Modelling nitrogen uptake, fish growth, plant dry matter and water quality in an aquaponic system producing *Lactuca sativa* and *Leuciscus idus*

MSc Thesis Plant Production Systems

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ABSTRACT

LEPAS (Lettuce Production in Aquaponic systems) is a model developped to simulate lettuce and fish growth and nutrient concentrations in plant and water in an aquaponic system under different environmental conditions (air temperature and radiation). The LEPAS model was not yet tested with low air temperature and radiation. An experiment was conducted during 28 days in the Netherlands. Lettuce (*Lactuca sativa*) was produced in one aquaponic system in combination with “winde fish” (*Leuciscus idus*). Fish fresh weight was recorded at the beginning and at the end of the experiment. An average fish feed (12.99 g / m^2 / day) was calculated. A 12 plants / raft was used to cover the 8.7 m^2 of the plant basin with a density of (20.5 plant / m^2). Model inputs were calculated including SLA (0.05 m^2), LUE (0.193 molC / MJPAR) and FCR (0.7) among other inputs. The measurements were done twice per week. Three lettuce plants were harvested to measure the chlorophyll, fresh weight, dry weight and leaf area (without root). During the whole experiment a ratio of 4.44 kg of fish / m^2 area covered by crop was established. The model outputs for plant dry weight are coherent but not in total agreement with the observed values. The leaf area outputs maintain a discrepancy after day 12 with the measurements till day 28. Fish growth model outputs are considered in good agreement, considering the actual fish conversion ratio (0.7) and no limitation by ammonia. Furthermore, water quality (ammonium and nitrate) calculated by the LEPAS model fit with the measurements and reflecting the bacteria sub-model reflect the rapid conversion as in reality. LEPAS is a sensible model related to the LUE and the C : N assimilation pathway, which is the main part of the LINTUL and Nicolet model to predict plant dry weight and leaf area index.

1 INTRODUCTION

Agricultural and livestock activities are considered the biggest consumers of fresh water. Estimations reveal that 85% of the global fresh water consumption is for agriculture (Hoekstra and Chapagain, 2007) and nearly one-third of the total water footprint of agriculture in the world is used for livestock products (Mekonnen and Hoektra, 2012). Moreover, the rising global food consumption and the intensification of animal and crop production systems will increase the pressure on the global freshwater resources in future years. Additionally, fertilizer has a cost range between 5-10% of total crop production and is expected to increase, due to the raising prices of the oil. Therefore, the expenses in inputs for intensive systems such as fertilizer will gradually reduce the gross margin profit of the farmer (Hochmuth and Hanlon, 2013). Moreover, intensive and improper application of chemical compounds is causing environmental and health problems (Alexandratos et al., 2012; Bindraban et al., 2012). Thus, as agriculture became a global business, conventional systems will definitely face challenging situations in the economic, social and environmental domains. Therefore, researchers, government, NGO's, universities and organizations all over the world, are looking for more sustainable practices in food production in order to reduce the negative effect on natural resources such as water.

The total global population is expected to reach 8.9 billion people by 2050. Continents such as Asia, Africa and Latin America are expected to have the highest percentages of growth (United Nations Department of Economic and Social Affairs 2004). Moreover, in the last 30 years the increase in the income of the population in developing countries led to an increase in fish consumption from 25.0 to 104.3 million ton fish per year (FAO, 2014). Due to the depletion of marine resources the FAO predicts that in the future the supply of fish for the population will be entirely dependent on fish production in aquaculture systems.

The increased demand for fish, water and fertilizer for crop production and the concerns about environment and health are motivations to test innovative farming systems such as "aquaponics" as viable systems for sustainable fish and crop production (FAO, 2014).

Aquaponics is an integrated and intensive fish-crop farming system under constant recirculation of water through interconnected devices. It is considered a promising technology, which is highly productive under correct set up and proper management (Lal 2013; Orsini et al., 2013). First, fish feed is eaten by fish and converted into ammonia (NH_3). Some ammonia ionizes in water to ammonium (NH_4^+). Then, bacteria (*Nitrosoma*) convert ammonia into nitrite (NO_2^-) and consequently bacteria (*Nitrobacter*) oxidize nitrite into nitrate (NO_3^-) (Tyson et al., 2011). Finally, the water delivers nutrients and oxygen to promote plant growth. Graber and Junge (2009), found similar yields between hydroponic systems and aquaponic

systems. Finally, it is important to establish systems under “smart water” use and to balance nutrient concentrations in water to ensure maximum fish and plant growth.

Aquaponics is considered a method where water and nutrients are efficiently used and maintained within the system (Liang & Chien, 2013). In aquaponics it is possible to reduce daily water loss to 2% of the total water volume of the system. Due to the constant recirculation of water it is also possible to maintain evenly distributed high nutrient concentrations in the water (nitrate) as the small addition of water to compensate the daily loss will not dilute the nutrients (Rakocy, 2006). The “water smart” approach makes aquaponics an alternative systems to produce food under sustainable practices in areas where water is scarce (Essa et al., 2008).

Therefore, food productions systems such as aquaponics to produce food, optimize nutrient use and nutrient use efficiency and to save water would be relevant as an alternative to fulfill the food demand of an increasing global population with minimal negative effects to the environment. Moreover, developing an accurate and practical tool to predict plant and fish growth and monitor nutrient concentrations in water, will improve the adoption and implementation (small or commercial scale) of aquaponic systems under several settings such as urban farming in developed countries or as a business model for household food security in developing countries.

The objective of this study is to test a combined model that predicts plant and fish growth and net ammonium and nitrate concentrations in water in an aquaponic system. This is done by comparing the model outputs with measurements under controlled conditions in order to assess the accuracy of the tool to simulate nutrient concentrations in water and fish and plant biomass production of the system.

2 AIM AND HYPOTHESIS

The aim of this study was to test the LEPAS model (Perini, 2014), developed for aquaponic growers to ensure consistent plant and fish growth by monitoring ammoniunmand nitrate concentrations in the water, under low radiation and low temperature conditions.

An accurate prediction of the model, with low radiation and temperature in our experiment compared to the experiments with which the model was calibrated, will enhance the value of the tool to monitor nutrient concentrations in water and the ability to determine crop and fish production under diverse environmental conditions.

3 AQUAPONICS

Principles

Aquaculture production can potentially cause environmental pollution due to the nutrients content in the water discharged to the soil, underground water and other water sources (Edwards, 2015). By adding the plant component, the nutrients concentrated in the water will be taken up through the plant roots and enhance plant growth, reducing the need of fertilizer. Furthermore, the constant recirculation of water through interconnected devices, maintains and delivers resources such as nutrients and water to all system components. Finally, the fact that aquaponics systems do not need soils, makes them suitable to be built in small household areas in developing countries or within the cities as urban farming (De Bon, 2010).

The nutrients such as nitrogen in particular, start the flow from feed intake by the fish and excretion into the water. The feaces are rich in ammonia (NH_3) and dependent on parameters such as pH and temperature, this is partly or completely converted into ionized ammonium (NH_4^+). The combination of both forms is referred to as total ammonia nitrogen (TAN) (Francis-Floyd et al., 2010). In reality, water is filtered through bio-filters containing bacteria which nitrify the TAN into nitrite (NO_2-) and afterwards into nitrate (NO_3-) according to the following equations:

Equation 1: $NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$

Equation 2: $NH_4^+ + 1.5O_2 => NO_2^- + 2H^+ + H_2O$

Equation 3: $NO_2^- + 0.5\ O_2 => NO_3^-$

At high pH the balance in equation 1 lies at the left (NH_3) and with low pH at the right ($NH4^+$). Both active forms of nitrogen (NH_4^+ and NO_3-) can be absorbed by the plant, with preference to nitrate as the active form to enhance plant growth (Andriolo et al., 2006). Additionally, waste water from fish contains macronutrients such as phosphorous, potassium and micronutrients such as iron that are important through the growing cycle of the crop (Diver, 2006). Nevertheless, previous studies report nutrient deficiency in plants grown in the aqua pool after the use of commercial fish feed over long periods (Roosta, 2014). Therefore, addition of amendments such as Iron (Fe) is a common practices to supply the nutrient deficit caused by the fish feed. Additionally, water exchange is adapted according to nutrients concentrations to avoid any toxicity (ammonia) and salinity such as sodium (Na) above 50 mg/l, and to minimize denitrification (Ako and Baker, 2009).

In aquaponics systems the ratio between fish feed delivered per day and the area covered by crops is essential to provide enough nutrients for plants and avoid toxicity levels from nitrate and ammonia for fish. Additionally, accurate amounts of fish feed per day will avoid accumulation of organic matter in the

systems, reducing potential denitrification sources by anaerobic conditions (Seawright et al., 1998). Rakocy et al. (2006) established a ratio between 60-100 g feed per m^2 of crop area for leafy crops such as lettuce, spinach, basil and cabbage.

The approach of aquaponics can also be seen as a weakness of the system. The susceptibility of the fish to chemical compounds such as pesticides, increases the complexity of aquaponics and forces it to rely on integrated pest management practices to avoid any negative effect on the crop yield. Finally, according to the definition of Lehman et al., (1993) aquaponics are considered sustainable food production systems which do not compromise any natural resource and are free of any potential harmful chemical for humans and the environment (Somerville, 2014).

Fish

Fish are dependent on the external temperature to regulate their metabolic functions and rate of activity that affect feed intake, digestion and oxygen consumption. However, fish have a range of temperatures they can tolerate according to species (warm and cold water fish). Therefore, assessing the environmental conditions (temperature) of a region is essential to choose the fish species to be produced.

Aquaponics systems are complex and sensible food productions system which demand daily maintenance and monitoring. Furthermore, parameters such as water temperature, dissolved oxygen (DO), pH, and nutrient levels must be monitored frequently to avoid inefficient performance (Bernstein 2011). Rakocy et al. (2006) recommended DO concentration in water of 6 mg / l in order to provide enough oxygen for plants, fish and bacteria. Moreover, a high ammonia concentration in water is toxic for fish, decreasing their growth (feeding and digestion) and eventually can cause death. Therefore, the suggested maximum concentration of ammonia (1 mg / l) should be maintained in water (Ebeling et al. 2012). Additionally, The European Inland Fishery Advisory Commission (EIFAC) established a maximum ammonia (NH_3) concentration of 0.25 mg / l in water. Nitrate concentrations should be kept lower than 50 mg / l to avoid negative effect in the fish immune system and prevent algae bloom in the system in order to avoid the reduction in the oxygen concentrations in water (Watson and Hill, 2006). Finally, other macro and micronutrients are not often measured individually in aquaponics. Any concentration above 200 mg / l of total dissolved solid in water should be avoid (Rakocy et al. 2006).

Biofilter

In order to avoid accumulation of toxic compounds in water such as ammonia and nitrite, which can cause fish death, it is necessary to decompose these compounds into more favorable compounds and promote the growth of plant and fish. The main purpose of the bacteria (*Nitrosoma* and *Nitrobacter*) present in the bio filter is to convert (TAN), essentially the -ionized fraction (NH_3), into nitrate (NO_3^-). Additionally, the

larger the amount of oxygen and ammonium present in the water, the higher the expected nitrification rate (Lucas and Southgate, 2003).

Crop

Lettuce is the most common leafy crop grown in aquaponics systems, due to its low nutrient demand and short growing cycle (5 weeks). Moreover, the constant recirculation of water in aquaponics systems provides a permanent supply of nutrients to the root zone and therefore no depletion on nutrients is visible (Tyson et al., 2011). Savidov (2004) suggests to maintain a pH between 7.5 and 8.0 to promote nitrification and availability of nutrients as phosphorous, calcium and magnesium. Nevertheless, Rakocy et al., (2006) state that such high pH values affect negatively the solubility of essential micronutrients such as iron, manganese, copper, zinc and boron. Therefore, a pH between 6.5 and 7.0 is acceptable for the three main components of the aquaponic system (plant, fish and bacteria).

Lettuce is considered a cold season crop and temperatures below 7˚C and above 25˚C will result in physiological disorders and unmarketable quality products. Providing the crop with a proper environment will achieve greater biomass production.

4 MATERIALS AND METHODS

The current research was performed in the Aquavita farm located in Driel, the Netherlands. The experiment was conducted from 3th of October until 2th of November 2016.

A drawing of the aquaponics system in Driel in shown in figure 1.

An aquaponics batch production was designed and implemented where 179 lettuce plants with same stage of growth (4-6 leaves) were cultivated in the plant basin at the same time. A 12 plants / raft was used for the lettuce plants. Finally, three lettuce plants were randomly selected and harvested twice per week (between 10:00am and 11:00am) and afterwards, transported to Wageningen University for analysis. Lettuce plants grown outside the system but of the same age and development stage (defined by the number of leaves) were used as replacement to maintain plant density (20.5 plants / m^2) in the plant basin throughout the trial.

