Growing the Future: Exploring Vertical Farming from a Plant Science Perspective

A pilot study in hydroponic controlled environment agriculture using *Ocimum basilicum*, *Lactuca sativa* var. *romana*, and *Lactuca sativa* L.

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Popular Science Summary

New food production methods: Vertical farming. As cities confront the dual challenges of climate change and population growth, innovative solutions are essential for food security and sustainable development. One such promising approach is vertical farming, a method that involves cultivating crops in stacked layers within controlled environments. Converting abandoned buildings or unused land in urban spaces into green oases for food production improves urban aesthetics and contributes to city sustainability while supplying the residents with fresh, nutritious food. Vertical farming as a future food production alternative that uses significantly less water and resources than traditional outdoor farming methods and with lower greenhouse gas emissions is notably supporting the United Nations Sustainable Development Goals No. 2 (Zero Hunger), No. 9 (Industry, Innovation, and Infrastructure), No. 11 (Sustainable Cities and Communities), and No. 15 (Life in Land). Cutting-edge technology such as robotics and Artificial Intelligence are promoting the next generation of vertical farming for increased autonomy and sustainability of vertical farms.

Insights from a pilot study. In this thesis project, a collaboration between SLU and SweGreen AB, we aim to contribute to the next generation of vertical farming with the expertise of a plant scientist. We tested the performance of basil, romaine, and oakleaf lettuce using two irrigation systems, ebb and flow (EF) and nutrient film technique (NFT). The findings indicate that lettuce crops perform better and have a more robust prediction in the NFT system, demonstrating the system's efficiency. The research also explores the potential of image analysis to estimate chlorophyll levels or use the association of leaf temperature and plant growth. While promising, these methods need refinement to make robust statements and utilize them for more accurate predictions and understanding of the mechanisms related to leaf temperature. Interestingly, plant growth seemed less dependent on leaf temperature when plants grew in the NFT system.

Looking Ahead. In essence, my research aims to contribute to the autonomy and increased sustainability of vertical farms for food production and underscores the importance of plant science for the next generation of vertical farming. As urban areas grow, vertical farming emerges as a key innovation, ensuring food security in urban spaces or for regions where outdoor agriculture is not feasible, i.e. food deserts. However, there is still much work to be done. Enhancing the vertical farming system in terms of sustainability and viability for a large number of people should be the aim of future studies, highlighting the crucial role that our academic and industry professionals play in shaping the future of food production.

Abstract

By introducing high-density crop production in controlled environments, vertical farming (VF) offers a sustainable solution to problems with urban food security. Incorporating plant science is necessary to improve an already functioning VF system after technological improvements and breakthroughs like robotics. This is where the following pilot study steps in: examining the output of the hydroponic vertical farming system at SweGreen AB, Stockholm, where we planted basil (*Ocimum basilicum*), romaine lettuce (*Lactuca sativa* var. *romana*), and oakleaf lettuce (*Lactuca sativa* L.). One objective is to compare plant performance, post-transplant recovery support, and growth forecast across ebb and flow (EF) and the nutrient film technique (NFT) irrigation system. Further, image analysis and chlorophyll contents were used to see whether digital images can be used as a substitute for direct measurements of chlorophyll. Leaf temperature was monitored as a proxy for plant growth that is fueled by photosynthesis. NFT was found to outperform EF for the lettuce species for plant performance and prediction accuracy. Image analysis algorithms for leaf color in RGB color channels were shown to need improvement, e.g. by machine learning, to make robust statements on the correlation to chlorophyll. For leaf temperature, it was found that in NFT leaf temperature has less influence on plant growth but additional studies are needed to fully understand the mechanisms behind it. With this study, I intend to contribute to the autonomy and sustainability of vertical farms to supply people with nutritious, fresh food in the future.

Keywords: *urban agriculture, smart farming, plant growth, AI, autonomous farming*

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Abbreviations

AI	Artificial Intelligence
BAS	basil, *Ocimum basilicum* Genovese
BM	vegetative biomass
BM(t)	predicted BM over time defined by **Formula 2**
CEA	controlled environment agriculture
CHL	chlorophyll content
EF	ebb and flow irrigation system
FIJI	Fiji is just ImageJ
GHG	greenhouse gas
GNI	greenness index
NFT	nutrient film technique irrigation system
OAK	oakleaf lettuce, *Lactuca sativa* L.
PPFD	photosynthetic photon flux density
RGB	red green blue color channels
RGR	relative growth rate
ROM	romaine lettuce, *Lactuca sativa* var. *romana*
RMSE	root mean squared error
SLU	Swedish University of Agricultural Sciences
SPAD	values of measurements taken with the SPAD meter
VF	vertical farming

1. Introduction

I was fascinated when I first saw the vertical farm SweGreen AB in the basement of Fotografiska in Stockholm and contacted them as the industrial partner for my master thesis. During this collaborative project between SweGreen AB and the Swedish University of Agricultural Sciences (SLU), we grew and monitored oakleaf lettuce, romaine lettuce, and basil plants in two hydroponic irrigation systems. Traditional agriculture has developed tools to capture parameters for field crops, and I wanted to see if we can use these advancements within the highly controlled conditions of vertical farms and thereby incorporate the plant science perspective. I use the captured data, compare two hydroponic irrigation systems concerning plant performance and prediction accuracy, and analyze correlations between vegetative biomass and morpho-physiological parameters. With this study, I aim to contribute to a sustainable and, in the future, autonomous alternative food production method for fresh, nutritious food - in vertical farms.

Food production in a changing climate

Food production has always been a major concern for humankind and developments in food production made the society that we live in today possible in the first place. To supply the growing population with food was the main goal for technological and biological innovations in agriculture. Improved varieties and practices were achieved with better knowledge about plant performance and techniques. However, some practices were not beneficial in the long run, for instance, the excessive use of synthetic fertilizer during the Green Revolution (John & Babu 2021). New environmental and/or societal conditions demand improved food production in agricultural systems, and today, we are facing a challenge that requires adaptation and rethinking - climate change.

Climate change is endangering agriculture in the form we use it right now. Drought periods, floods, or high temperatures demand higher inputs such as fertilizer and water while still resulting in reduced yield (Reidsma et al. 2009). However, the agricultural sector is not solely the victim but also contributes significantly to the total greenhouse gas emissions (GHG) and water usage (see **Figure 1A.** + **B.**).

Some causes are the emissions of machinery, transportation, and the production of mineral fertilization (Ortiz et al. 2021; Liaqat et al. 2022).

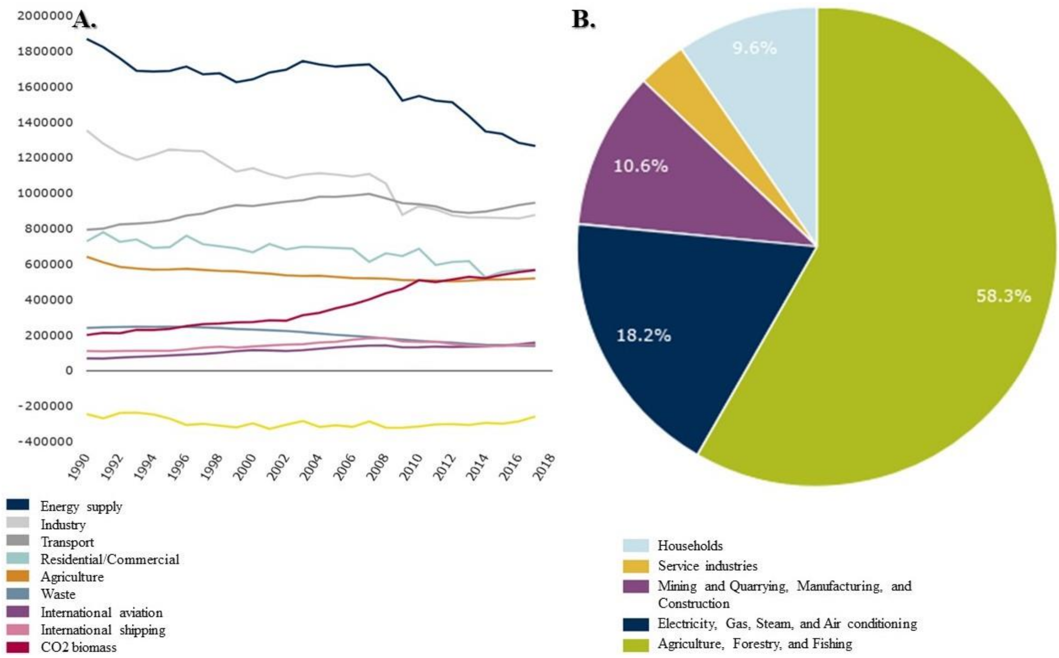

Figure 1. Overview of the contribution of agriculture to greenhouse gas emissions and water use.
***A.** Greenhouse emissions for each economic sector from 1990 to 2018; the agricultural sector is shown in orange. (EEA 2019)* ***B.** Annual water use by economic sector of 2017; the agricultural sector is shown in green (EEA 2022).*

To cover sufficient food production, most consumers used to tolerate agriculture's impact on the environment and climate change (Luís et al. 2018). With an increased awareness of climate change and the motivation to produce food with a low climate impact, consumers' awareness and innovators' interest in alternatives grew (Baiardi & Morana 2021; Califano et al. 2024). Not least because people experienced a higher frequency of droughts and heat waves even in temperate regions, and education about climate change, people became aware of the subject of research (Foley et al. 2011; Semida et al. 2019; Anderson et al. 2020; Malhi et al. 2021). One of the approaches aims to produce food independently of environmental conditions indoors in a controlled environment system. This system has the potential to also produce food in food deserts and climatic regions that are not favorable to outdoor agriculture. Especially, low- and middle-income countries will profit from an improved system and decreasing costs due to innovation (Mir et al. 2022).

