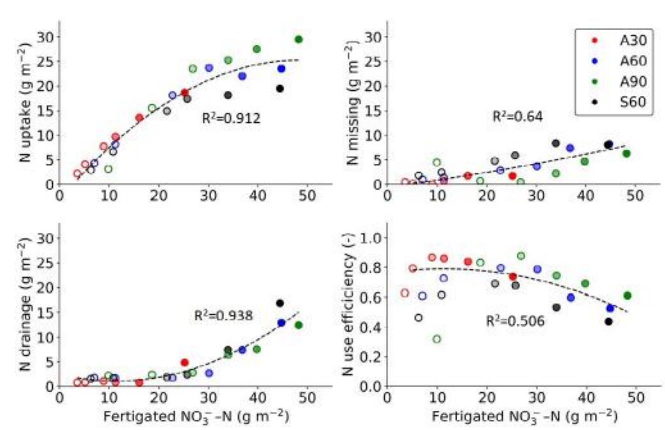


**Fig. 6.** N taken up by the plant, drained out of the root zone, missing from the balance and the NUE per N treatment as a function of the amount of N irrigated. The darker hue indicates the higher I/T treatment. All trend lines are all second order polynomials with $R^2$ values are reported on the graph, excluding the lowest I/T treatment of each N treatment as discussed in the text.

calculated increase in gaseous nitrogen loss was the amount of available nitrogen.

The drainage water was not reused in this experiment, but the remaining water and nitrogen could be utilized to irrigate a third crop of higher salinity tolerance. At the highest fertigation rates, the nitrogen in the drainage water would be about 12% of the total nitrogen fed to the fish. Other sources of available nitrogen include the sludge removed from the system, which constituted 5–9% of the total nitrogen fed to the fish, and the nitrogen in the non-fruit vegetative plant material, which was about 7% of the total nitrogen fed to the fish. With some preparation, these nitrogen sources could be reused in the IAAS, or applied elsewhere as fertilizer, compost, or animal feed. The sludge also has potential value in terms of biogas (Yogev et al., 2016), as well as a source of TAN (Palada et al., 1999), phosphorus (Cerozi and Fitzsimmons, 2017) and possibly micro-nutrients, but it was not used in this study.

After normalizing the data to correspond to the 40-day length of this experiment, results from Daum and Schenk (1996) show that cucumber plants grown on rock-wool in the Netherlands took up 13.2 of the 17.6 $g\,m^{-2}$ nitrogen applied, and those from Grewal et al. (2011) show that the uptake of cucumbers grown by means of the nutrient film technique (NFT) in Australia was 17 out of 42 $g\,m^{-2}$ nitrogen applied; both of these values are in the same range of plant nitrogen uptake in relation to the applied nitrogen recorded in the present study (Fig. 6). The total amount of nitrogen calculated to have been emitted in gaseous form (including that from the RAS mentioned in section 3.1) was between 17 and 30%, which is similar to the 25% estimated by Fang et al. (2017).

The optimal I/T for different N concentrations will depend on the value assigned to plant yield and the environmental damage due to $NO_3^-$ leaching and gaseous emissions of certain forms of nitrogen, such as $N_2O$, which was not measured in this experiment. The fraction of $N_2O$:($N_2O + N_2$) can also be affected by the soil type as the same I/T would results in different levels of oxygen availability in each soil type, or by use of bio-char which is also reported to reduce the $N_2O$:($N_2O + N_2$) ratio (Cayuela et al., 2013) although there is a lack of understanding concerning the mechanisms involved (Sánchez-García et al., 2014).

## 4. Conclusions

The optimal amount of nitrogen to apply depends on the value of the additional crop versus the cost of the extra nitrogen and water applied, and of the environmental cost of nitrogen leaching and gaseous nitrogen emissions. The yield increase per increase in $NO_3^-$–N applied became very small for the higher applications, both the Quadratic plateau (QP) and Mitscherlich exponential (ME) models describing yield response to nitrogen fertigation fit the data well (respective $R^2$ values of 0.953 and 0.945). According to the QP model the fertigation amount that achieved a near-to-maximal yield with limited nitrogen leaching was 30 $g\,m^{-2}$, which corresponded to an I/T level 2.3 for the A90 treatment, 3.0 for the A60 treatment and wasn't reached for the A30 treatment. According to the ME model the same yield was reached only at 39 $g\,m^{-2}$, and the maximum yield was not reached. These values are however dependent on multiple parameters and highlight the need for more inclusive modelling.

Increased fish density makes the RAS more sensitive to technical failure, so that higher density systems should be designed with automated back-up systems in place. Irrigation with water from aquaculture resulted in a slightly higher yield than synthetically fertilized water with a similar nutrient composition. The potential additional benefit due to the DOM or bacterial community associated with the aquaculture water poses an interesting subject for further investigation. Further research into the fate of the DOM-N in the root zone and quantification of gaseous nitrogen emissions and their forms is recommended in order to further improve the sustainability of integration of aquaculture and agriculture.

## Declarations of interest

None.

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## Appendix 1

The average nutrient concentrations of the 4 treatments during the experimental period. Ca, Mg, S, Na, Cl, B naturally occurred at these concentrations in the water used and Fe, Mn, Zn, P, K were added daily.

| Nutrient | A30 | A60 | A90 | S60 | unit |
|---|---|---|---|---|---|
| P | 36 | 41.25 | 44 | 39.5 | mg/L |
| K | 102 | 106 | 98 | 104 | mg/L |
| Ca | 169 | 173 | 174 | 139 | mg/L |
| Mg | 89 | 95 | 98 | 83 | mg/L |
| $SO_4$ | 764 | 808 | 836 | 612 | mg/L |
| Na | 252 | 277 | 286 | 232 | mg/L |
| Cl | 405 | 449 | 471 | 369 | mg/L |
| Cu | 0.03 | 0.04 | 0.05 | 0.03 | mg/L |
| B | 0.49 | 0.52 | 0.47 | 0.53 | mg/L |
| Fe | 1.4 | 1.56 | 1.46 | 1.41 | mg/L |
| Mn | 1.41 | 1.52 | 1.5 | 1.1 | mg/L |
| Zn | 0.5 | 0.5 | 0.5 | 0.5 | mg/L |
| pH | 6.5 | 6.5 | 6.4 | 6.5 | |
| EC | 2.72 | 2.84 | 3.03 | 2.72 | $dSm^{-1}$ |

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