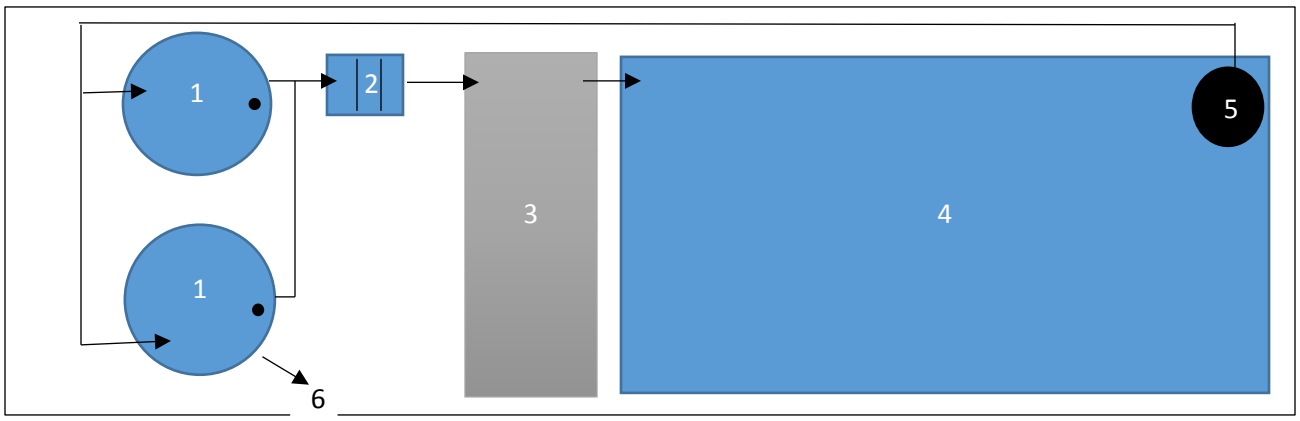

Figure 1. Schematic figure of the Aquaponic system located in Driel.

1. Fish tanks for fish production (1000 l of water per tank)
2. Filter with membranes for solids
3. Bio filter (2.3 m^2 surface area and 0.7 m^3 of volume) containing lava rocks and bacteria.
4. Plant basin (9.5 m^2 surface area and 1.8 m^3 of volume) were all rafts (15) contain each one 12 plants and float on the water surface (20.5 plants / m^2).
5. Water pump for constant recirculation of water within the system.
6. Aerator for constant supply of oxygen for fish, crop and bacteria.

The system build in Driel had been working for the last months prior to the experiment. Therefore, a good bacterial biomass and performance is assumed in the bio filter. Nevertheless, after these months the filter and the plant basin were cleaned from sludge, organic residues and algae in order to minimize any negative effect such as denitrification during the experiment. Moreover, 38.66 kg of fish and 8.7 m^2 of

area was covered by lettuce at trial establishment. Thus, a fish/plant ratio of 4.44 kg fish / m^2 of crop was used during the experiment.

Figure 2. Aquaponic pool with 8.7 m^2 of lettuce in a density of 20.5 plant / m^2 and 12 lettuce plants per raft.

4.1 PLANT AND FISH

Lactuca sativa

Lettuce is considered a leafy crop and is the most popular crop produced in aquaponic systems. An advantage of lettuce over other crops is the fast growing cycle of only 4-5 weeks, low nutrient demand and permanent vegetative state which makes lettuce an ideal crop to test in a model and profitable when grown in aquaponics (Rakocy, 2006).

The lettuce plants were bought in a horticulture nursery on 22-October-2016 in polystyrene trays, using peat soil as substrate and with four to six leaves per plant. After complete removal of all the peat soil in the root zone an average mean weight of five grams per plant was estimated. All lettuce plants were transferred to the Aquavita greenhouse for an acclimatization period (radiation and temperature). On Wednesday-26- October all lettuce plants (180) were transplanted into polystyrene rafts and moved to the aquaponic basin (see figure 2). Additionally, replacement plants remained inside greenhouse on a plastic tray with water to maintain the substrate moist.

During five weeks three lettuce plants were randomly selected from the rafts and harvested twice a week (Monday and Thursday) and replaced with replacement plants. Moreover, just after harvest an SPAD-502Plus (Konika Minolta) chlorophyll meter was used to determine the nitrogen content in leaves per plant. For each lettuce plant three measures were taken from three different leaves on the distal margin with a total of nine measurements per plant. Afterwards, plants were transport to Wageningen University

where all lettuce plants were cleaned of any substrate residue and also, roots were removed. Furthermore, plants leaves were counted including leaves with >1cm length. Finally, using an LI-COR LI-3100C the leaf area of each plant was determined. Fresh weight and dry weight per plant was determined using a gravimetric scale. Lettuce dry weight per plant was determined after 24 hours inside a Binder® oven at 75 ˚C.

Additionally, in order to calibrate the SPAD values with real amounts of nitrogen in lettuce, plants were delivered to Wageningen University laboratory located in Radix building for nitrogen extraction. Total N content was determined using the Dumas Method with a CHN1110 Element Analyzer (CE instruments, Milan, Italy).

Aquaponics systems are highly dependent on the fish feed quality to provide enough nutrients to the plant. Usually commercial fish feed does not provide enough Iron to the system and amendments are supplied to avoid yellowing of leaves. Therefore, 45g of Ferro Plus® was added once per week during four weeks.

Leuciscus idus

"Winde" fish were produced during the four weeks of experimentation. Fish tank number one registered 61 fish with a total fresh weight of 20.44 kg and fish tank number two registered 57 fishes with a total fresh weight of 18.22 kg. Both fish tanks start the experiment with an average fish weight of 327 g / fish.

Additionally, fish feed was recorded and delivered daily according to standard dietary tables, depending on fish mass and feeding behavior. The commercial pellets selected as fish feed contain nutritional values of 49% protein, 11% crude fat, 1.8% crude protein, 8% ash, 1.5% calcium, 0.5% sodium and 1.2% phosphorous.

Water

Determination of nitrogen and ammonium concentration in water, were obtained using the segmented-flow analysis (SFA) system. Every week (Monday) 50 ml of water was taken from the water flowing from the bio filter to the plant basin (figure 1). Afterwards, the water samples were delivered to Wageningen University laboratory (in the Radix building) for analysis. Only nitrate and ammonium concentrations were determined in the laboratory, as references of the nutrient content in water in the aquaponic system.

4.2 MODEL INPUTS

As the LEPAS model responds to the environmental parameters these (Appendix 2) are imperative as inputs for the model. The light transmission coefficient (TAO) of the greenhouse and of the shading net was measured using a LI-1400 LI-COR radiation sensor (PAR sensor). Daily total radiation values were download from (KNMI, 2016) Deelen station (#275). Air temperature (minimum and maximum) was

measured with a thermal-hygro meter inside the greenhouse but outside temperatures were downloaded from the weather station. Finally, a thermometer was used to measure water temperature at 10 cm below water surface.

The daily total radiation from KNMI was used in the weather file for the model (Appendix 5). Water temperature data were provided in the FEEDDATA file (Appendix 6) including total amount of fish feed (feed) given to the fish tanks.

4.3 MODEL LANGUAGE

The model language used is FST : FORTRAN simulation translator. The FST software is downloaded from the Plant Production Systems, Wageningen University Research Group, website and it is freely available (http://models.pps.wur.nl/node/970).

5 CALIBRATION AND VALIDATION

Recorded data (Appendix 1) were used for validation of the model.

Data collected during the experiment (Appendix 1) were used as parameters: the specific leaf area (SLA), the light use efficiency (LUE), and a coefficient used in the calculation of the maintenance respiration (CSUNLIT). The specific leaf area (0.05 m^2 / g DW) was measured from the collected values of the dry weight per plant (g DW / plant) and leaf area per plant (m^2 / plant). The light use efficiency was first calculated as g DW / MJPAR with the measured data and then converted to molC / MJ PAR by using a coefficient from Linker et al., (2004, αC=30 g DM / molC). The radiation was corrected to intercepted radiation by using the following formula:

LI = 1-exp (-k * LAI)

With LI =light intercepted; k = extinction coefficient which can vary but we used k= 0.7; LAI = leaf area index in (m^2 l / m^2 g) at the different sample moments

This factor of LI was added in order to accurately predict the radiation capture by the plant below the net, as the plants in the initial stage only have four to six leaves, the radiation lost in the surface area is high. Therefore, the more the development of the plant is advanced, the higher is the area covered by the leaves and finally, the captured radiation. The calculated light use efficiency equaled 0.193 molC / MJPAR (equivalent to 5.78 g DW / MJPAR). The same αC coefficient (Linker et al., 2004) was used in the calculation of CSUNLIT (1.20 m^2 / molC) by converting the measured DW per plant (g DW / plant), according to the specific plant density (plant / m^2).

Other parameters, such as the plant area (AREAP), the initial plant fresh weight (FW) and dry weight (DW) and fish body weight (BW) and the initial water volume in the system (WATVOLI), were measured and used according to run the model. A feed conversion ratio (FCR) of 0.70 was calculated from the amount of feed given to fish and their gained weight at the end of the experimental period (Appendix 4).

Values of parameters not specific to this model, or not inferable from the collected data, were obtained from literature. All the parameters used with their units and source can be found in Appendix 2.

SPAD measurements could only be calibrated with the amount of organic nitrogen in the plant. Since the lab results showed the amount of total nitrogen in the plant, while the SPAD indirectly measures only the organic nitrogen content, a further step was needed to accomplish the calibration. According to Henriques and Marcelis (2000), after a certain amount of organic nitrogen is reached, lettuce plants start accumulating nitrate. At low light condition, which is the case in the current experiment (daily total radiation of about 5.78 MJ / m^2 / d), the accumulation response is represented by a specific function. By

knowing the relationship between the amount of total nitrogen (gN / kg DM) and the nitrate nitrogen (gN / kg DM) it is possible to deduce the plant organic nitrogen concentration (gN / kg DM).

The model was run in order to test its performance by comparing the simulation with the measured plant dry weight, organic nitrogen concentration in the plant and ammonium and nitrate concentration in the water. Final fish fresh weight was simulated with one feed input (total feed given) (Appendix 6).

6 RESULTS

After five weeks of experiment in the Aquavita farm and several measurements from the aquaponic system, the model was run and both results (model and experiment) are presented in the next chapter.

Plant dry weight

The applied harvesting method implies three plants harvested twice per week from different trays in the aquaponic pool and replaced with lettuce plants of similar age and development stage. During the trial several harvest dates can be distinguished (03/10/2016, 06/10/2016, 10/10/2016, 13/10/2016, 17/10/2016, 20/10/2016, 24/10/2016, 27/10/2016, 31/10/2016, and 02/11/2016). Those dates represent the harvesting 1 to 10 respectively.

Figure 3 presents the average dry weight per plant (dots) calculated from measured (means) harvest and simulated by the model with light use efficiency values of 0.193 molC / MJPAR (measured value). The model outputs remain inside the confidence intervals within the first 17 days, and only in day 19 a minimal discrepancy is visible. Finally, the model accurately predicts the final dry weight per plant after 28 days.

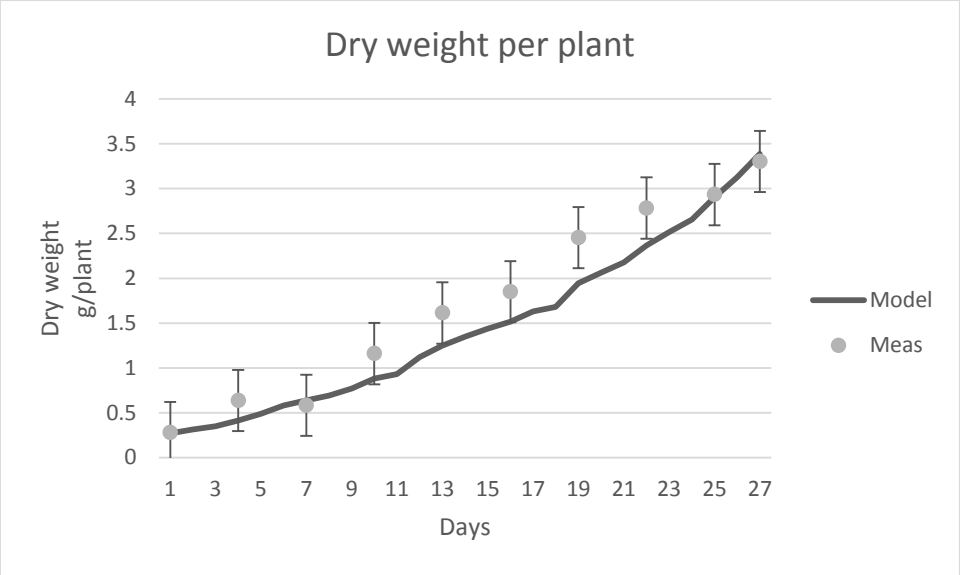

Figure 3. Model simulation for plant dry weight (g / plant) in a ratio of 20.5 plant per m^2 and LUE of 0.193 molC / MJPAR and specific leaf area of 0.05 m^2 leaf / g DW. The model was run from day 1 to day 28. The line represent the model outcomes. Dots represent the measured plant growth as average of three harvested plants for each of 10 harvests.