By taking into account these factors, this indoor farming approach can promote the achievement of UN Sustainable Development Goals No. 2 Zero Hunger, No. 9 Industry, Innovation, and Infrastructure, No. 11 Sustainable Cities, and No. 15 Life on Land by 2030. This approach is relatively new compared to traditional outdoor production and is described by the term controlled environment agriculture (CEA). Additionally, to a lower impact on the climate by reducing GHG emissions, water usage through recirculation, and reduced pesticide/insecticide application, CEA provides locally produced fresh food independently from environmental conditions or climate change events (Kozai et al. 2020). Additionally, CEA allows large-scale production while using minimal space, for example in urban spaces (Armanda et al. 2019; Martin & Molin 2019; Kozai & Niu 2020; Huang et al. 2024).

New developments in food production

With increasing urbanization, the idea of growing food in the city directly instead of in rural regions or importing goods becomes increasingly attractive to supply more people while gaining self-sufficiency and providing fresh food (Martin & Molin 2019). Old buildings, rooftops, or even residential areas present possible spaces for growing large amounts of food in a minimal area (Specht et al. 2014).

CEA makes this possible by producing indoors!

CEA is a data-driven closed agricultural method where plants grow mostly without soil, for example in rock wool as a substrate, and do so in hydro-, aqua-, or aeroponic systems indoors under highly controlled conditions (air temperature, humidity, CO_2 content) (Mir et al. 2022). Artificial lighting using light-emitting diodes (LED) makes the production of fresh food independent of natural light and allows for year-round production. Additionally, through recirculation in the CEA system, the loss of water and nutrients (e.g. leaching) is reduced significantly compared to open-field production (Cetegen & Stuber 2021). Different forms of CEA production systems are on the market: greenhouse production and container farm production but also plant factory production (Ting et al. 2016; Butturini & Marcelis 2020). The CEA production system of vertical farming (VF) is characterized by the vertical multi-layered setup for growing plants.

A specific case of controlled environment agriculture: Vertical farming

The foundation for the modern VF was set by the proposition of soilless growing agriculture but the idea of VF was already present in the Hanging Gardens of Babylon of 600 BC, one of the world wonders of ancient times (Al-Kodmany 2018). In the 1960s, Othmar Ruthner revived the idea of Gericke to construct the first hydroponic towers for growing food (Kleszcz et al. 2020). However, at that time, VF was not appealing for common use as high costs for the energy supply of the system and associated costs for maintenance were limiting factors. In the 2000s, Dickson Despommier and Toyoki Kozai reformed the previous ideas and developed independently from each other a multilayered system to grow plants in urban spaces and thereby improve food safety and security (Despommier 2010, 2013; Kozai et al. 2020; Kozai & Niu 2020). Recent acceleration of technological advancements, sustainability interest, and investments in renewable energy production brought back the attention to VF systems as the hurdles of costs and energy supply got lower and made VF feasible, profitable, and more sustainable.

Growing plants under artificial lights in controlled environments and systems like VF is “the future food production perspective”, especially in urban spaces. Typical fast- and short-growing species for VF cultivation are leafy vegetables, e.g. lettuce and kale cultivars, as well as herbs. But also other more sophisticated species such as strawberries are grown commercially in hydroponic VFs. The advantages of VF are the independence of environmental conditions, resource efficiency, and high yields per area, e.g. SweGreen AB (68 plants per day on 6 m^2) or Grönska Stadsodling (18 times more per m^2 than outdoor production)(Molin & Martin 2018). These examples highlight the need for rules to prevent the greenwashing of vertical farms and the continued ambiguity about claims concerning absolute quantities.

Different hydroponic irrigation systems to supply the plants with water-based nutrient solution were developed with the ebb and flow (EF) and the nutrient film technique (NFT) being the most frequently installed systems (see **Figure 2**). Both are circular systems to reuse nutrients and water and work with a pump system. Whereas in EF the nutrient solution is pumped to the plant roots periodically in specific intervals, the shallow flow and the nutrient film that it forms in NFT supplies the plant with recycled nutrients and water continuously (Santosh & Gaikwad 2023). Considering the continuous supply of nutrients and water and the ability of plants to adapt to changing conditions, here excess and limited water supply, the EF system might have a constraining impact on the growth of plants as different signals induce adaptation mechanisms that are energetically favored over biomass accumulation (Voesenek & Bailey-Serres 2015; Robbins & Dinneny 2018). I wanted to examine if NFT results in better plant performance, i.e. vegetative biomass, acclimation after transplantation, and improved prediction due to more stable and constant conditions.

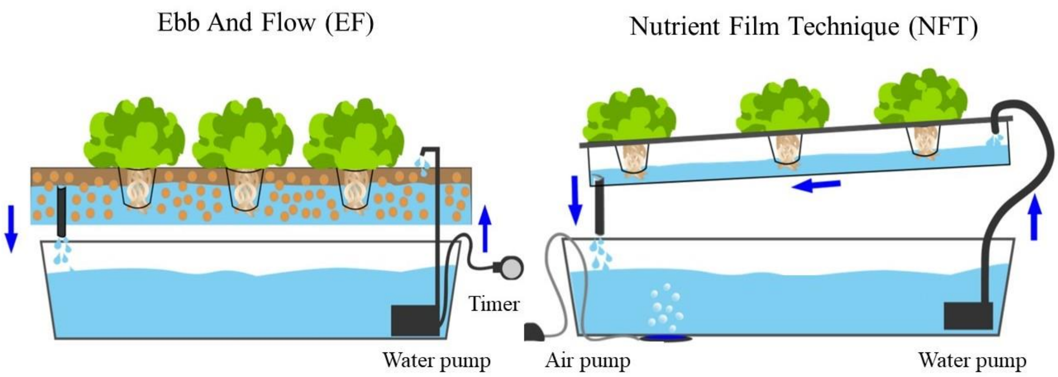

Figure 2. Illustration of ebb and flow and nutrient film technique irrigation systems for growing hydroponics.

Both hydroponic irrigation systems are circular (arrows in blue) and use a water pump system to transport the nutrient solution (light blue) from the tank to the growing platform where it passes the plant roots. Whereas the ebb and flow system floods the roots periodically on a leveled platform, the nutrient film technique constantly supplies the plant roots with nutrient solution as it creates a nutrient film. Adopted and modified from NoSoilSolutions (https://www.nosoilsolutions.com/6-different-types-hydroponic-systems/, last accessed 23/05/2024).

Cities, regions, and counties can, with the right infrastructure in place, produce high-quality fresh food on-site and thereby reduce transportation and food waste (Lovell 2010; Specht et al. 2014; Martin & Molin 2019). For governments and policymakers, VF is interesting as this growing system presents an opportunity to become less dependent on food imports and thereby, contributes to the independence of cities and entire countries. Despite these advantages, policymakers and consumers are still hesitant to integrate growing systems in residential areas and use the spaces in cities and population-dense regions as well (Benis & Ferrão 2018; Van Gerrewey et al. 2021). Reasons may be the uncertainties in the regulation of reusing urban spaces and the lack of information and education about food production which may cause hesitation among consumers (Ares et al. 2021; Califano et al. 2024).

Nevertheless, VF attracted developers and investors like Jeff Bezos (investment in Plenty VF 2017) or the European Investment Bank (EIB 2021) resulting in many start-ups and a huge interest of stakeholders. Funding and the attraction of tech companies made new technological advancements possible for second-generation technologies in vertical farms. Robots with sensors, Artificial Intelligence (AI), and imaging techniques such as digital and infrared cameras allow real-time insight into the system. Additionally, improved models for growth in VF with optimal conditions will be useful to predict plant growth and further improve quality.

The plant perspective on vertical farming

While the technological advancements have been rapid, the expertise of plant scientists is now needed to interpret the outputs of technology and subsequently use these new tools for high yields of high-quality plants and increased resource efficiency in VF (Beacham et al. 2019; Van Delden et al. 2021; Van Gerrewey et al. 2021). In this pilot project, I combine biological concepts such as photosynthesis and the importance of leaf temperature with imaging techniques, infrared camera measurements, and methods used in the field on plants growing in a vertical farm. For this purpose, I examine several correlations of plant parameters: the correlation of leaf chlorophyll content and the leaf color index from digital images as well as the leaf color index with vegetative biomass measurements, and the association between leaf temperature and plant growth.

For the biological background, I considered the concepts of photosynthesis (please find more detailed explanations in **Supplement S1**) and leaf temperature. In brief, photosynthesis as one of the major biochemical pathways for energy metabolism is the process happening in green plants in both light-dependent and light-independent reactions. The light-dependent reaction is responsible for providing energy for the light-independent reaction in the Calvin Cycle that produces sugar from fixed CO_2, which is taken up from the atmosphere through leaf openings, the stomata. The general formula for the light reaction part of photosynthesis describes this process (**Formula 1**).