Leaf area index

The model simulations for leaf area index (figure 4) after week three slightly differ from the measurements. The increase of leaf area depend on factors such as light and nutrients. Thus, a low light use efficiency (0.193 molC / MJPAR) will cause an underestimation of the model. These results for leaf area have a strong correlation with the values obtained for dry matter production per plant and in both cases the model underestimates the measurement with stronger negative effect for leaf area index.

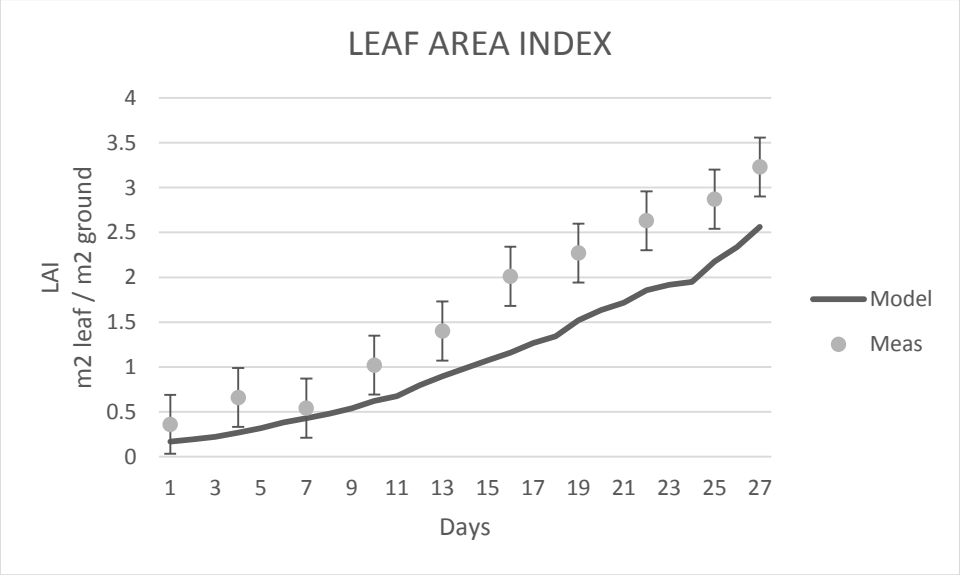

Figure 4. Leaf area index simulation (m^2 leaf / m^2 ground) run for 28 days. Dots represent the measured plant growth as average of three harvested plants for each of 10 harvests. The continuous line represent the model prediction with LUE of 0.193 molC / MJPAR, and initial leaf area of 0.05 m^2.

Fish body weight

Fish growth (figure 5) was simulated based on the total amount of fish reared in both tanks. Moreover, due to the low temperature which affected feeding behavior some days no feed was delivered to the fish tanks (Appendix 4). Those days were also included in the FEEDDATA file (Appendix 6). The simulation is represented for two situations. The continuous line shows the model predictions calculated from the model equations where fish growth is linked to feeding ratios, temperature and ammonia concentrations in water. The model simulation for fish growth show an agreement with the measured final fish fresh weight. Finally, the dashed line shows the results of fish growth calculated with the conversion rate of 0.7 and real amount of feed given to the fish tanks during the whole experiment.

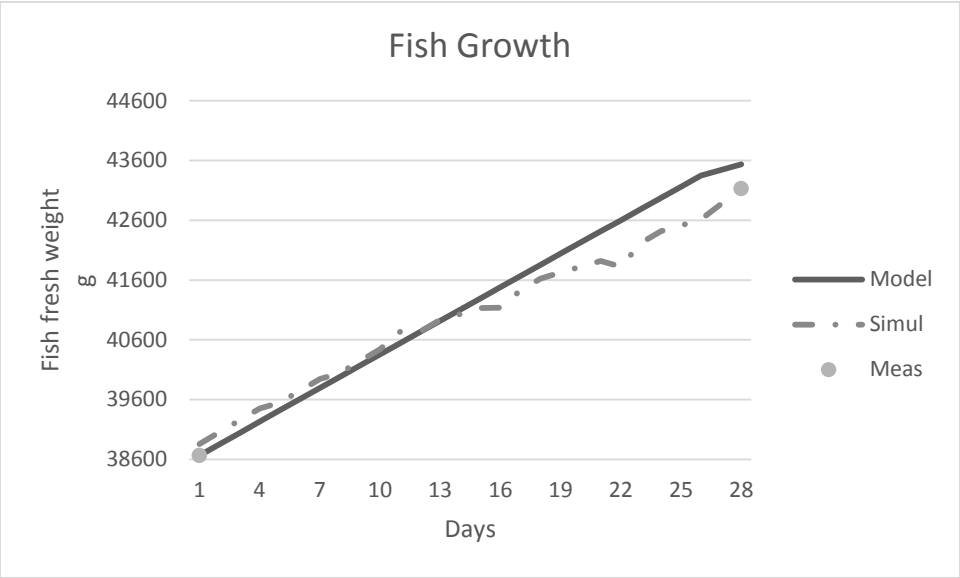

Figure 5. Results for fish growth in fresh weight (g) during 28 days. Dots show the measured fish growth, while lines indicated model growth designed on mathematical equations. The dashed line shows predictions using real amounts of daily feeding ratios. There are only two measured points, at day 1 and day 28.

Ammonium and nitrate in water

The measurements for ammonium concentrations in the aquaponic water were always lower than 0.25 mg / l. Moreover, the model predicts a stable ammonium concentration over time remaining below the 0.25 mg / l. The reduction of the ammonium concentration in water during the first week was expected. During the first week bacteria will start demanding oxygen and ammonium, in order to build up nitrate as is visible between figure 6 and 7. These reduction in ammonium and increase in nitrate concentrations refers the well performance of the bacteria in the bio filter since the beginning of the trial till the end after 28 days.

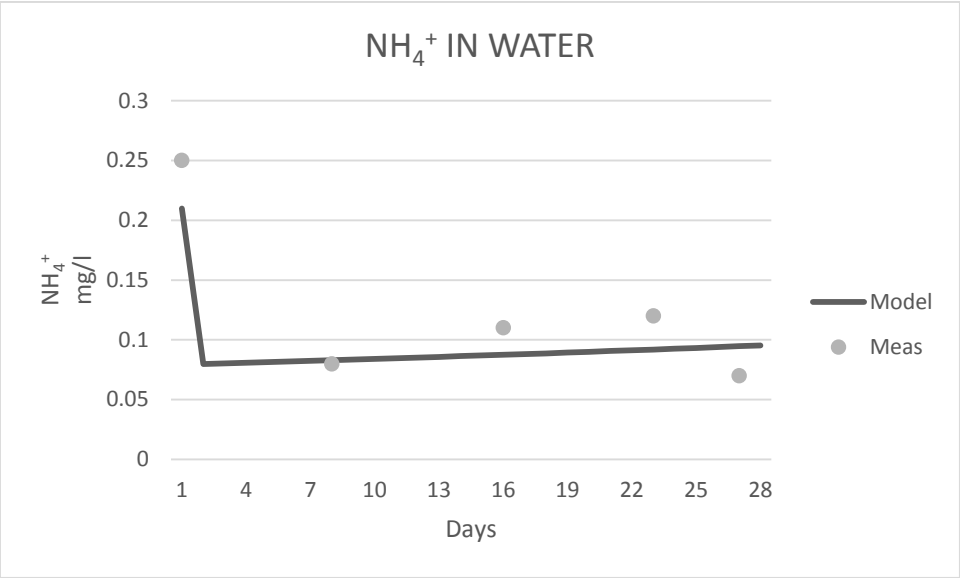

Figure 6. Results for ammonium concentration in water (mgN_NH_4^+/l) during 28 days. Ammonium concentrations were simulated using total amount of daily feed (feed) as input. Dots represent the measured concentrations of ammonium and the line indicates the model predicted concentrations for NH_4^+ in water.

Figure 7 present the model simulations for nitrate (NO_3^-) concentrations in water. Furthermore, the model was run with the real amount of feed delivered to the fish tanks, due to the importance of achieve a positive balance between the amount of feed given and the nitrate concentrations accumulate in the water. The continuous line represent the nitrate concentration predicted by the model and the dots represent the nitrate concentration from the water samples analyzed in laboratory. Thus, the model results underestimate the measurements from the first and second week. Nevertheless, the drastic reduction of nitrate concentrations in week three is mainly explain by the unexpected replacement of water (day 16) to the system and as a consequences the reduction of the achieved concentration and thereafter, untrusty measurements. Nitrate concentration were expected to increase as ammonium decrease and the system mature, therefore, according to the model predictions after 28 days a nitrate concentration of 25 mg / l can be achieved compared with the measurements 27 mg / l two days before.

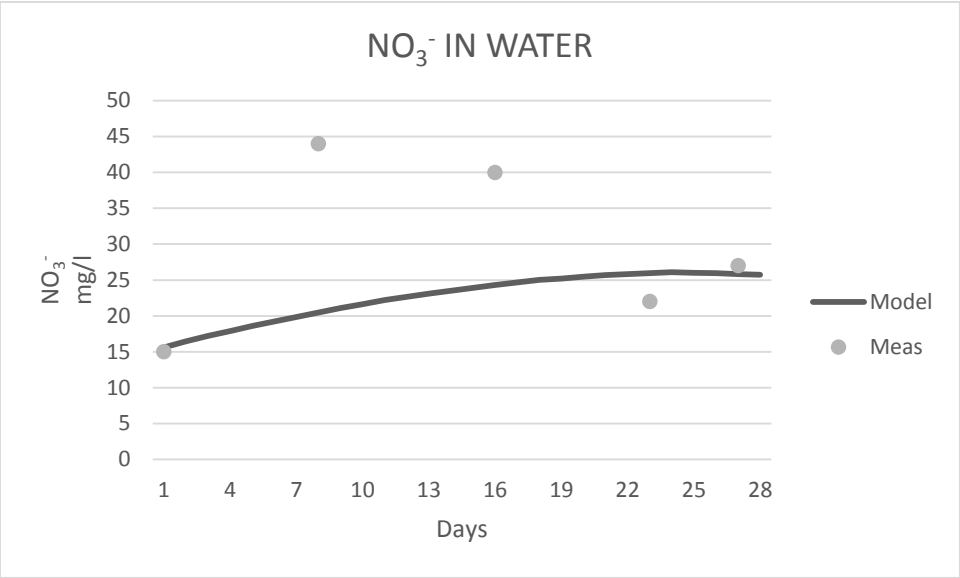

Figure 7. Results for nitrate concentration achieved in water (mgN_NO_3 / l) during 28 days. Dots show the measurements obtained from laboratory and the lines indicated the model predictions using real values of daily feed.

Plant nitrogen concentrations

The model predictions for plant nitrate concentrations are shown in figure 8. Moreover, no measurements were done for nitrate concentrations in plant. According to the model equations the NO_3^- concentrations are calculated from the initial amount of nitrogen in the structure (MNSI) which is calculated by the initial fresh and dry weight of the plant. Plants in a low light use efficiency (0.193 molC / MJPAR) (continuous line) accumulate high concentrations of nitrate in the tissues. Moreover, low radiation and therefore light use efficiency (see figure 8) will tremendously exceed the maximum level of 3500 mg NO_3^- / kg FW for lettuce allowed according the European Food Safety Community (EFSA).

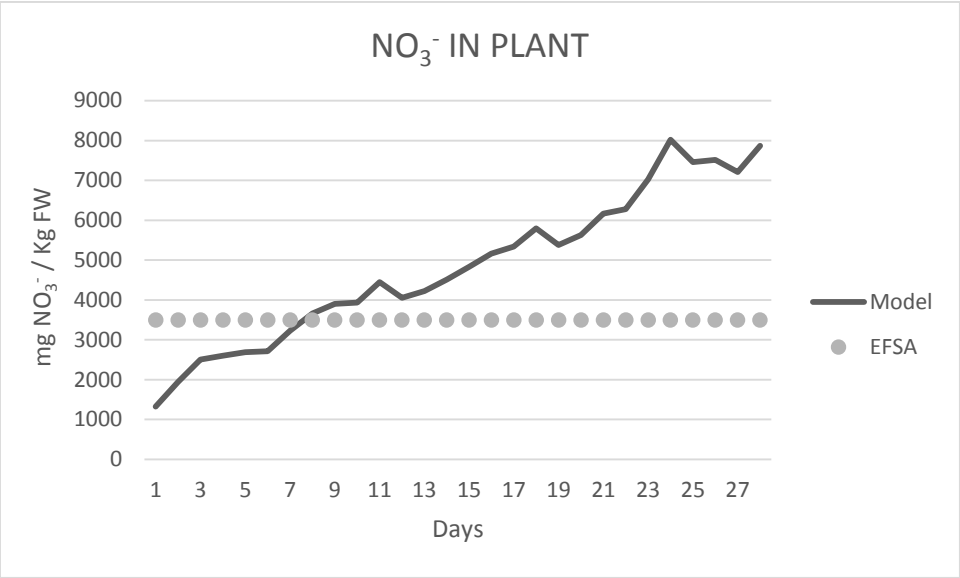

Figure 8. Results for plant nitrate concentrations (mg NO_3^- / kg FW) during 28 days. The continuous line represents the model prediction in a LUE of 0.193 molC/MJPAR. Dots represent the maximum concentration imposed by EFSA for lettuce under cover (3500 mg NO_3^- / kg FW).

Results regarding plant organic nitrogen concentrations (reduced nitrogen) are present in figure 9. The model concentrations at the beginning of the trial show a large deviation from the measurements. After the day 19 the plant organic nitrogen concentrations simulated by the model start decreasing and maintains a discrepancy. Nevertheless, at day 28 similar plant organic nitrogen concentrations are achieved between model outputs and measurements.

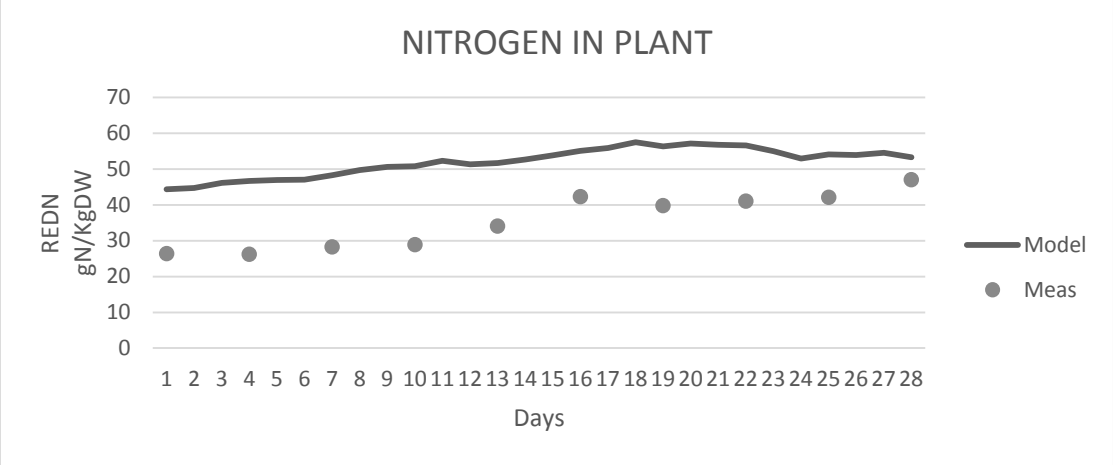

Figure 9. Results for plant reduced nitrogen content (g N / kg DW) for 28 days. Dots represent the measured reduced nitrogen (average of three plants) and the line indicates the model simulation with LUE of 0.193 molC / MJPAR.

Daily temperature

Figure 10 present the measurements (twice per week) for minimum and maximum air temperature inside the greenhouse compared with the air temperature downloaded from KNMI weather station #275 in Deelen Netherlands. The measured minimum temperature inside the greenhouse remain close to the outside temperature from the weather station. The measured maximum temperatures inside the greenhouse show discrepancy with the temperature from the weather station. These differences are mainly explained by the close shape of the greenhouse which avoids external air flow, achieving higher maximum temperatures inside the greenhouse especially on sunny days.

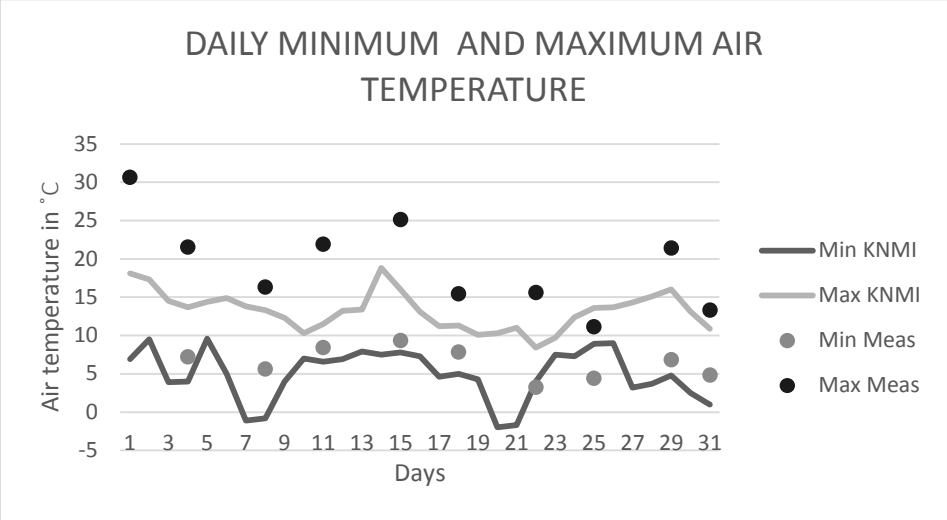

Figure 10. Comparison between minimum and maximum air temperatures. Continuous line refer the downloaded temperature from the weather station in Deelen, Netherlands. Dots refer the air temperature measured twice per week inside the green house.

7 DISCUSSION

The LEPAS model presents a way to combine several components (bacteria, plant and fish) and relate them to environmental factors such as radiation, temperature, feed given, nitrate and ammonium concentrations, in order to assess the performance within the system and achieve optimal plant and fish growth. In reality, aquaponics systems are by default complex and to transform the processes taking place in these systems to mathematical equations seem to be as complicated as reality. The aim of this study was to validate a recently developed model (Perini, 2014), changing basic input parameters of light and temperature, predicting nitrate and ammonium concentrations and comparing them to nitrate and ammonium concentrations found in the experiment. But as several process are involved and strongly linked the model predictions resulted to be partly underestimatingd compared with the measured values, notably in the case of leaf area index. The model showed to be very sensitive to changes in LUE and as the extinction factor k was not measured but estimated the potential error in k could account for the differences.

The function in the fish component relating water temperature to feeding ratio as a percentage of the fish mass (FCONV) had a subroutine adapted to lower temperature factors which was used to fit with the measured water temperature (14.4˚C). The subroutine to calculate FCR depending on ammonium concentrations in the water (FCRO) was present in the model. The ammonium concentrations in water (FCRO) that determine the (FCR) infers negatively in the FCR only when the limit of 1 mg / l (Somerville et. al, 2014) of ammonium is reached in an aquaponic system. In our case no adaptation was needed.
The model simulations according the “FEEDATA” file combining fish feed given (g / day) and water temperature fit with the simulated fish growth and measurements for final fish fresh weight (figure 5).
The calculated FCR is 0.7 which equals the measured data.
In order to simplify the model, the (FCR) could be adapted as an initial parameter according the fish type.

The bacteria functions and the ammonium and nitrate concentrations calculated in water resulted to be coherent with the measurements. It is visible (figure 6 and 7) that as ammonium decrease, nitrate increase and during the five weeks of the experiment nitrate was building up reaching 25 mg / l while ammonium remained below 0.1 mg / l. These refer in reality to the performance of the nitrifying bacteria and in the model to the well correlated function to the different variables that infer the transformation of fish feed into nutrients concentrations in water. Nevertheless, the nitrate concentrations achieved in the system are low for commercial production of lettuce in an aquaponic system at scale with a minimum of 37 mg / l. This is because the low amount of feed given to the fish (12.99 g / m^2 / d) compared with the recommended value for leafy crops (60-100 g / m^2 / d) by Rakocy (2006).

The measurements for plant dry weight and leaf area were used as an estimation of the model output with the aim of understanding the factors which determine an accurate result of the model under different growing conditions (radiation and temperature). Moreover, dry weight per plant and leaf area are highly influenced by the light use efficiency and carbon assimilation pathway as is show in the model diagram (appendix 7). The fact that K was not measured but estimated, leaves for slight under or over estimations of LUE that have large effect on LAI and plant DM. The model outputs are within an acceptable range so the model can be seen as "fit"' to the tested alternative environmental conditions.

According to the literature, after certain amount of organic nitrogen is reached, lettuce plants start accumulating nitrate at low light condition which is the case of the current experiment with low total radiation (5.78 MJ/ m^2/ day) compared to the values referred to by Henriques and Marcelis (2000) with a daily total radiation of about (10.3 MJ /m^2 /day). Additionally, simulations show a high accumulation of nitrate (figure 8) under low light conditions (LUE 0.193 molC / MJPAR). Thus, results show that the growing conditions of the experiment were not favorable to prevent nitrate accumulation in plant tissues and are not recommended for aquaponic production. In order to test if measured temperature inside the greenhouse has a different output from the values downloaded from KNMI a run was performed with each of them and results show the same output (results not shown in this report). Therefore, the "weather file" does not need to be combined with the measurements for air temperature inside green house. Is recommended to use in the weather file, only the air temperature and daily total radiation downloaded from the closer weather station (Deelen) from the website (KNMI).

Determining accurate nitrate concentrations in plants using the LEPAS model, can support decisions taken by the farmer regarding the need for additional radiation (light), feeding regimes, replacement water, ventilation, shading and heating.

The water replenishment even can be modified according the location and environmental conditions. The replacement of water is done to reduce accumulation of uneaten fish feed in order to avoid denitrification. Nevertheless, if an accurate amount of feed is delivered and the recirculation system works well, an aquaponics systems can remain over long periods without replacement of water and achieve high concentrations of nutrients in water which positively affect plant growth. Finally, replacement of water must be adapted to the maximum possible time in order to save water but without affect or compromise the fish growth

Certain parameters such as pH are not used as input, which is one of the most important to achieve correct functioning of the three main components (plant, fish and bacteria) and is recommended to be maintained between 6.8 to 7.5 (Tyson et al., 2011). By ignoring pH the model does not allow for change in ammonia concentrations leading to suboptimal fish or plant growth when pH is outside the advised range.

It is interesting to translate an ecologically complex system (aquaponics) into a mathematical model, able to predict results that can positively or negatively affect the productivity of an aquaponic system. Nevertheless, an aquaponic system is seen as a system that can produce food not only under high resource use efficiency but also with low economic investment. Therefore, it is important to not only focus in the understanding of nitrate balance in the system but it is even more important to find accurate predictions of fish and plant growth that can be applied in the reality and can be used to achieve optimal performance of the system under diverse environmental conditions. According to the results obtained, the most important factor is the light availability which is strongly related to the plant growth and nitrate and nitrogen concentrations in the plant. Moreover, a correct fish/plant ratio must be determined in order to reduce any negative concentration of ammonium and nitrate in water and maintain a proper concentration of available nutrients to be taken up by the plant.

Finally, aquaponics are seen as a sustainable but complex food production system. Therefore, testing and calibrating the LEPAS model is important as it may simulate accurate results in many different environmental circumstances and fish/feed/plant combinations without investing money and time into many large experiments in the field. Doing this, the tool could be friendlier for farmers and researcher around the world to produce food in aquaponics systems under correct practices and resource use efficiency.

8 CONCLUSION

LEPAS model does not predict exactly the dry matter, leaf area and organic nitrogen according the measurements in a situation with low radiation and low temperature but the outputs are within acceptable range. Outputs are highly sensitive to light use efficiency, hence the need to measure as good as possible to accurate predict leaf area index and plant growth. Moreover, LEPAS is an interesting and complex tool which combines all components and factors in a combined model. Thereby, it is able to predict plant dry matter, fish growth in fresh weight, leaf area, nitrate in plant and water and nitrogen in plant, the most basic and important parameters to assess the performance of a system, using simple measurements from real experiments, and compensating the lack of measurements by literature review. The LEPAS model could be used as a tool to understand more deeply the relations between the main components of an aquaponic system (plant, bacteria and fish) and how different parameters (radiation, temperature and feed) can affect positively or negatively the nutrient balance in water and the productivity of the system.

After making first a very detailed model one can decide which factors can be simplified or left out for use in practice without compromising the outcomes. Or perhaps a simple model can be used only within

certain environmental thresholds. Or the model can be used by researchers to generate input output tables that can be used as guidelines by practitioners.

As LEPAS is the first model created for an aquaponic system, could be useful to improve the tool by testing and calibrating the model for plant nitrate concentrations. Also, reduce the complexity and make the model easier to run and to interpret the results.

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11 APPENDIX 1

Summary of the measured parameters in Aquavita farm and laboratory according the harvest date. Each value presented is the average of three plants harvested per date.

Measured data from (3/October/2016) until (2/November/2016).

	DW	FW	SLA	LAI	DMC
	g/plant	g/plant	m^2leaf/gDW	m^2leaf/m^2area	gDW/gFW
03/10/2016	0.27	5.08	0.07	0.36	0.05
06/10/2016	0.64	9.24	0.05	0.66	0.07
10/10/2016	0.58	8.26	0.05	0.54	0.07
13/10/2016	1.16	16.73	0.04	1.02	0.07
17/10/2016	1.61	23.12	0.04	1.40	0.07
20/10/2016	1.85	34.34	0.05	2.01	0.05
24/10/2016	2.45	40.44	0.05	2.27	0.06
27/10/2016	2.78	48.79	0.05	2.63	0.06
31/10/2016	2.93	49.93	0.05	2.87	0.06
02/11/2016	3.30	62.07	0.05	3.23	0.05

Parameters	Description
DW	Average dry weight per plant
SLA	Average Specific leaf area per plant = m^2 leaf/g DW
LAI	Average leaf area index per plant = m^2 leaf/m^2 growing space
DM	Dry matter per plant = g DW/g FW

12 APPENDIX 2

Summary table including the model abbreviations linked to a function in the model as in reality, data measured in the experiment and literature review used as a constant or function to run the model.