Formula 1: Extended general formula for photosynthesis. Adapted Taiz et al. (2015)

$$6\ CO_2 + 12\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$$

Leaf temperature and photosynthesis are interconnected as the enzyme catalyzing the CO_2 fixation, RuBisCO, has an optimal temperature for catalysis which lies between 20 to 25 °C (Kobza & Edwards 1987). Higher temperature reduces the activity of RuBisCo and therefore high leaf temperatures reduce photosynthesis rates significantly (Kobza & Edwards 1987). The stomata play a major role in controlling leaf temperature, as transpiration of water vapor as a byproduct of photosynthesis ensures optimal temperatures in the leaf plus CO_2 is taken up for fixation through these leaf openings. In conditions with restricted water supply, e.g. a drought period, however, the plant closes the stomata to reduce water loss, i.e. reducing transpiration, which, in turn, means no leaf temperature regulation is possible, nor any CO_2 fixation can be accomplished. So, a double effect in these conditions is influencing photosynthetic efficiency and plant growth and, in the long run, plant performance, i.e. biomass accumulation. Recent studies considering leaf temperature, among other factors, are conducted in CEA and smart farming to monitor plant performance in real time and support mathematical models for plant growth with the ultimate goal of building self-sufficient farms (Amitrano et al. 2020; Avgoustaki et al. 2022; Son et al. 2023).

To contribute to autonomous VF by including plant science concepts for interpretation, I define three working hypotheses for this thesis project based on literature and the possibilities at SweGreen AB:

(I) plants (basil, romaine lettuce, and oakleaf lettuce) growing in the NFT irrigation system a) perform better, i.e. accumulate more vegetative biomass, b) have a better recovery, and c) have a higher accuracy for plant growth prediction modeling compared to the EF irrigation system.

(II) image analysis is a method for monitoring plants regarding morpho-physiological parameters and plant performance in VF.

(III) leaf temperature is associated with the plant growth rate.

The thesis work will cover the material and methods (section 2) that were used to explore the working hypotheses, present the results (section 3), and discuss the results in a larger context (section 4). It will conclude with the main remarks and prospects.

2. Material and methods

Definition of parameters

According to Violle et al. (2007), plant performance is the outcome of functional traits, i.e. morphological, physiological, and phenological, and performance traits, i.e. vegetative biomass, reproductive output, and plant survival. For simplicity and because of the ambiguous usage and definitions of the term "trait", I will use the term parameter instead. For this project, morphological and physiological parameters as functional parameters were examined: Morphological parameters refer to physical attributes such as leaf color; physiological parameters include internal functions and processes in the metabolism and biochemical pathways. In plants, photosynthesis plays a major role in the energy metabolism of the plant, and therefore, I examined parameters related to this biochemical pathway such as leaf chlorophyll content.
The plant performance parameter vegetative biomass (BM) can be determined destructively by harvesting and weighing the sample or non-destructively by modeling (**Formula 2).** Starting from the initial vegetative biomass (*BM(0)*) and considering exponential growth and the relative growth rate (RGR, **Formula 3**) the vegetative biomass can be calculated at any time point (BM(t)).

Formula 2: Vegetative biomass to any time point t (BM(t)) as a function of the initial biomass and exponential to the product of relative growth rate RGR at t.

$$BM(t) = BM\ (0) * e^{RGR*t}$$

Formula 3: Relative growth rate as the difference between the natural logarithm of the vegetative biomass at time points 2 (*BM2*) and 1 (*BM1*) divided by the difference of time points t2 and t1.

$$RGR = \frac{\ln(BM2) - \ln(BM1)}{t2 - t1}$$

Other performance parameters such as reproductive output and plant survival were not the subject of this project due to the study design. For a summary of examined functional and performance parameters that are contributing to plant performance, the framework of Arnold's framework (Arnold 1983) for animals adapted by Violle et al. (2007) for plants was adjusted for this project (**Figure 3**).

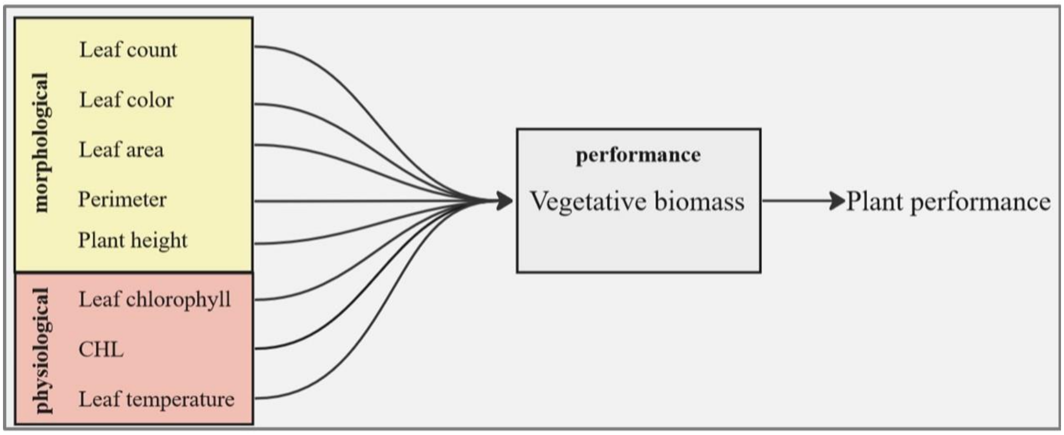

***Figure 3*. Overview of the morphological, physiological, and performance parameters examined in this project.**

The figure was adapted from Violle et al. (2007) and adjusted to represent the parameters examined in this project. Morphological parameters (yellow box) include physical attributes and physiological parameters (red box) pathways include internal functions, biochemical pathways, and metabolism that describe photosynthesis in this project. Performance parameters are the result of the morphological and physiological parameters and are a way to measure plant performance. CHL, chlorophyll content.

Experimental setup and plant material

Data collection was conducted at the innovation laboratory facilities of SweGreen AB (SweGreen X, Stockholm) in a three-layer vertical farm situated in a closed, climate-controlled hydroponic system for growing plants in an artificial environment without soil by using water-based mineral nutrient solutions. Controlled temperature and humidity inside the system were achieved by a built-in split air conditioner (SC-JA4819, Qlima, Netherlands), and air circulation on all layers was ensured by one 15-watt electrical ventilator per layer. Plants were grown under LED lighting "Siera" (Heliospectra, Sweden) with a photoperiod of 18 h. The experiment was conducted on the upper layer (layer 3) where two different hydroponic irrigation systems, that are recirculating nutrient solution using a timed pump system, were installed next to each other with the same light and ambient conditions. The ebb-and-flow irrigation system (EF), where plant roots are periodically flooded every hour with nutrient solution, and the nutrient film technique (NFT), where the plant roots are covered in a nutrient film. Flow rates for NFT were 100 mL/min. EF had issues for a total of two days between day 11

and day 14 meaning that the overflow of the nutrient solution was followed by a period of drying out completely. However, the plants did not dry completely as the rock wool growing media were still moist.
The nutrient solution for both irrigation systems was prepared according to the specific recipe of SweGreen with nutrient ratios of N:P:K:Ca ratio of 7:1:9:5. Average air temperature and relative humidity were captured for the whole vertical farm and saved to a cloud system. Local conditions on a plant level such as air humidity and air temperature were measured with a handheld device (testo 605i, Germany) and data was exported to the mobile application testo Smart (testo, Germany). The average photosynthetic photon flux density (PPFD) of 95 µmol m^{-2} s^{-1} on the plant level was measured with PAR200 Quantum Spectrometer (UPRtek, Taiwan) coupled to the mobile application PAR200 Plus v1.0.0 (UPRtek, Taiwan).

Seeds for the species basil (BAS) "Basilika Storbladig" (*Ocimum basilicum* Genovese, Olssons Frö AB, Helsingborg, Sweden), romaine lettuce (ROM) "Romansallat Patrona RZ(41-123) (*Lactuca sativa* var. *romana*, Semenco, Sweden), and oakleaf lettuce (OAK) "Ekbladsallat Freelou" (*Lactuca sativa* L., Ollsons Frö AB, Sweden) were sown on soaked 36 x 36 x 40 mm rock wool growing medium plugs (Grodan, The Netherlands) using one seed per plug for OAK and ROM and approximately 15-20 for BAS. Plugs with seeds (n = 30 plugs/species) were placed in the nursery and transplanted to layer 3 after 2.5 weeks for BAS, 3 weeks for ROM, and 3.5 weeks for OAK after sowing, respectively. Plants of each species were transplanted to the EF and NFT irrigation system (n = 15 plants/species/treatment). The total period of data collection was two weeks which is the recommended time of final growth by SweGreen AB.

Parameter measurements

To assess plant performance, morphological and physiological parameters were captured using destructive and non-destructive methods. Destructive methods are defined as methods that were assessed on harvested plants (e.g. for imaging or vegetative biomass). Non-destructive methods are defined as methods where the plant was assessed inside the growing system and was not harvested. Five time points (day 00, day 03, day 07, day 11, day 14) were measured at each sampling event, and randomly selected representative plants (n = 3 plants/species/treatment) were used for destructively assessed parameters. For BAS, plants that did not experience shading at the time of transplantation to the experimental setup were selected and monitored throughout the data collection; for OAK and ROM, the whole plant was assessed.

Morphological parameters

In this project, plant height and leaf area (for BAS) were determined non-destructively. Plant height was measured using a caliper (Burgwächter KG, Germany); for selected BAS plants, individual height was measured. The leaf area of the first set of true BAS leaves of the two selected plants per plug (plant 1 and plant 2) was calculated after measuring the leaf length and width with a caliper (Burgwächter KG, Germany). An average of the leaf length and width were calculated and used as an input for a model for the leaf area of the basil variety Genovese (Mousavi Bazaz et al. 2011). Both plant height and leaf area were excluded from analyses as inaccuracies made the measurements unusable.