Abbreviation	**Meaning**	**Unit**	**Value**	**Source**
ALFAC	α C	gDM/molC	30	Linker *et al*., 2004
ALFAN	α N	gDM/molN	101	Linker *et al*., 2004
ALNU	equivalent of chosen ALNUCORR by function	1/gDW		
ALNUCORR	α	1/gDW	0.148c1, 0.151c2, 0.161c3	Zhang *et al.*, 2008
AREAF	biofilter surface area	m2	188	
AREAP	area of growing plants	m2	8.7	Measured
AVDTR	acerage DTR	MJ/m2/d	5.78	Measured
BATCHN	number of batches	1		
BC	β C	m3*kPa/molC	0.6	Seginer, 2003
BN	β N	m3*kPa/molN	6	Seginer, 2003
C	c	1/°C	0.0693	Seginer, 2003
CASS	gross carbon assimilation	molC/m2/d		
CBAL	carbon balance	molC		
CDEF	deficient carbon in vacuole	molC/m2/d		Zhang *et al.* (2008)
CSUNLIT	coefficient for respiration calculation	m2/molC	1.20	Measured
CSUR	surplus carbon in vacuole	molC/m2/d		
DELT	delta time	d	1	
DENSW	water density	g/m3	999190	Calculated

DM	total plant dry matter	kg DM		
DMC	dry matter content of plant	g DM/g FM		Measured
DMPL	dry matter per plant	g DM /plant		Measured
DTR	daily total radiation	MJ/m2/d		KNMI, 2016
DWH	initial dry weight of new plantings	g/plant		Measured
DWI	initial dry weight of first plantings	g/plant		Measured
FCONV	function to determine amount of feed to give to fish depending by TW	g feed / 100 g fish /d		Feeding Company ('SKRETTING')
FCONVCOR R	function to determine amount of feed to give to fish depending by MAW	g feed / 100 g fish /d		
FCR	feed conversion ratio	g feed/g fish growth	0.7	Calculated
FCRESP	molC/molC		0.30	Seginer, 2003
FCRO	feed conversion ratio depending by MAW	g feed/g fish growth		
FEED	Average amount of feed given per day	g feed/d	113.07	Calculated
FEEDDIGES T	feed digestibility	g feed/g feed	0.9	Seawright *et al*., 1998
FEEDNC	feed nitrogen content	gN /gDM	0.0784	Feeding Company ('SKRETTING')
FISHNC	fish nitrogen content	gN /g fish	0.0368	Ogino and Saito, 1970
FM	total plant fresh matter	kgFM		
FMPL	fresh matter per plant	g FM/plant		
FNUP	function to limit photosynthetic activity by nitrogen availability			Zhang *et al.*, 2008
FWH	initial fresh weight of new plantings	g/plant		

FWI	initial fresh weight of first plantings	g/plant	0.27	Measured
GCSTR	gross carbon accumulation as structure	molC/m2/d		
GRNW	gross nitrate accumulation in water	gN/d		
HARVESTDAYS	days in which harvest occurs	d		
JBMAX		g N/m3 film	1.55, 1.69,1.72,1.86	Zhu and Chen, 2002
JNMAX		mmol/h/gDW		
JNMAXO		mmol/h/gDW		
JNMAXOCORR		mmol/h/gDW	0.0349c1, 0.0374c2, 0.0505c3	Zhang *et al.*, 2008
K	light exctinction coefficient	m2ground/m2leaf	0.7	Constant
KMO		g N / m3	5.5, 2.0, 2.0, 2.0	Zhu and Chen, 2002
KNU		gN/m3		
KNUCORR		gN/m3	0.28c1, 0.28c2, 0.56c3	Zhang *et al.*, 2008
KR		molC/m2/d	0.0216	Seginer, 2003
LAI	leaf area index	m2/m2		
LAMBDA	λ	m3/molC	0.0009	Seginer, 2003
LIMITN	nitrate concentration in plant	mg NO3/kg FM		
LUE	light use efficiency	molC/MJPAR	0.193	Measured
MAW	ammonia in water	g N-NH4+		
MAWI	initial ammonia in water	g N-NH4+	0.78	Measured
MCEXC	carbon in excess compartment	molC/m2		
MCEXCH	carbon in excess compartment of new	molC/m2		

	plantings			
MCEXCI	carbon in excess compartment in first plantings	molC/m2		
MCS	carbon in structure	molC/m2		
MCSH	carbon in structure in new plantings	molC/m2		
MCSI	carbon in structure in first plantings	molC/m2		
MCV	carbon in vacuole	molC/m2		
MCVH	carbon in vacuole in new plantings	molC/m2		
MCVI	carbon in vacuole in first plantings	molC/m2		
MDENITR	amount of nitrogen lost by denitrification	g N		
MFSH	fish weight	g (body weight)		
MFSHI	initial fish weight	g (body weight)	38666	Measured
MMO	nitrogen converted by bacteria	g N		
MNETASS	net carbon assimilation	molC/m2		
MNFAECES	nitrogen in faeces	g N		
MNFEED	nitrogen in feed	g N		
MNFSH	nitrogen in fish	g N		
MNFSHI	initial nitrogen in fish	g N	1,422.90=(38666g* 0.0368g)	Calculated
MNS	nitrogen in structure	molN/m2		
MNSH	nitrogen in structure of new plantings	molN/m2		
MNSI	nitrogen in structure of first plantings	molN/m2		
MNU	nitrogen uptaken by plant	molN/m2		
MNV	nitrogen in vacuole	molN/m2		

MNVH	nitrogen in vacuole of new plantings	molN/m2		
MNVI	nitrogen in vacuole of fisrt plantings	molN/m2		
MNW	nitrate nitrogen in water	g N-NO3		
MNWI	initial nitrate nitrogen in water	g N-NO3	58	Measured
MRESP	carbon lost by maintenance respiration	molC/m2/d		
MTOTNU	total nitrogen uptaken by plant	g N		
N	nitrogen atomic weight	gN/molN	14	
NBALP	nitrogen balance in plant	g N		
NBALW	nitrogen balance in water	g N		
NC	r	molN/molC	0.16	Seginer, 2003
NDENITR	denitrification rate	gN/d		
NITRN	nitrate nitrogen in plant	g N-NO3/100g DM		
NNHWAT	ammonia concentration in water	g N-NNH_3 / m3		
NNO3WAT	nitrate concentration in water	g N-NO3 / m3		
PAR	photosyintetically active radiation	MJ/m2/d		
PARINT	PAR intercepted	MJ/m2/d		
PDENS	average plant density	plants /m2	20.5	Measured
PI	π	kPa	580	Seginer, 2003
RAW	rate of ammonia accumulation in water	gN/d		
RCEXC	rate of carbon accumulation in excess compartment	molC/m2/d		
RCSTR	rate of carbon accumulation in structure	molC/m2/d		
RCVAC	rate of carbon accumulation in vacuole	molC/m2/d		

RDENIT	fraction of nitrogen lost by denitrification	1/d	0.05	Calculated (Chiara)
REDN	amount of organic nitrogen in plant	g N/100g DM		
REFILLDAY	day of water refill in the system	30		
RESP	amount of carbon lost by total respiration	molC/m2/d		
REXP	rate of nitrogen expelled by fish	g N/d		
RFSH	growth rate of fish	g fish/d		
RMO	rate of microbiological nitrogen conversion in a m2 of biolfilm surface	g/d		
RMOTT	rate of microbiological nitrogen conversion	g/m2/d		
RNETASS	net carbon assimilation rate	molC/m2/d		
RNFAECES	rate of nitrogen expelled as faeces	gN/d		
RNFEED	rate of nitrogen input as feed	gN/d		
RNFSH	rate of nitrogen accumulated in fish	gN/d		
RNSTR	rate of nitrogen accumulation in structure	molN/m2/d		
RNU	rate of nitrogen uptaken by plant	molN/m2/d		
RNUAREA	rate of nitrogen uptaken in a m2 of plant growing area	molN/d		
RNUPL	rate of nitrogen uptaken per plant	molN/pl/d		
RNUTOT	rate of nitrogen uptaken by all batches	gN/d		
RNVAC	rate of nitrogen accumulation in vacuole	molN/m2/d		
RNW	rate of nitrate nitrogen accumulation in water	molN/m2/d		
RRESP	rate of carbon lost by growth respiration	molC/m2/d		
RWATVOL	fraction of water lost by evapotraspiration	m3/d	-0.02	

SLA	specific leaf area	m2 leaf/g DM	0.05	Measured
T	daily average air temperature	°C	5.33 (minimum) 12.63 (maximum)	KNMI,2016
TAO	greenhouse	%	40	Measured
TB	Tb	°C	20	Seginer, 2003
TOTAREAP	total area of growing plants	m2	8.7	Measured
TOTN	total nitrogen content in plant	gN tot/ 100 g DM plant		
TRG	absolute value of turgor demand	kPa*m3/m2/d		
TRGDEF	turgor demand	kPa*m3/m2/d		
TW	daily average water temperature	°C	14.4	Measured
V	amount of water in plant	m3/m2		
WATVOL	amount of water in the system	m3		
WATVOLI	initial amount of water in the system	m3	3.719	
Y	fraction of given feed according to FCONV function	g feed / 100 g fish /d		

13 APPENDIX 3

Summary table with the measurements obtained from field work using the SPAD meter, data obtained from the laboratory and literature as a reference to calibrate the model. The SPAD values represent the average of nine measurements per plant. The laboratory and calculations are based per single plant.

	MEASURED	Radix own data	from Henriques & Marcelis, 2000	CALCULATED
	SPAD	**TOT N**	**NO3-N**	**RED**
		gN/kgDW	gN-NO3/kgDW	gN-org/kgDW
sample1	20.34	27.40	0.48	26.92
sample2	19.78	34.30	1.56	32.74
sample3	21.76	0.00	0.00	0.00
sample4	22.36	17.40	0.00	17.40
sample5	24.72	23.60	0.00	23.60
sample6	20.06	26.60	0.35	26.25
sample7	22.80	28.30	0.62	27.68
sample8	20.38	21.10	0.00	21.10
sample9	19.92	20.00	0.00	20.00
sample10	20.88	43.60	3.01	40.59
sample11	23.23	23.70	0.00	23.70
sample12	21.63	34.10	1.53	32.57
sample13	19.06	28.80	0.70	28.10
sample14	22.59	30.60	0.98	29.62
sample15	21.82	29.50	0.81	28.69
sample16	23.84	42.00	2.76	39.24
sample17	24.61	36.60	1.92	34.68
sample18	23.91	49.20	3.89	45.31
sample19	22.86	40.90	2.59	38.31
sample20	20.88	45.70	3.34	42.36
sample21	25.28	34.90	1.65	33.25
sample22	23.28	38.70	2.25	36.45
sample23	23.89	40.40	2.51	37.89
sample24	24.19	37.40	2.04	35.36
sample25	24.96	45.30	3.28	42.02
sample26	24.48	44.40	3.14	41.26
sample27	24.71	39.20	2.32	36.88
sample28	22.49	42.70	2.87	39.83
sample29	22.34	42.80	2.89	39.91
sample30	24.08	47.30	3.59	43.71

14 APPENDIX 4

Summary table of the total feed delivered to the fish tanks in grams per day and the fish growth based on real amount of fish feed given.

DAY	FISH FEED g/day	REAL FISH GROWTH (initial fish weight + grams feed day / FCR / 1000)
03-oct	140	20.64
04-oct	140	20.84
05-oct	140	21.03
06-oct	140	21.23
07-oct	70	21.33
08-oct	140	21.53
09-oct	140	21.72
10-oct	70	21.82
11-oct	140	22.02
12-oct	140	22.22
13-oct	210	22.52
14-oct	0	22.52
15-oct	140	22.71
16-oct	70	22.81
17-oct	70	22.91
18-oct	140	23.11
19-oct	70	23.21
20-oct	140	23.40
21-oct	70	23.50
22-oct	70	23.60
23-oct	70	23.70
24-oct	140	23.90
25-oct	70	24.00
26-oct	140	24.20
27-oct	70	24.29
28-oct	70	24.39
29-oct	140	24.59
30-oct	70	24.69
31-oct	70	24.79
01-nov	86	24.91
02-nov	3166g	24.91

Fish (Tank 1) initial weight: 20.44kg Fish (Tank 1) final weight: 21.79

Fish (Tank 2) initial weight: 18.22kg Fish (Tank 2) final weight: 21.34

Plant area covered by crop: 8.7m^2 Fish conversion ratio: 0.7

Actual ratio: 12.99g (feed/m^2plant area)

15 APPENDIX 5

Summary of downloaded data from KNMI used as an input file for the model. Due to the model functions dependent on the radiation and temperature to predict plant growth.

```
*       Country:  Netherlands
*       Station:  Deleen
*       Year:     2016
*       Source:   KNMI
*       Author:
*       Longitude:          E
*       Latitude: N
*       Elevation:          50      m.
*       Comments:           Deleen:  "data,"  vap.  pressure  calculated  from  rel.  humid.
Columns:
*       ========
*       station   number
*       year
*       day
*       irradiation (kJ     m-2     d-1)
*       minimum temperature         (degrees Celsius): RECORDED DATA
*       maximum temperature         (degrees Celsius): RECORDED DATA
*       vapour    pressure (kPa) : NOT DOWNLOADED
*       mean wind speed (m s-1) : NOT DOWNLOADED
*       precipitation (mm d-1) : NOT DOWNLOADED
5.67    51.97     7       0       0
275     2016      277     7560    4       13.7    0       0       0
275     2016      278     5430    9.6     14.4    0       0       0
275     2016      279     8700    5       14.9    0       0       0
275     2016      280     8750    -1.1    13.8    0       0       0
275     2016      281     9220    -0.8    13.3    0       0       0
275     2016      282     4260    4       12.3    0       0       0
275     2016      283     3940    7       10.3    0       0       0
275     2016      284     5510    6.6     11.5    0       0       0
275     2016      285     7080    6.9     13.2    0       0       0
275     2016      286     2010    7.9     13.4    0       0       0
275     2016      287     10090   7.5     18.8    0       0       0
275     2016      288     6150    7.8     16      0       0       0
275     2016      299     4050    7.3     13.1    0       0       0
275     2016      290     3010    4.6     11.2    0       0       0
275     2016      291     2330    5       11.3    0       0       0
275     2016      292     3790    4.3     10.1    0       0       0
275     2016      293     250     -2      10.3    0       0       0
275     2016      294     8930    -1.7    11      0       0       0
275     2016      295     2930    4       8.4     0       0       0
275     2016      296     2090    7.5     9.7     0       0       0
275     2016      297     5240    7.3     12.4    0       0       0
275     2016      298     2560    8.9     13.6    0       0       0
275     2016      299     1540    9       13.7    0       0       0
275     2016      300     7380    3.2     14.3    0       0       0
275     2016      301     5400    3.7     15.1    0       0       0
275     2016      302     6980    4.8     16      0       0       0
275     2016      303     2550    2.5     13.1    0       0       0
275     2016      304     6220    1       10.9    0       0       0
```

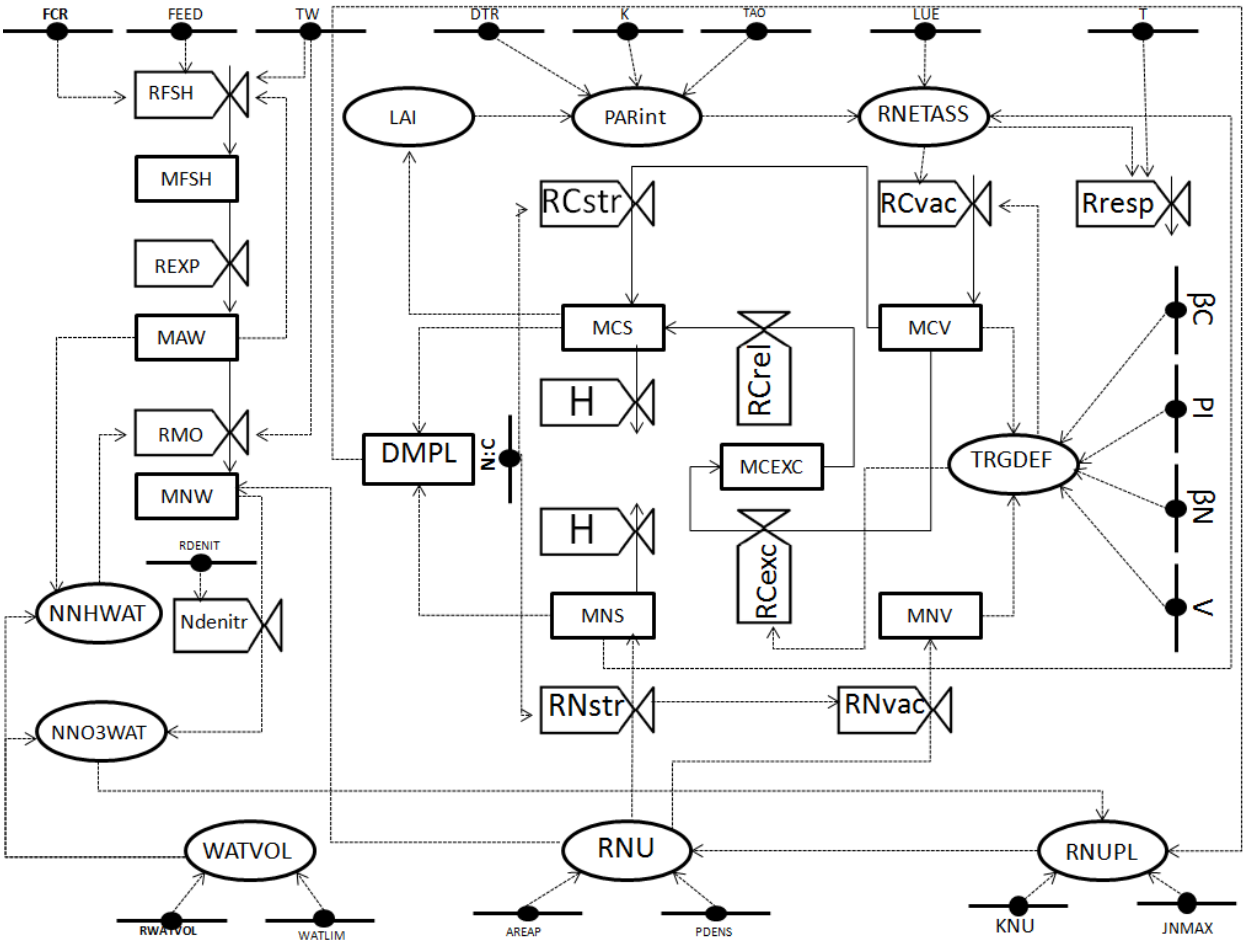
16 APPENDIX 6

Present the real amounts of feed given to the fish tanks and temperature in water recorded from the field experiment and combined in a file use as input for the model to predict nitrate concentrations, fish and plant growth as a relation of the amount of feed given.

date	time	feedp	feedt		tempwat
2016-10-03	00:00:00	0.	140.		21.2
2016-10-04	00:00:00	0.	140.		-9999.
2016-10-05	00:00:00	0.	140.		-9999.
2016-10-06	00:00:00	0.	140.		17.2
2016-10-07	00:00:00	0.	70.		-9999.
2016-10-08	00:00:00	0.	140.		-9999.
2016-10-09	00:00:00	0.	140.		-9999.
2016-10-10	00:00:00	0.	70.		15.6
2016-10-11	00:00:00	0.	140.		-9999.
2016-10-12	00:00:00	0.	140.		-9999.
2016-10-13	00:00:00	0.	210.		14.6
2016-10-14	00:00:00	0.	0.		-9999.
2016-10-15	00:00:00	0.	140.		-9999.
2016-10-16	00:00:00	0.	70.		-9999.
2016-10-17	00:00:00	0.	70.		15.9
2016-10-18	00:00:00	0.	140.		-9999.
2016-10-19	00:00:00	0.	70.		-9999.
2016-10-20	00:00:00	0.	140.		12.6
2016-10-21	00:00:00	0.	70.		-9999.
2016-10-22	00:00:00	0.	70.		-9999.
2016-10-23	00:00:00	0.	70.		-9999.
2016-10-24	00:00:00	0.	140.		10.1
2016-10-25	00:00:00	0.	70.		-9999.
2016-10-26	00:00:00	0.	140.		-9999.
2016-10-27	00:00:00	0.	70.		12.9
2016-10-28	00:00:00	0.	70.		-9999.
2016-10-29	00:00:00	0.	140.		-9999.
2016-10-30	00:00:00	0.	70.		-9999.
2016-10-31	00:00:00	0.	70.		12.6
2016-11-01	00:00:00	0.	86.		-9999.
2016-11-02	00:00:00	0.	0.		11.

17 APPENDIX 7

System diagram of an aquaponic system build on mathematic functions. The meaning of the variables combined in the model can be found in Appendix 2.



TITLE LEPAS

* Model Author: Chiara Perrini (2014).

* MSc. Thesis student: Luis Rosado (2017)

*January 2017

*Wageningen University

* Supervisor: Maja Slingerland

* Examiner: Tom Schut

DEFINE_CALL
INITCN(INTEGER_INPUT,INTEGER_INPUT,INPUT_ARRAY,INPUT_ARRAY,OUTPUT_ARRAY,
OUTPUT_ARRAY,OUTPUT_ARRAY,OUTPUT_ARRAY,...

OUTPUT_ARRAY,REAL_INPUT,REAL_INPUT,REAL_INPUT,REAL_INPUT,REAL_INPUT,REAL
_INPUT,REAL_INPUT,REAL_INPUT,INPUT,INPUT)
DEFINE_CALL FNU (INTEGER_INPUT, INPUT_ARRAY, OUTPUT_ARRAY)
DEFINE_CALL NEWPLANT(INTEGER_INPUT,INTEGER_INPUT, INPUT_ARRAY,
INPUT_ARRAY, INPUT_ARRAY, INPUT_ARRAY, INPUT_ARRAY, ...
INPUT_ARRAY, INPUT_ARRAY, INPUT_ARRAY, REAL_INPUT, REAL_INPUT,
REAL_INPUT, OUTPUT_ARRAY, OUTPUT_ARRAY,...
OUTPUT_ARRAY, OUTPUT_ARRAY, OUTPUT_ARRAY, OUTPUT, INPUT,
INPUT,INPUT,INPUT,INPUT,INPUT)

ARRAY
MNS(1:J),MNSI(1:J),MNV(1:J),MNVI(1:J),MCS(1:J),MCSI(1:J),MCV(1:J),MCVI(1:J),MCEXC(1:J),M
CEXCI(1:J),PARINT(1:J),CASS(1:J),...
MRESP(1:J),RRESP(1:J),RESP(1:J),
MNW(1:J),JNMAX(1:J),NNO3WAT(1:J),RNETASS(1:J),RNUPL(1:J),RNU(1:J),RNW(1:J),RCVAC(1:J
), ...

RNVAC(1:J),RCEXC(1:J),RCSTR(1:J),RNSTR(1:J),CSUR(1:J),CDEF(1:J),V(1:J),TRGDEF(1:J),TRG(1
:J),LAI(1:J),DM(1:J),DMPL(1:J),...

FM(1:J),FMPL(1:J),NITRN(1:J),REDN(1:J),TOTN(1:J),DWI(1:J),FWI(1:J),DMC(1:J),GCSTR(1:J),NDENITR(1:J),RNUAREA(1:J),FNUP(1:J),...

CBAL(1:J),MNETASS(1:J),MNU(1:J),NBALP(1:J),NBALW(1:J),MDENITR(1:J),MTOTNU(1:J),LIMITN(1:J), NITRNSEG(1:J), ...

DWH(1:J),FWH(1:J),MCSH(1:J),MCVH(1:J),MCEXCH(1:J),MNSH(1:J),MNVH(1:J),HARVESTDAYS(1:J)

* A(1:J), B(1:J), D(1:J), E(1:J), F(1:J)

ARRAY_SIZE J=1

INITIAL

**********CONSTANTS**********

***** PLANT *****

** BC (m3*kPa/molC) and BN (m3*kPa/molN) are the osmotic value of carbon and nitrogen, NC (molN/molC), N (g/molN)

CONSTANT BC=0.6; BN=6.; NC=0.16; N=14.

** Plant respiration: KR a respiration coeff (molC/m2/d), C respiration increase per degree (1/°C), TB the basal temperature (°C)

CONSTANT KR=0.0216; C=0.0693; TB=20.

** LAMBDA is a coefficent relating carbon to water in plant (m3/molC), PI the osmotic turgor needed in the vacuole (kPa)

CONSTANT LAMBDA=0.0009; PI=580.

** ALFAC coefficient relating DM to mol C (gDM/molC)

CONSTANT ALFAC=30.

** ALFAN coefficient relating DM to mol N (gDM/molN)

CONSTANT ALFAN=101.

***** WATER *****

** water density at 14.4°C (g/m3)

CONSTANT DENSW=999190.

**********PARAMETERS**********
****

***** FISH *****

** N content of fish, gN /g fish = 1/6.25 (gN / g protein) * 0.23 g protein / g fish

PARAMETER FISHNC = 0.0368

** N content of feed, gN /gDM = 1/6.25 (gN / g protein) * 0.49 g protein / gDM

PARAMETER FEEDNC = 0.0784

** Digestibility of the feed N (fraction)

PARAMETER FEEDDIGEST = 0.9

***** BACTERIA *****

** AREAF is the surface area (m2) of biofilter

PARAMETER AREAF=188.