Additionally, leaf color and perimeter around the plant – for OAK and ROM, and leaf count were measured destructively. Leaf color, here used as a synonym for the whole plant (ROM and OAK) or the BAS "canopy", and the perimeter was assessed with image analysis using the open-source software FIJI Is Just ImageJ (FIJI, Schindelin et al. 2012) with images of the size 1567 x 2100 pixels. Images were taken under the same light conditions outside of the growing system. The background of the plant images was removed and modified images were used as input for a plugin to extract basic color metrics from images in FIJI (Strock 2021). The plugin was modified to the "Huang" method so the thresholding for accurate selection of the plant and conversion into 8-bit type images. Then, leaf color metrics for the Red Green Blue (RGB) color space, which range from 0 to 255 for each channel, i.e. Red, Green, and Blue, were extracted and the output was exported in a CSV file.
The RGB color channels were used as an input for calculating the Greenness Index (GNI, Sonnentag et al. 2012) shown in **Formula 4**:

Formula 4: Greenness index GNI calculated with the values of RGB channels Red, Green, and Blue.

$$GNI = \frac{Green}{(Green + Red + Blue)}$$

For the perimeter of OAK and ROM, the same pictures were used for leaf color measurements. Using FIJI, perimeter values in pixels were measured and converted to millimeters with the "Set scale" function of FIJI. Leaf count was determined by separating leaves from the plant and counting fully developed true leaves of the plants.

Physiological parameters

Indicators for photosynthesis capacity were estimated by measuring leaf chlorophyll content and calculated chlorophyll content (CHL) as well as leaf temperature using non-destructive methods.
Leaf chlorophyll content was measured with the soil plant analysis development (SPAD-502) meter (Konica Minolta, Japan). For OAK and ROM, 6 measurements were done on leaves of different stages, and the average was calculated; for BAS, the first set of fully developed leaves of selected plants was used. These leaves were monitored throughout the entire data collection. CHL was calculated using the SPAD values in the generalized formula by Markwell et al. (1995) (**Formula 5**). For analyses, the raw SPAD values were used for better accuracy.

Formula 5: Chlorophyll content CHL in $\mu mol\ m^{-2}$ calculated from SPAD values.

$$CHL = 10.6 + 7.39 * SPAD + 0.114 * SPAD^2$$

Leaf temperature was captured by the FLIR infrared camera model TG267 (FLIR Systems, USA) (**Figure 4A. + B.**). Emissivity was set to ε = 0.90 after calibration according to the manufacturer's instructions. Average temperatures of non-shaded leaves of the whole plant for OAK and ROM were captured and saved in the mobile application METERLiNK provided by the manufacturer (**Figure 4C.**). The leaf temperature of BAS was measured on the first set of true leaves and the average was calculated. Intervals of two consecutive sampling time points were defined (interval 1: day 00 to day 03, interval 2: day 03 to day 07, interval 3: day 07 to day 11, interval 4: day 11 to day 14) to calculate the RGR of BM (**Formula 3**) as well as average leaf temperature.

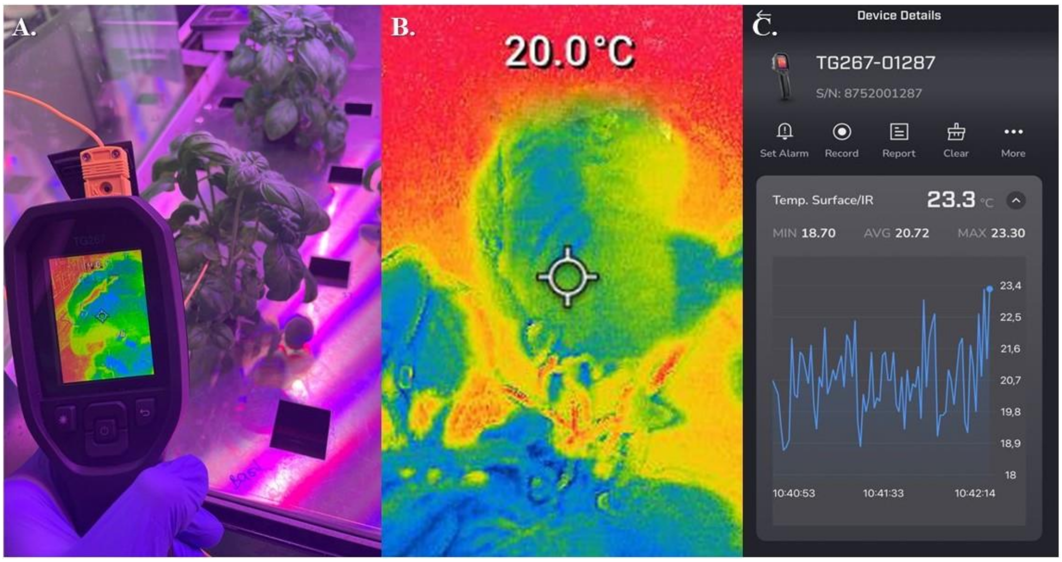

Figure 4. Visualization of the process to capture leaf temperature exemplary for basil.

A. *Handheld FLIR infra-red camera was aimed toward the plant and was supported by an integrated laser and K-type thermocouple was placed next to the targeted area to determine the emissivity.* ***B.*** *Image of infrared measurements on a BAS leaf, red to blue colors show warm to cold temperatures.* ***C.*** *Mobile application interface for the FLIR infrared camera was used to determine the average leaf temperature for the whole plant.*

Performance parameters

The performance parameter above ground BM was captured post-harvest. Roots and rock wool growing medium were cut off and BM in fresh weight was determined with a fine scale (accuracy 0.01 g). Selected BAS plants were weighted individually after harvest.

Statistical analysis and modeling

Data collection and processing were done with Excel (MS Office 12) and tables were converted to text files for statistical analyses and modeling with R (v4.3.3) using RStudio with the packages dplyr, ggplot2, ggpubr, pacman, minpack.lm for, and broom (R Core Team 2024). The Student's t-test was performed for the significance of BM differences. The Pearson correlation test was performed for the significance of correlations. Statistical significance for Student's t-test and Pearson correlation test is indicated by asterisks: * for statistical significance at a 90 % level ($p < 0.1$), ** for statistical significance at a 95 % level ($p < 0.05$), and *** for statistical significance at a 99 % level ($p < 0.01$). For the modeling approach, **Formula 2** for BM modeling and **Formula 3** for RGR were considered. Regressions between the fitted curve and the model were run and R-squared values and Root Mean Square Error (RMSE) were considered for evaluating the prediction accuracy.

3. Results

Plant performance

Vegetative biomass accumulation for each species

Results of the comparison between the irrigation systems, EF and NFT, for each species, BAS, ROM, and OAK, separately are shown in **Figure 5**. BAS plants had a lower weight than ROM and OAK. No significant differences between EF and NFT were observed at any time points for BAS (results of Student's t-test in **Supplement S2**). On day 03 and day 11, statistically significantly higher performance of ROM plants in NFT was found compared to EF. OAK plants performed better in NFT compared to EF on day 11 and day 14 which was statistically significant.

With time, the variance of BM (visualized using standard error bars) for BAS and ROM became larger, and for OAK remained small. This variation around the medians is pronounced for BAS predominantly in EF, whereas for ROM it occurs in NFT.

Recovery support after transplantation

The first 7 days were considered based on previous observations (see **Figure 5**: day 00 to day 07 and **Supplement S2**). For BAS, the plants showed no significant difference between BM when grown in NFT. ROM plants performed better in NFT on day 03 which is statistically significant. However, the BM of ROM plants growing in EF caught up on day 07. OAK plants grown in NFT show the same tendency as ROM plants: more BM on day 03 in NFT, which was not statistically significant, and the same BM on day 07.

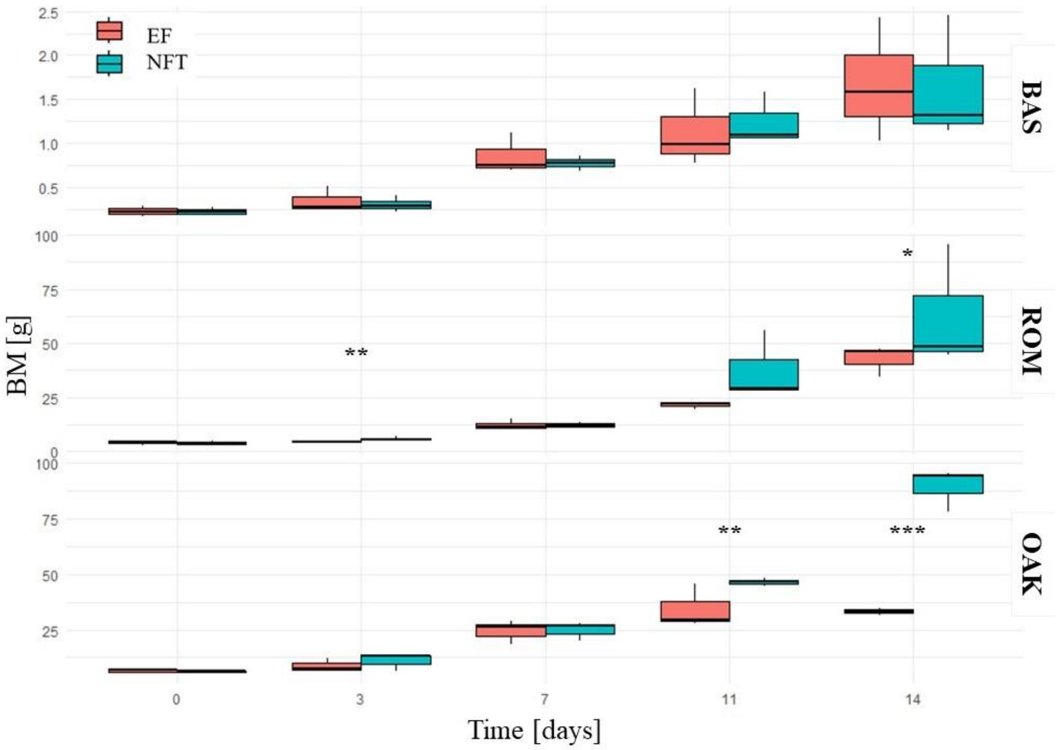

Figure 5. Result of the comparison of ebb and flow and nutrient film technique irrigation systems of basil, romaine, and oakleaf lettuce using vegetative biomass.