** DENITR is a fixed rate of nitrogen lost my denitrification (5% of total nitrate) (1/d)

PARAMETER RDENIT=0.

***** PLANT *****

** K is the light exctinction coefficient(m2ground/m2leaf),LUE the light use efficiency(molC/MJPAR),TAO the gh+net transmissivity(%)

PARAMETER K=0.7

PARAMETER LUE=0.193

PARAMETER TAO=0.4

** SLA is the specific leaf area (m2 leaf/g DM)

PARAMETER SLA=0.05

** PDENS is the average plant density (plants /m2)

PARAMETER PDENS=20.5

** AREAP is the area (m2) of plant grown per batch

PARAMETER AREAP=8.7

** BATCHN is the number of batches

PARAMETER BATCHN=1.

** FRESP is the fraction of C lost as growth respiration

PARAMETER FCRESP=0.30

** CSUNLIT is the area of sunlit per molC of plant material (m2 / molC)

```
PARAMETER CSUNLIT= 1.20

***** WATER *****
** RWATVOL is the change in (m3/d) watervolume due to evapotranspiration
PARAMETER RWATVOL=-0.02
** REFILLDAY is the day when water is refilled to intial value
PARAMETER REFILLDAY=30.
**********************************************************SETTINGS**********
****************************************
*********************************************************************************
****************************************
** Periodic harvesting simulation parameters (days)
PARAMETER HARVESTDAYS(1:J)=1.
** Initial harvest number
SETTING HARVNR=0.
** Values for intial weights of plants replacing harvested plants at times defined in HARVESTDAYS (g/plant)
PARAMETER DWH(1:J)=0.27
PARAMETER FWH(1:J)=5.08
**************************************************
** Periodic harvesting on fresh weight          **
*PARAMETER FIRST=1.; PERIOD=1.
**************************************************

** initial and final time for computation
TIMER STTIME=277.; FINTIM=304.; DELT=0.1; PRDEL=0.1

** specified outputs
PRINT  MFSH, NNO3WAT , NNHWAT, DMPL, LAI , REDN , NITRN, RNSTR, RNVAC, LIMITN


TRANSLATION_GENERAL DRIVER = 'RKDRIV'
*********************************************************************************
****************************************
```

**********INITIALS**********
****

** Call subroutine which calculates initial carbon and nitrogen content in the 3 compartments as function of the given initial DW&FW

CALL
INITCN(1,J,DWI,FWI,MCSI,MNSI,MCVI,MNVI,MCEXCI,LAMBDA,BC,BN,PI,NC,DENSW,ALFAC,ALFAN,PDENS,N)

INCON ZERO=0.

***** FISH *****

** initial fish weight in g fish (MFSHI) and g N in fish = MFSHI*FISHNC

INCON MFSHI=38666.; MNFSHI =1422.91

***** PLANT *****

** DM and FM initial in g/m2

PARAMETER DWI(1:J)=.27

PARAMETER FWI(1:J)=5.08

***** WATER *****

** WATVOLI is the initial amount of water (m3) recirculating in the system

INCON WATVOLI=3.719

** initial amounts of ammonia and nitrate in the water (gN)

INCON MAWI=0.78; MNWI=58.

**********FUNCTIONS**********
****

***** FISH *****

** function to relate water temperature to feeding ratio as percentage of the fish mass

*FUNCTION FCONV=16.,0.9, 18.,1., 20.,1.2, 22.,1.3, 24.,1.2, 26.,1.1, 28.,0.8

FUNCTION FCONV=12.,0.7,14.,0.8,16.,0.9, 18.,1., 20.,1.2, 22.,1.3, 24.,1.2, 26.,1.1, 28.,0.8

** function to calculate FCR depending by ammonia concentration in the water

FUNCTION FCRO=0., .7, 2., 1.8, 3., 1.9, 4., 2., 5., 3.0, 6., 5.0

** correction of feed supplied for reduced growth as function of [NH4]

```
FUNCTION FCONVCORR=0., 1.0, 1., 1.0, 1.5, 1.0, 2., 0.8, 2.5, 0.6, 3., 0.1

***** BACTERIA *****
** Monod JBMAX (g N/m3 film) with K  value for removal rate of nitrate KMO (g N / m3)
FUNCTION KMO=  8.,  5.5, 14., 2.0,  20., 2.0,  27., 2.0
FUNCTION JBMAX=8., 1.55, 14., 1.69, 20., 1.72, 27., 1.86

***** PLANT *****
** function to correct KNU, ALNU and JNMAXO values according to daily average total radiation inside the gh
FUNCTION KNUCORR=3.7, 0.28, 5., 0.28, 7.1, 0.56
FUNCTION ALNUCORR=3.7, 0.148, 5., 0.151, 7.1, 0.161
FUNCTION JNMAXOCORR=3.7, 0.0349, 5., 0.0374, 7.1, 0.0505

**********************************************************WEATHER**********************
*****************************************
WEATHER CNTR='NLD'; ISTN=275; IYEAR=2016

** Measurement file for the REAL amount of feed given to healthy fish (feedp), totally (feedt) and measured TW (tempwat)
MEASUREMENTS Datafile='FEEDDATA.txt'
MEASURED feedp,        feedt,     tempwat
FEEDIN = feedt

DYNAMIC

** J/M2/d to MJ/m2/d
DTR=RDD/1.E+6
** average daily total radiation INSIDE the gh (MJ/m2/d)
AVDTR=DTR*TAO
** average temperature (°C)
T=0.5*(TMMN+TMMX)
** Photosynthetically active radiation (MJ/m2/d)
PAR=0.5*DTR
```

```
** TW is the temperature of the water (°C)
** TW=tempwat
TW = INSW(tempwat, 20., 21.)

**********************************************************STATES**************************
****************************************
***** FISH *****
*Mass of fresh fish,  g (body weight)
MFSH=INTGRL(MFSHI,RFSH)

***** BACTERIA *****
*Mass of nitrogen converted to nitrate, g N
MMO=INTGRL(ZERO,RMO)

***** PLANT *****
*Mass of carbon in structural material, molC/m2
MCS(1:J) =INTGRL(MCSI(1:J),RCSTR(1:J))
*Mass of carbon in the vacuolar compartment molC/m2
MCV(1:J) =INTGRL(MCVI(1:J),RCVAC(1:J))
*Mass of carbon in the excess compartment molC/m2
MCEXC(1:J)=INTGRL(MCEXCI(1:J),RCEXC(1:J))
*Mass of nitrogen in structural material molN/m2
MNS(1:J)  =INTGRL(MNSI(1:J),RNSTR(1:J))
*Mass of nitrogen in the vacuolar compartment molN/m2
MNV(1:J) =INTGRL(MNVI(1:J),RNVAC(1:J))

***** WATER *****
*Mass of nitrogen in form of ammonia in water   g N-NH4
MAW  =INTGRL(MAWI,RAW)
*Mass of nitrogen in form of nitrate in water   g N-NO3
MNW =INTGRL(MNWI,RNW)

**********************************************************************************
****************************************
```

**********FISH MODEL**********

**growth rate of the fish indirectly dependent by water temperature through FEED calculation

* g fish/d = (g feed/d) / (g feed/g fish growth)

*RFSH=FEED/FCR

**FCR is in g feedin/g fish growth

FCR=AFGEN(FCRO, NNHWAT)

**growth rate of the fish dependent by MEASURED amount of feed given to the fish

* g fish/d = (g feed/d) / (g feed/g fish growth)

RFSH=FEEDIN/FCR

**nitrogen expelled by the fish (g N/d)

REXP=RNFEED-RNFSH-RNFAECES

**nitrogen in feed (g/d)

* gN/d = g feed/d * g N/ g feed

*RNFEED = FEED * FEEDNC

**nitrogen in feed (g/d) according to MEASURED amount of feed given to the fish

RNFEED = FEEDIN * FEEDNC

**nitrogen retained in the fish (g/d)

* gN/d = g fish/d * g N/ g fish

RNFSH = RFSH * FISHNC

**nitrogen excreten in faeces and removed from the system consdering indigestibility(g/d)

* gN/d = g feed/d * fraction

RNFAECES = RNFEED*(1. - FEEDDIGEST)

**simulated feeding ration

* g feed/d = g feed / g fish/d * g fish

```
FEED    = Y * MFSH
**percentage to calculate the daily feed ration
* g feed / 100 g fish /d
Y=(AFGEN(FCONV, TW) / 100.) * AFGEN(FCONVCORR, MAW/WATVOL)

**WATVOL (m3) calculated daily to adjust the concentration of ammonia and nitrate in the water
WATVOL=INTGRL( WATVOLI , RWATVOL)

*************************************************************************************************
****************************************
*****************************************************BACTERIA
MODEL*********************************************************
*************************************************************************************************
****************************************
**rate of ammonia nitrogen supply in the water (g/d)
RAW=REXP-RMO

**rate of ammonia conversion using the Monod approach:
* g/m2/d  =        g/m2/d   * g N/m3 / (g N/m3 + g N/m3)
RMOTT   =   AFGEN(JBMAX, TW) * NNHWAT / (NNHWAT + AFGEN(KMO, TW))

**concentration of ammonia in water (g N-NNH4 / m3)
NNHWAT= MAW/WATVOL

**rate of ammonia conversion: in a time step cannot be larger than amount available(and it is constrained into only positive values)
* g/d =    gN/d            gN/d   ,   m2  * g/m2/d
RMO=INSW(MAW/DELT, 0., MIN(MAW/DELT, AREAF * RMOTT))

*************************************************************************************************
****************************************
*****************************************************PLANT
MODEL*********************************************************
```

```
**********************************************************************************************
**************************************
**call subroutine which adjusts the photosynthetic rate depending by the nitrogen availability
CALL FNU(J, TOTN, FNUP)

**********************************************************CARBON:
** intercepted PAR in MJ/m2/d
PARINT(1:J)=TAO*PAR*(1.-EXP(-K*LAI(1:J)))

**gross carbon assimilation (molC/m2/d)
* molC/m2/d= MJ/m2/d*molC/MJ
CASS(1:J)=PARINT(1:J)*LUE*FNUP
**net carbon assimilation (molC/m2/d)
RNETASS(1:J)=CASS(1:J)-RESP(1:J)

**carbon lost by maintenance respiration (molC/m2/d), which is function of temperature and sunlit area
MRESP(1:J)=KR*EXP(C*(T-TB))*(1.-EXP(-CSUNLIT*MCS(1:J)))
**carbon lost by growth respiration (molC/m2/d), wich is function of growth (gross)
RRESP(1:J)=INSW(GCSTR(1:J), 0., FCRESP * GCSTR(1:J))
**gross rate of carbon partitioned in the structure (molC/m2/d)
GCSTR(1:J)=MIN(CASS(1:J), RNU(1:J)/NC)
**total carbon lost by respiration (molC/m2/d)
RESP(1:J)=MRESP(1:J)+RRESP(1:J)

**********************************************************NITROGEN:
**N uptake limited by nitrate availability in water and constrained into only positive values
* molN/m2/d=            gN /( d * gN/molN * m2), molN/pl/d * pl/m2
RNU(1:J)= INSW (MNW, 0., MIN (MNW/(DELT*N*TOTAREAP), RNUPL(1:J) * PDENS))
**N uptake per plant
* molN/pl/d  =  mmol/h/gDW   *  gN/m3 /(gN/m3 + gN/m3) * gDW/pl    * h/d * mol/mmol
RNUPL(1:J)   =   JNMAX (1:J) * NNO3WAT/(KNU + NNO3WAT) * DMPL(1:J) * 24. / 1000.
**N uptake per batch
* molN/d    = molN/m2/d * m2
RNUAREA(1:J)= RNU(1:J)  * AREAP
```

```
**total area covered with plants (m2)
TOTAREAP=AREAP*BATCHN
**maximum uptake rate corrected for plant size(mmol/h/gDW)
JNMAX(1:J)=JNMAXO*EXP(-ALNU*DMPL(1:J))
**concentration of nitrate in water (g N-NO3 / m3)
NNO3WAT =MNW / WATVOL
**M.M. constant for nitrogen uptake as function of daily amount of light (gN/m3)
KNU=AFGEN(KNUCORR, AVDTR)
**coefficent for nitrogen uptake as function of daily amount of light (1/gDW)
ALNU=AFGEN(ALNUCORR, AVDTR)
**maximum uptake rate as function of daily amount of light (mmol/h/gDW)
JNMAXO=AFGEN(JNMAXOCORR, AVDTR)

**gross rate of nitrate supply in the water, not considering denitrification rate
* gN/d = gN/d -          molN/d    *  gN/molN
GRNW  = (RMO  - ARSUMM(RNUAREA,1,J)*   N)
**nitrate nitrogen in the water considering a spontaneous denitrification rate
* gN/d = gN/d * gN/gN
RNW    = GRNW  * (1.-RDENIT)
**rate of nitrogen lost by denitrification
* gN/d = gN/d * gN/gN
NDENITR= GRNW * RDENIT

**********************************************structure formation:

**RCVAC: rate of carbon to vacuole (molC/m2/d)
**   if turgor deficiency is negative there is surplus of carbon(CSUR)and it is diverted into excess compartment (negative RCVAC(*))
**  if trgdef is 0 no need of RCVAC
**  if trgdef is positive, rate of carbon into vacuole equals deficit (CDEF)
RCVAC(1:J)=FCNSW(TRGDEF(1:J), -CSUR(1:J), 0., CDEF(1:J))
**rate of nitrogen to vacuole (molN/m2/d)
RNVAC (1:J)= RNU (1:J)- RNSTR(1:J)
```