*Box plots of species-specific comparison between ebb and flow (EF, red) and nutrient film technique (NFT, blue) irrigation systems for the two-week data collection period in terms of vegetative biomass [g]. Variation is shown in standard error bars and significance was tested by the Student's t-test and indicated by asterisks: *, $p < 0.1$; **, $p < 0.05$; ***, $p < 0.01$. BM, vegetative biomass; BAS, basil; ROM, romaine lettuce; OAK, oakleaf lettuce.*

Prediction of plant performance

The logistic fit of the data points (formula and parameters in **Supplement S3**) revealed a pattern similar to the predicted BM using the function BM(t) for all species and irrigation systems (**Figure 6)**. This resulted in R-squared values close to 1; except for OAK in EF (R-squared 0.76). The OAK EF logistic fit is an S-shaped curve, BM(t) follows the exponential curve and predicts a lower BM accumulation after day 03. The reduced BM accumulation between day 11 and day 13 can be explained by the issue in the EF system.

Percentages of error rates are calculated on the dependent variable BM using RMSE values and are listed in the following: for BAS EF 5 %, BAS NFT 6 %, ROM EF 51 %, ROM NFT 17 %, OAK EF 38 %, and OAK NFT 31 %. Apart from BAS, the lettuce species in NFT had a lower error rate of predictions which may be associated with the continuous supply of nutrients and water and more stable conditions.

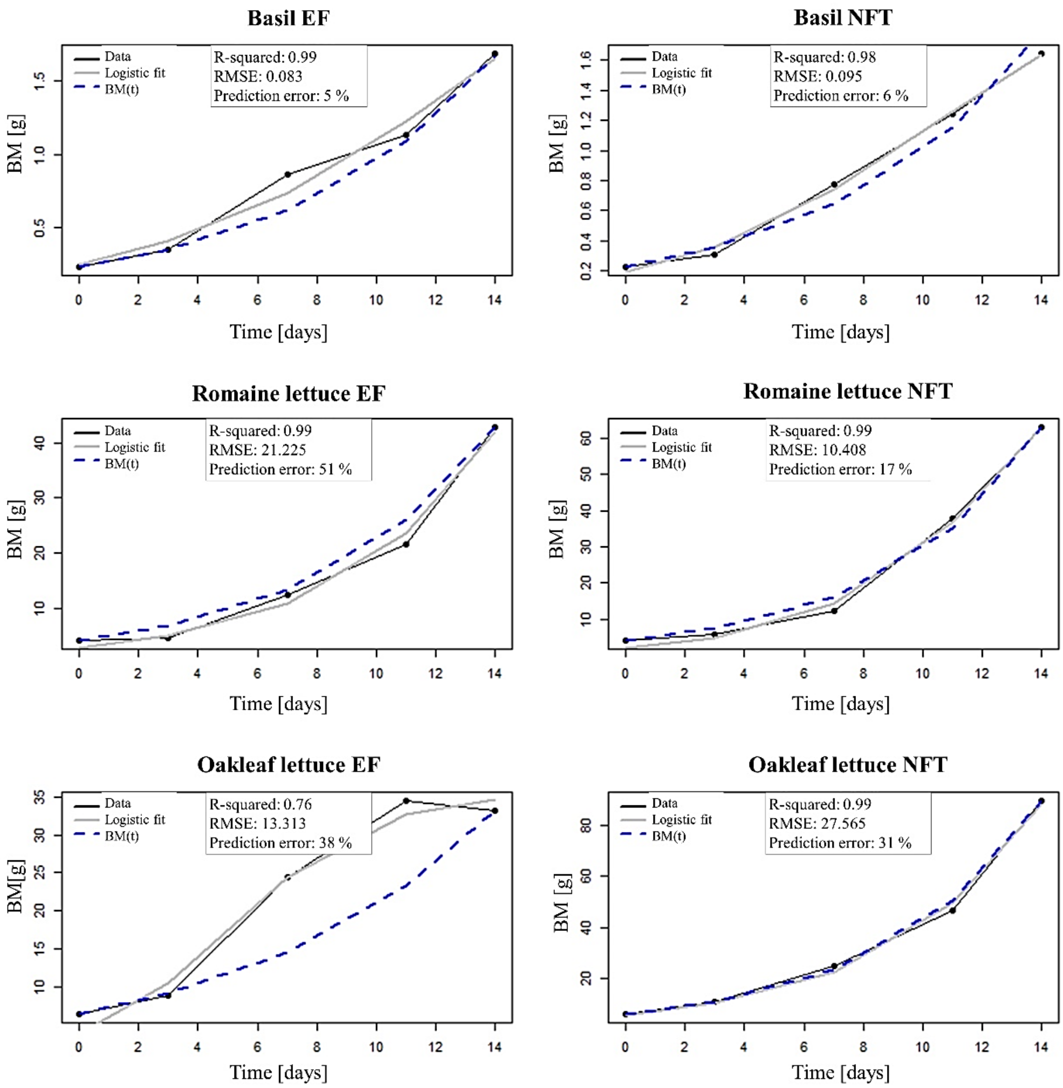

Figure 6. Data points, logistic fit, and modeled growth for basil, romaine, and oakleaf lettuce in ebb and flow and nutrient film technique irrigation systems.

Connected data points (black) show the mean of the BM of 3 plants per data point and the logistic fit (grey) was done considering the data points. Modeled vegetative biomass accumulation using BM as a function of time (blue-dashed) considers relative growth rate and initial biomass at day 00. R-squared and RMSE values are displayed for each species and irrigation system for accuracy comparison. EF, ebb and flow; NFT, nutrient film technique. BM(t), vegetative biomass as a function of time (Formula 2); RMSE, root mean squared error.

Leaf color, chlorophyll content, and plant performance

Greenness index versus chlorophyll content

The CHL values between the species vary due to their morphology and range from SPAD 31 to 41 (CHL: 344 - 538 µmol m^{-2}) for BAS, SPAD 32 to 50 (CHL: 362 - 682 µmol m^{-2}) for ROM, and SPAD 13 to 18 (128 to 176 µmol m^{-2}) for OAK. For analysis, the SPAD values were used for accuracy reasons.

Species showed different directions of correlation for GNI compared to SPAD values (**Figure 7**). For BAS the correlation was negative, ROM showed no correlation, and for OAK there was a positive correlation. All of the samples showed scattered data points, indicating a high variance and consequently low R-squared values of 0.17 for BAS, 0.01 for ROM, and 0.13 for OAK. Nevertheless, a tendency toward correlations was visible for each species. The Pearson correlation test was performed to examine the statistical significance of the correlation for each species separately (see table in **Supplement S4**).

For BAS, a negative correlation (cor = -0.413**) was found, indicating that the higher GNI, the lower SPAD. In the case of BAS, it is important to note that the whole "canopy" of basil plants was included in the image analysis. No correlation between SPAD and GNI was found for ROM (p = 0.544; cor = 0.115). OAK had a positive correlation between SPAD and GNI (cor = 0.359*) indicating that the higher GNI of the leaf, the higher SPAD.

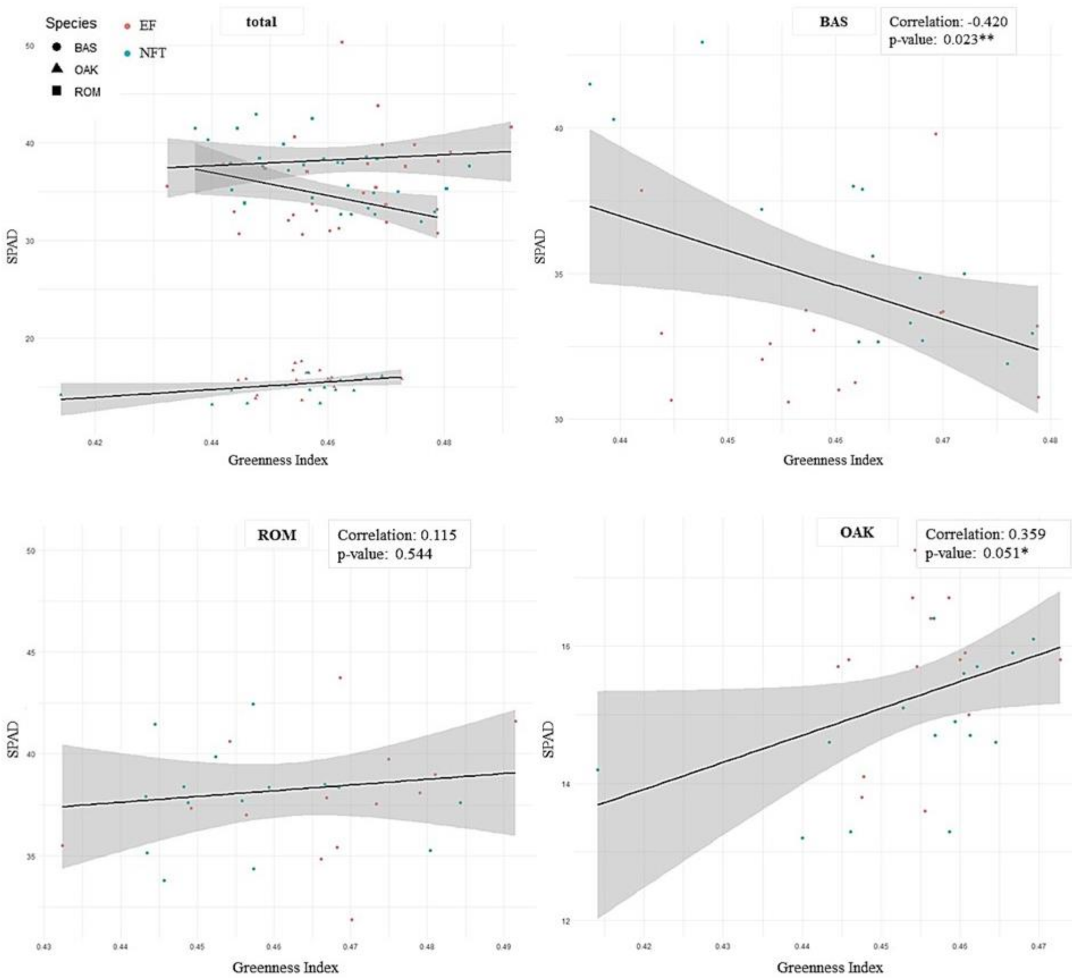

Figure 7. Scatter plot for correlation of greenness index and chlorophyll content for each species. *Greenness index was calculated from RGB channel values and correlated with SPAD measurements for BAS, ROM, and OAK. Trend lines are included and data points are distinguished for EF (red) and NFT (blue). Correlation and p-values are displayed: *, p < 0.1; **, p < 0.05. SPAD, leaf chlorophyll content measured with SPAD meter; BAS, basil; ROM, romaine lettuce; OAK, oakleaf lettuce.*

Chlorophyll content and greenness index versus plant performance

Differences between the irrigation systems became apparent in the correlations between SPAD versus BM and between SPAD versus BM (**Table 1,** scatter plots in **Supplement S5**). There was a negative correlation between SPAD versus BM when calculating for total species and irrigation systems. Looking at each species and irrigation separately, the results revealed a negative correlation for BAS in EF and BAS in NFT, the latter being statistically significant. A positive correlation was found in ROM when comparing SPAD versus BM, which was statistically significant for ROM in EF. A positive correlation for SPAD versus BM in OAK is found for EF and NFT.

Weak positive correlations were found between GNI versus BM for aggregated data on all species for both irrigation systems. When testing correlations for each species and irrigation system separately, a positive correlation for GNI versus BM was found for BAS in both EF and NFT. For GNI versus BM in ROM, there was a weak negative correlation which is not statistically significant in either EF or NFT. For OAK, there was a statistically significant positive correlation in the interaction of GNI versus BM. The tests for correlation between SPAD and GNI, respectively, and BM show that the method cannot be used in the same way for all species and instead, the species morphologies for robust statements should be considered.

Table 1**. **Results of Pearson correlation test of biomass to chlorophyll content and greenness index for basil, romaine, and oakleaf lettuce separately for each irrigation system.

Comparison of calculated leaf chlorophyll content measured with SPAD meter (SPAD) and greenness index (GNI) as independent variables to vegetative biomass (BM) as a dependent variable. Both irrigation systems, ebb and flow (EF) and nutrient film technique (NFT), were tested separately for statistical significance in each species. P-values and correlation values are indicated. SPAD x BM, SPAD measurements versus vegetative biomass, GNI x BM, greenness index versus vegetative biomass.

	Irrigation system	p-value	Correlation (cor)
SPAD x BM			
total		0.021**	-0.244
BAS	EF	0.473	-0.201
	NFT	0.001***	-0.763
ROM	EF	0.038**	0.540
	NFT	0.121	0.418
OAK	EF	0.893	0.038
	NFT	0.443	0.214
GNI x BM			
total		0.811	0.026
BAS	EF	0.136	0.403
	NFT	0.025**	0.574
ROM	EF	0.895	-0.037
	NFT	0.789	-0.076
OAK	EF	0.032**	0.556
	NFT	0.081*	0.465

Statistical significance is marked with * $p < 0.1$; ** $p < 0.05$; and *** $p < 0.01$.

Leaf temperature and plant performance

The relationship between leaf temperature (21.4 °C ± 1.1) and plant growth (shown by RGR) for BAS, ROM, and OAK under EF and NFT is displayed in **Figure 8**. For BAS and ROM in EF, a negative correlation between leaf temperature and RGR was found, suggesting that as leaf temperature increases, RGR decreases. OAK in EF showed a positive correlation, indicating that higher leaf temperatures might enhance growth which contrasted the findings for BAS and ROM.

In the NFT irrigation system, BAS and OAK exhibit a rather stable RGR across the observed leaf temperature ranges, indicating that there is no significant impact of leaf temperature on growth in this system. For ROM, a slight negative correlation between leaf temperature and RGR was observed which is however not significant. P-values obtained from the Pearson correlation test for species grown in NFT are closer to 1 compared to EF which underlines this finding.

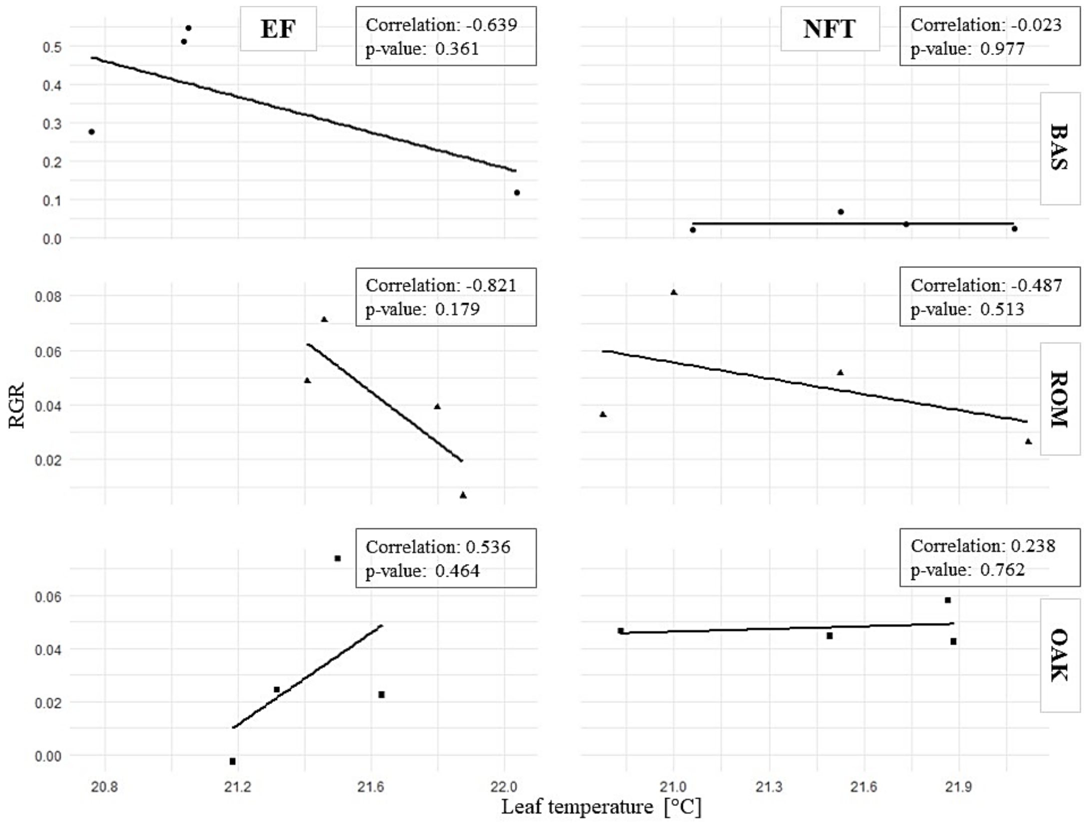

***Figure 8. Comparison of leaf temperature and relative growth rates over four intervals for basil, romaine, and oakleaf lettuce for each irrigation system**.*

The figure displays the relationship between leaf temperature and relative growth rate (RGR) for different plant species under two irrigation systems, ebb and flow (EF) and nutrient film technique (NFT). Each panel represents a species in each irrigation system indicating individual data points and lines to show regression trends. Correlation values for direction and p-values for correlation significance of Pearson correlation test are indicated for each plot. BAS, basil; ROM, romaine lettuce; OAK, oakleaf lettuce.

4. Discussion

I developed a method using the available instrumentation, captured and evaluated data, and will now discuss the results regarding my working hypotheses:

(I) Growing BAS, ROM, and OAK in NFT will result in a) better plant performance, b) better recovery after transplantation, and c) higher accuracy for plant growth predictions compared to the EF irrigation system.

(II) Image analysis is a useful, non-destructive tool for monitoring plants regarding their morphology, physiology, and performance.

(III) Leaf temperature and the plant growth rate are associated.