**rate of carbon going into excess compartment (molC/m2/d)
**if rcvac positive is going to be subtracted;if negative(*)it means that there is surplus in the vacuole and it is added into RCEXC
RCEXC(1:J)=RNETASS(1:J)-RCVAC(1:J)-RCSTR(1:J)

**rate of carbon and nitrogen in the structure (molC(N)/m2/d)
**minimum value between the nitrogen or carbon availability relativily to the ratio needed to build structure (NC)
**carbon available is the sum of net assimilation, (-,+) carbon in the vacuole, and what stored in the EXC compartment
**nitrogen available is the sum of nitrogen uptaken and what stored in the vacuole
RCSTR(1:J)=MIN(RNETASS(1:J)-RCVAC(1:J)+MCEXC(1:J)/DELT,
(RNU(1:J)+MNV(1:J)/DELT)/NC)
RNSTR(1:J)=MIN(RNU(1:J)+MNV(1:J)/DELT, NC*RCSTR(1:J))

**csur, cdef in kPa*m3/m2/d
**surplus of carbon: the amount that goes out is limited by the C availability into the vacuole and it equals the surplus(TRG)

*TS: molC/m2/d = molC/m2 , (kPa m3 m-2 * m-3 kPa-1 molC * d-1)
CSUR(1:J) = MIN(MCV(1:J) / DELT , TRG(1:J) / (BC * DELT))

**deficiency of carbon: the amount that goes into the vacuole is limited by the maximum amount available and it equals the demand
*TS: molC/m2/d = molC/m2/d + molC/m2 * 1/d , kPa m3 m-2 * m-3 kPa-1 molC * d-1)
CDEF(1:J) = MIN(RNETASS(1:J)+ MCEXC(1:J)/ DELT, TRG(1:J) / (BC * DELT))

**TRGDEF: turgor deficiency
*kPa m3 m2 = kPa * m3 m-2 - molC m-2 * kPa m3 molC-1 - molN m-2 * kPa m3 molN-1
TRGDEF(1:J) = PI * V(1:J) - MCV(1:J) * BC - MNV(1:J) * BN

*kPa m3 m2 = kPa * m3 m-2
TRG(1:J) = ABS(TRGDEF(1:J))
**volume of water (m3/m2) in the plant is related to carbon assimilated in the structure

* m3/m2= molC/m2 * m3/molC
V(1:J) = MCS(1:J)* LAMBDA

**leaf area index (m2leaf/m2ground)
* m2/m2 = molC/(m2ground) * (m2leaf/g DM) * gDM/molC
LAI(1:J)= MCS(1:J) * SLA * ALFAC

**********Dry matter:
* kg DM = (molC/m2 * gDM/molC + molN/m2 * g DM/molN) * m2 * kg/g
DM(1:J) =((MCS(1:J)+ MCV(1:J) + MCEXC(1:J))* ALFAC + MNV(1:J)* ALFAN) * TOTAREAP /1000.
* g DM /plant = kgDM * m2/plant * 1/m2 *g/kg
DMPL(1:J) = DM(1:J)/ PDENS / TOTAREAP *1000.

**********Fresh matter:
* kgFM =g/plant * plant/m2 * kg/g
FM(1:J)=FMPL(1:J)*PDENS / 1000.
* g FM/plant= g DM /plant + gH2O/m3 * m3/m2 * m2/plant
FMPL(1:J) = DMPL(1:J) +DENSW * V(1:J) / PDENS

**dry matter content (g DM/g FM)
DMC(1:J)=DMPL(1:J)/FMPL(1:J)
**********N in plant:
**NITRN: nitriate-N concentration in plant material
*g N-NO3/100g DM = molN / m2 / (gDM/pl *plant/m2) * gN/molN * g /100 g
NITRN(1:J) = MNV(1:J) / (DMPL(1:J) * PDENS) * N * 100.
*molN-NO3/kg DM (as in Seginer, 2003)
NITRNSEG(1:J) = MNV(1:J) / (DMPL(1:J) * PDENS) * N * 100. /14. *10.
*mg NO3/kg FM = molN / m2 / (gFM/pl *plant/m2) * gN/molN * gNO3/gN * 1000 gFM/kgFM *1000 mgNO3/gNO3
LIMITN(1:J)= MNV(1:J) / (FMPL(1:J) * PDENS) * N * 4.43 *1000. *1000.
**REDN: organic-N concentration in plant material
*g N/100g DM = molN / m2 / (gDM/pl *plant/m2) * gN/molN * g /100 g
REDN(1:J) = MNS(1:J) / (DMPL(1:J) * PDENS) * N * 100.

```
**gN tot/ 100 g DM plant
TOTN(1:J)=NITRN(1:J)+REDN(1:J)
**********************************************************REFILL
WATERTANK*****************************************************************
EVENT
  FIRSTTIME REFILLDAY
  NEWVALUE WATVOL = WATVOLI
ENDEVENT
**********************************************************HARVEST**********
****************************************
**harvest EVENT based on time of harvest controlled by HARVESTDAYS
EVENT
  FIRSTTIME HARVESTDAYS(1)

  CALL
NEWPLANT(J,NINT(HARVNR)+1,HARVESTDAYS,MCS,MCV,MNS,MNV,MCEXC,FWH,DWH,PD
ENS,DENSW,LAMBDA,MCSH,MCVH,MNSH,MNVH,MCEXCH,HD,...
            PI,BC,BN,NC,ALFAN,ALFAC)
** molC(N)/m2
  NEWVALUE MCS(1:J)= MCSH
  NEWVALUE MCV(1:J)= MCVH
  NEWVALUE MNS(1:J)= MNSH
  NEWVALUE MNV(1:J)= MNVH
  NEWVALUE MCEXC(1:J)= MCEXCH

  NEXTTIME  HD
  NEWVALUE HARVNR=HARVNR + 1.

ENDEVENT

*****************************************************
**harvest EVENT based on FW (new plant values from J)  *
**invert row 26 and 27                          *
**call out rows: 90,92,94,95 and 389 to 404          *
```

```
**call in rows: 98 and 411 to 424                    *
*EVENT
*   FIRSTTIME STTIME + FIRST
*   NEXTTIME  TIME + PERIOD
*   NEWVALUE MCS(1:J)= INSW(FMPL(1:J)-120.,A,MCSI(J))
*   A(1:J)=MCS(1:J)
*   NEWVALUE MCV(1:J)= INSW(FMPL(1:J)-120.,B,MCVI(J))
*   B(1:J)= MCV(1:J)
*   NEWVALUE MNS(1:J)= INSW(FMPL(1:J)-120.,D,MNSI(J))
*   D(1:J)= MNS(1:J)
*   NEWVALUE MNV(1:J)= INSW(FMPL(1:J)-120.,E,MNVI(J))
*   E(1:J)=MNV(1:J)
*   NEWVALUE MCEXC(1:J)=INSW(FMPL(1:J)-120.,F,MCEXCI(J))
*   F(1:J)=MCEXC(1:J)
*ENDEVENT
**                                   *
**                                   *
**                                   *
*******************************************************

**********************************************************BALANCES**********
***********************************************
*Carbon Balance, molC.
CBAL (1:J) = AREAP * (MCS + MCV + MCEXC - MNETASS -MCSI -MCVI -MCEXCI)
MNETASS=INTGRL(ZERO, RNETASS)

*Nitrogen balance, gN.
*N balance in PLANT
NBALP (1:J) = AREAP * (MNSI + MNVI + MNU - MNS - MNV) * N
MNU=INTGRL(ZERO, RNU)
*N balance in WATER
NBALW(1:J)=MAWI+MNFEED+MNWI+MNFSHI-  MAW-MNW-MTOTNU-MNFSH  -MNFAECES-
MDENITR
MNFEED=INTGRL(ZERO, RNFEED)
```

```
MNFAECES=INTGRL(ZERO, RNFAECES)
MNFSH=INTGRL(MNFSHI, RNFSH)
MDENITR(1:J)=INTGRL(ZERO, NDENITR)
RNUTOT=ARSUMM(RNUAREA,1,J)*N
MTOTNU(1:J)=INTGRL(ZERO,RNUTOT)

**********************************************************RE-
RUNS*************************************************************************
END

STOP

**********************************************************SUBROUTINE_INITCN***********
*****************************************
**initial carbon and nitrogen in structural, vacuolar and excess compartment
      SUBROUTINE                                                                INITCN
(L,K,DWI,FWI,MCSI,MNSI,MCVI,MNVI,MCEXCI,LAMBDA,BC,BN,PI,NC,DENSW,ALFAC,ALFA
N,PDENS,N)

      IMPLICIT REAL (A-Z)
      INTEGER L,J,K
      REAL DWI(K), FWI(K), MCSI(K), MNSI(K), MCVI(K), MNVI(K), MCEXCI(K), NC
      DO J=1,K
*        molC/m2=       gW/plant * plant/m2* m3/gW * molC/m3
        MCSI(J)= (FWI(J)-DWI(J))* PDENS   / DENSW / LAMBDA
*        molN/m2= molC/m2*molN/molC
        MNSI(J)= MCSI(J)*NC
*        molN/m2=gDW/plant * plant/m2 * gN/gDW * molN/gN  - molN/m2
        MNVI(J)= (DWI(J)  * PDENS    * 0.05   / N)       - MNSI(J)
*             molC/m2=  m3/molC  *  kPa  *  molC/m2  *  molC/m3*kPa     -  m3*kPa/molN  *  molC/m3*kPa
*molN/m2
        MCVI(J)= LAMBDA  * PI  * MCSI(J) / BC           - (BN         / BC          *MNVI(J))
*        molC/m2= gDW/plant* plant/m2 - gDW/molN*molN/m2 *molC/gDW -  molC/m2
        MCEXCI(J)= (DWI(J)* PDENS    - ALFAN   *MNVI(J))/ALFAC    - (MCSI(J)+MCVI(J))
```

```
      END DO

      RETURN
      END
**********************************************************SUBROUTINE_FNU***************
*****************************************
**limiting function for carbon assimilation
      SUBROUTINE FNU (JJ,REDN, FNUP)
      IMPLICIT REAL (A-Z)
      INTEGER K, JJ
      REAL REDN(JJ),FNUP(JJ)

      DO K=1,JJ
        FNUP(K)=MAX(0., MIN(1., 1./3.*(REDN(K) - 2.)))
      END DO

      RETURN
      END
**********************************************************SUBROUTINE_NEWPLANT*******
*********************************************
**Resetting initial value of C and N after harvest (for units see SUBROUTINE_INITCN)
      SUBROUTINE NEWPLANT (K,HN,HARVESTDAYS,MCS,MCV,MNS,MNV,MCEXC,FWH,
     $                DWH,PDENS,DENSW,LAMBDA,MCSH,MCVH,MNSH,MNVH,
     $                MCEXCH,HD,PI,BC,BN,NC,ALFAN,ALFAC)
      INTEGER K,HN
      REAL HARVESTDAYS(K),MCSH(K),MCVH(K),MNSH(K),MNVH(K),MCEXCH(K),NC,
     $     PDENS,DENSW,LAMBDA,HD,FWH(K),DWH(K),MCS(K),MCV(K),MNS(K),
     $     MCEXC(K),MNV(K)

      HD=HARVESTDAYS(HN+1)
      MCSH= MCS
**TS:  molc /m2   g water/plant     * plant/m2 *   m3/g *  molc/m3
      MCSH(HN)= (FWH(HN)-DWH(HN)) *PDENS     / DENSW /LAMBDA
      MCVH = MCV
```

```
      MCVH(HN)= LAMBDA*PI*MCSH(HN)/BC-(BN/BC*MNVH(HN))
      MNSH = MNS
      MNSH(HN)= MCSH(HN)*NC
      MNVH = MNV
**TS: 0.01 gN/gDM in vacuole as default. Should be parameter.
*      molN /m2 = (molN/gDM=gN/gDM * molN/gN) * (gDM/m2=molN / m2 *  gDM/molN)
      MNVH(HN) = (0.01   / 14.)            *        (MNSH(HN)  *  ALFAN)
** LUIS FINAL report:
*MNVH(HN)=(DWH(HN)*PDENS*0.05/14.) - MNSH(HN)

     MCEXCH = MCEXC
      MCEXCH(HN)=(DWH(HN)*PDENS-ALFAN*MNVH(HN))/ALFAC-( MCSH(HN)+MCVH(HN))

      RETURN
      END
```