NFT irrigation supports plant performance, recovery, and accurate prediction for plant growth

The irrigation systems of EF and NFT differ in their way of supplying the plant roots with the nutrient solution and water. EF is widely used and was one of the first commercial hydroponic irrigation systems to produce many different plant species (Mir et al. 2022). Whereas EF periodically floods the roots with nutrient solution and water, NFT supplies the roots continuously as a nutrient film is created. This constant supply of nutrients and water could be the reason for better plant performance, i.e. BM accumulation, which reduces plant stress and allows the plant to acclimatize. Plant stress can be provoked by, for instance, incompatible flooding intervals in EF for the lettuce species (Nielsen et al. 2006; Santosh & Gaikwad 2023) but also by changing conditions, i.e. flooding and ebbing in intervals of 1 hour, that the plants cannot adapt to (Athanasiou et al. 2009; Voesenek & Bailey-Serres 2015; Maurel & Nacry 2020). Reduced plant stress means that most energy acquired via photosynthesis goes to BM accumulation instead of mechanisms to cope with changing water levels. Reduced stress could also explain the higher accuracy or lower error rate, respectively, for the lettuce species (ROM and OAK) in the NFT irrigation system.

BAS performed similarly across EF and NFT regarding BM accumulation, recovery, and prediction accuracy. A possible explanation could be that BAS is considered to be comparably robust; especially in hydroponic cultivation where nutrient and water supply and air temperature are sufficient (Rakocy et al. 2004). However, only two representatives out of 15-20 plants per plug were examined for practical reasons. This selection of plants (plants that showed potential to survive during the data collection period) could distort the results for BM accumulation in basil, especially since during the 2-week data collection, BAS plants were mechanically stressed. Future research with more extensive set of samples and a more targeted data collection can account for this.

Regarding recovery support monitored from the day of transplantation, day 00, to day 07, the findings show that even though the lettuce species ROM and OAK showed better performance on day 03, NFT seems to have no impact on the recovery at the end of the recovery monitoring period. As this transition from nursery to the growing platform is crucial for plant survival and prediction and yield of BM, it is important to study this bottleneck. Closer monitoring of the first days after transplantation and a higher sample number for each time point may give more conclusive results.

Modeling for BM in a field setting, e.g. for prediction of yield, is already used as a tool today. However, fluctuations in environmental conditions and climatic events caused by climate change, such as long drought periods or high temperatures, make it difficult to rely on these computed predictions for yield. I saw the potential for a modeling approach in VF as conditions are highly controlled, well-monitored, and perfected for the plants thus fluctuations and plant stress conditions are extremely rare. All species except OAK in EF show an exponential growth pattern regardless of the irrigation system. Because the system is so well suited to modeling, the non-exponential behavior of the growth curve of OAK in EF became apparent. The repeated measurements in this study allow us to see exactly when situations arise, such as between day 11 and day 14 in the EF system. This indicates that OAK is perhaps more susceptible to water stress than ROM and BAS. Future studies could investigate this behavior further.

Image analysis to monitor morpho-physiological parameters and plant performance

Leaf color from image analysis and the correlation to (leaf) chlorophyll measurements using SPAD or chemical analysis have been done in field-grown soybeans and maize (Nguy-Robertson et al. 2015; Rigon et al. 2016). My aim was to test if this method could provide the opportunity to determine leaf chlorophyll and thereby estimate the nutrient status and the potential photosynthetic capacity of the plants by a simple digital picture. Linking image tools and leaf chlorophyll content presents a method for assessing leaf color by digital imaging and making it possible to monitor plants' nutritional status, health status, and potential photosynthetic capacity by determining leaf color and chlorophyll content (Tackenberg 2006; Nguy-Robertson et al. 2015; Singh et al. 2020; Ahmed & Yadav 2023).

Different color channels for pixels have been previously explored via image analysis. I tested Red Green Blue (for primary color components) channels for this thesis. However, the algorithm I used is not designated for plant color detection which made it difficult to account for morphological differences between species. For instance, shaded BAS leaves in the BAS "canopy" or the narrow growth and leaf venation of ROM plants were hard for the algorithm to pick up. In future research, other color channels may be explored, for example, HSB (intuitive color description), which is more suitable for color comparison (Rigon et al. 2016).

GNI indicates the proportion of the green color channel of the total RGB channel and is used for satellite image analysis, e.g. to evaluate the phenology of trees (Sonnentag et al. 2012). The GNI was adapted to quantify green leaf color for this project as the amount of chlorophyll pigment and the green appearance of the leaf correlate; chlorophyll reflects the green light and absorbs red and blue light for photosynthesis (Taiz et al. 2015). The usage of GNI is already a good way to determine how green the plant is and can give valuable monitoring information, however, it needs to be put into context to make color detection more accurate and reliable.

Leaf temperature as a proxy for plant growth

As early as 1987, Kobza and Edwards examined the effect of leaf temperature changes on the photosynthesis capacity of wheat under situations of water shortage. This study kept its relevance for decades and found application in recent studies, e.g. on coping mechanisms during heatwaves (Scafaro et al. 2023) and to support mathematical growth models in CEA (Son et al. 2023, Amitrano et al. 2023). This inspired me to use this biological concept in the vertical farm system for my thesis work.

Changes in leaf temperature can indicate physiological changes or changes in the ambient environment such as in levels of CO_2, air temperature, or humidity (Son et al. 2023). To account for the factor of air temperature, I also measured the ambient temperature on the plant level which was, as expected, relatively stable (23.5 °C ± 0.8). However, I could not consider humidity or CO_2 levels (not monitored in the data collection period) as I did not have enough data on stomatal conductance and transpiration rates.

When examining the relationship between leaf temperature and plant growth (indicated by RGR) in this study, the irrigation systems showed significant differences: EF showed a clear species-specific relationship whereas NFT showed a stable RGR regardless of leaf temperature. This is a interesting finding as the continuous irrigation pattern and overall more stable system in NFT seem to support stable plant growth even with fluctuating leaf temperatures. This finding highlights that appropriate irrigation systems based on species-specific sensitivities and growth characteristics are needed to better VF cultivation but also that the mechanisms of leaf temperature are not fully understood.

Practical implications and future applications

This pilot study showed the potential of incorporating plant science in vertical farms for future smart and autonomous farms. The results of this study can be used as a starting point for improvements of the method in a larger experiment with more samples.

Further analyses to enlighten the mechanisms behind the better performance in NFT considering nutrient use efficiency, hydrological estimates, and root architecture present another way to incorporate plant science in VF research. For a more robust prediction of BM accumulation, more sophisticated mathematical models can be built considering factors such as light intensity content and using the dry weight of the harvested plants (Huang et al. 2024; Rahimikhoob et al. 2024). As for this project, dry weight was not determined as harvested plants were frozen to conduct a metabolomics analysis for comparison regarding the metabolite profiles that can also be linked with nutritional and sensory benefits.

Improvements in image analysis algorithms need to aim for a more dependable, robust, and applicable method for a broad range of species that considers the variety in morphology of species. These implications enable the real-time monitoring of plants regarding the nutritional status and current and future performance accurately but also can be incorporated into disease monitoring. The results of improved image analysis algorithms together with chemical analysis via spectrophotometry as calibration for accurate chlorophyll content calculations can give insights and make resource use more efficient. Additionally, the results of image analysis can contribute to autonomous VF using machine learning, deep learning, and AI (Dey et al. 2016; Ahmed & Yadav 2023; Chen et al. 2023).

Image analysis as a tool for resource-efficient and autonomous VF can be complemented and used simultaneously, e.g. with multi- or hyperspectral cameras, with leaf temperature measurements for real-time monitoring of plant stress, as another dimension for growth prediction models (Avgoustaki et al. 2022). To be used as an additional input for mathematical models, the association between leaf temperature and photosynthesis needs to be explored further for accurate interpretation and towards understanding the underlying mechanisms by for example measurements of photosynthesis capacity with leaf gas exchange analysis. Regardless of the system, pest and disease detection and monitoring can be done using leaf temperature measurements to add to for example integrated pest management, early detection of infection or infestation, or targeted pesticide or fungicide application (Bernard et al. 2013; Singh et al. 2020).

5. Conclusion

In this master thesis, I used biological concepts to explore the plants growing in VF from the view of a plant scientist. I explained briefly the biological reasons for better performance and plant growth prediction in the NFT compared to EF. Even though my results from image analysis are inconclusive, they present a solid starting point for future improvements and extended practical work. The relationship of leaf temperature to plant growth showed interesting results when comparing the irrigation systems, which supported previous suggestions about better plant performance and reduced plant stress in NFT compared to EF.
The plant science perspective in VF is of great importance to ensure high quality and quantity in the system and promote AI-supported system autonomy. High quantity does not always mean high quality, so future research, including consumer participation, would make for better products. Improvements in crop quality can be achieved for VF in the future by using the understanding of plant metabolism.

Future studies on system autonomy, enhanced quality with high quantities, and the integration of AI in VF should prioritize the incorporation of biological measures and real-time plant performance. This approach is crucial for developing robust models and achieving high precision in decision-making. Moreover, researchers striving to make VF economically viable for those in need of cultivating nutritious, fresh food would significantly contribute to food security and sustainable food production.

References

Ahmed, I. & Yadav, P.K. (2023). Plant disease detection using machine learning approaches. *Expert Systems*, 40 (5), e13136. https://doi.org/10.1111/exsy.13136

Al-Kodmany, K. (2018). The Vertical Farm: A Review of Developments and Implications for the Vertical City. *Buildings*, 8 (2), 24. https://doi.org/10.3390/buildings8020024

Amitrano, C., Chirico, G.B., De Pascale, S., Rouphael, Y. & De Micco, V. (2020). Crop management in controlled environment agriculture (CEA) systems using predictive mathematical models. *Sensors*, 20 (11), 3110. https://doi.org/10.3390/s20113110

Anderson, R., Bayer, P.E. & Edwards, D. (2020). Climate change and the need for agricultural adaptation. *Current Opinion in Plant Biology*, 56, 197–202. https://doi.org/10.1016/j.pbi.2019.12.006

Ares, G., Ha, B. & Jaeger, S.R. (2021). Consumer attitudes to vertical farming (indoor plant factory with artificial lighting) in China, Singapore, UK, and USA: A multi-method study. *Food Research International*, 150, 110811. https://doi.org/10.1016/j.foodres.2021.110811

Armanda, D.T., Guinée, J.B. & Tukker, A. (2019). The second green revolution: Innovative urban agriculture's contribution to food security and sustainability – A review. *Global Food Security*, 22, 13–24. https://doi.org/10.1016/j.gfs.2019.08.002

Arnold, S.J. (1983). Morphology, performance and fitness. *American Zoologist*, 23 (2), 347–361. https://doi.org/10.1093/icb/23.2.347

Athanasiou, K., Dyson, B.C., Webster, R.E. & Johnson, G.N. (2009). Dynamic acclimation of photosynthesis increases plant fitness in changing environments. *Plant Physiology*, 152 (1), 366–373. https://doi.org/10.1104/pp.109.149351

Avgoustaki, D.D., Avgoustakis, I., Miralles, C.C., Sohn, J. & Xydis, G. (2022). Autonomous mobile robot with attached multispectral camera to monitor the development of crops and detect nutrient and water deficiencies in vertical farms. *Agronomy*, 12 (11), 2691. https://doi.org/10.3390/agronomy12112691

Baiardi, D. & Morana, C. (2021). Climate change awareness: Empirical evidence for the European Union. *Energy Economics*, 96, 105163. https://doi.org/10.1016/j.eneco.2021.105163

Beacham, A.M., Vickers, L.H. & Monaghan, J.M. (2019). Vertical farming: a summary of approaches to growing skywards. *The Journal of Horticultural Science and Biotechnology*, 94 (3), 277–283. https://doi.org/10.1080/14620316.2019.1574214

Benis, K. & Ferrão, P. (2018). Commercial farming within the urban built environment – Taking stock of an evolving field in northern countries. *Global Food Security*, 17, 30–37. https://doi.org/10.1016/j.gfs.2018.03.005

Bernard, F., Sache, I., Suffert, F. & Chelle, M. (2013). The development of a foliar fungal pathogen does react to leaf temperature! *New Phytologist*, 198 (1), 232–240. https://doi.org/10.1111/nph.12134

Butturini, M. & Marcelis, L.F.M. (2020). Vertical farming in Europe. In: *Plant Factory*. Elsevier. 77–91. https://doi.org/10.1016/B978-0-12-816691-8.00004-2

Califano, G., Crichton-Fock, A. & Spence, C. (2024). Consumer perceptions and preferences for urban farming, hydroponics, and robotic cultivation: A case study on parsley. *Future Foods*, 9, 100353. https://doi.org/10.1016/j.fufo.2024.100353

Cetegen, S.A. & Stuber, M.D. (2021). Optimal design of controlled environment agricultural systems under market uncertainty. *Computers & Chemical Engineering*, 149, 107285. https://doi.org/10.1016/j.compchemeng.2021.107285

Chen, Y., Huang, Y., Zhang, Z., Wang, Z., Liu, B., Liu, C., Huang, C., Dong, S., Pu, X., Wan, F., Qiao, X. & Qian, W. (2023). Plant image recognition with deep learning: A review. *Computers and Electronics in Agriculture*, 212, 108072. https://doi.org/10.1016/j.compag.2023.108072

Despommier, D. (2013). Farming up the city: the rise of urban vertical farms. *Trends in Biotechnology*, 31 (7), 388–389. https://doi.org/10.1016/j.tibtech.2013.03.008

Despommier, D.D. (2010). *The vertical farm: feeding the world in the 21st century*. 1. ed. Thomas Dunne Books [u.a.].

Dey, A.K., Sharma, M. & Meshram, M.R. (2016). An analysis of leaf chlorophyll measurement method using chlorophyll meter and image processing technique. *Procedia Computer Science*, 85, 286–292. https://doi.org/10.1016/j.procs.2016.05.235

EEA (2019). Greenhouse gas emissions by aggregated sector. https://www.eea.europa.eu/data-and-maps/daviz/ghg-emissions-by-aggregated-sector-5#tab-dashboard-02 [2024-05-22]

EEA (2022). Water use in Europe by economic sector, 2017. https://www.eea.europa.eu/data-and-maps/daviz/annual-and-seasonal-water-abstraction-7/#tab-dashboard-02 [2024-05-22]

EIB (2021). *EIB impact report 2020: Climate action, environmental sustainability and innovation for decarbonisation*. https://www.eib.org/attachments/publications/eib_impact_report_2020_en.pdf

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D. & Zaks, D.P.M. (2011). Solutions for a cultivated planet. *Nature*, 478 (7369), 337–342. https://doi.org/10.1038/nature10452

Huang, J.J., Tan, C.X. & Zhou, W. (2024). Universal modeling for optimizing leafy vegetable production in an environment-controlled vertical farm. *Computers and Electronics in Agriculture*, 219, 108715. https://doi.org/10.1016/j.compag.2024.108715

John, D.A. & Babu, G.R. (2021). Lessons from the aftermaths of green revolution on food system and health. *Frontiers in Sustainable Food Systems*, 5, 644559. https://doi.org/10.3389/fsufs.2021.644559

Kleszcz, J., Kmiecik, P. & Świerzawski, J. (2020). Vegetable and gardening tower of Othmar Ruthner in the Voivodeship Park of Culture and Recreation in Chorzów—the first example of verticalfarming in Poland. *Sustainability*, 12 (13), 5378. https://doi.org/10.3390/su12135378

Kobza, J. & Edwards, G.E. (1987). Influences of leaf temperature on photosynthetic carbon metabolism in wheat. *Plant Physiology*, 83 (1), 69–74. https://doi.org/10.1104/pp.83.1.69

Kozai, T. & Niu, G. (2020). Role of the plant factory with artificial lighting (PFAL) in urban areas. In: *Plant Factory*. Elsevier. 7–34. https://doi.org/10.1016/B978-0-12-816691-8.00002-9

Kozai, T., Niu, G. & Takagaki, M. (eds) (2020). *Plant factory: an indoor vertical farming system for efficient quality food production*. Second edition. Academic Press, an imprint of Elsevier.

Liaqat, W., Barutçular, C., Farooq, M.U., Ahmad, H., Jan, M.F., Ahmad, Z., Nawaz, H. & Li, M. (2022). Climate change in relation to agriculture: A review. *Spanish Journal of Agricultural Research*, 20 (2), e03R01. https://doi.org/10.5424/sjar/2022202-17742

Lovell, S.T. (2010). Multifunctional urban agriculture for sustainable land use planning in the United States. *Sustainability*, 2 (8), 2499–2522. https://doi.org/10.3390/su2082499

Luís, S., Vauclair, C.-M. & Lima, M.L. (2018). Raising awareness of climate change causes? Cross-national evidence for the normalization of societal risk perception of climate change. *Environmental Science & Policy*, 80, 74–81. https://doi.org/10.1016/j.envsci.2017.11.015

Malhi, G.S., Kaur, M. & Kaushik, P. (2021). Impact of climate change on agriculture and its mitigation strategies: A review. *Sustainability*, 13 (3), 1318. https://doi.org/10.3390/su13031318

Markwell, J., Osterman, J.C. & Mitchell, J.L. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research*, 46 (3), 467–472. https://doi.org/10.1007/BF00032301

Martin, M. & Molin, E. (2019). Environmental Assessment of an Urban Vertical Hydroponic Farming System in Sweden. *Sustainability*, 11 (15), 4124. https://doi.org/10.3390/su11154124

Maurel, C. & Nacry, P. (2020). Root architecture and hydraulics converge for acclimation to changing water availability. *Nature Plants*, 6 (7), 744–749. https://doi.org/10.1038/s41477-020-0684-5

Mir, M.S., Naikoo, N.B. & Kanth, R.H. (2022). Vertical farming: The future of agriculture: A review. 11 (2), 1175–1195

Molin, E. & Martin, M.A. (2018). Assessing the energy and environmental performance of vertical hydroponic farming. https://doi.org/10.13140/RG.2.2.28444.77442

Mousavi Bazaz, A., Karimian Fariman, Z. & Bannayan, M. (2011). Modeling individual leaf area of basil (*Ocimum basilicum*) using different methods. *International Journal of Plant Production*, 5 (4). https://doi.org/10.22069/ijpp.2012.753

Mu, X. & Chen, Y. (2021). The physiological response of photosynthesis to nitrogen deficiency. *Plant Physiology and Biochemistry*, 158, 76–82. https://doi.org/10.1016/j.plaphy.2020.11.019

Nguy-Robertson, A., Peng, Y., Arkebauer, T., Scoby, D., Schepers, J. & Gitelson, A. (2015). Using a simple leaf color chart to estimate leaf and canopy chlorophyll *a* content in maize (*Zea mays*). *Communications in Soil Science and Plant Analysis*, 46 (21), 2734–2745. https://doi.org/10.1080/00103624.2015.1093639

Nielsen, C.J., Ferrin, D.M. & Stanghellini, M.E. (2006). Efficacy of biosurfactants in the management of *Phytophthora capsici* on pepper in recirculating hydroponic systems. *Canadian Journal of Plant Pathology*, 28 (3), 450–460. https://doi.org/10.1080/07060660609507319

Ortiz, A.M.D., Outhwaite, C.L., Dalin, C. & Newbold, T. (2021). A review of the interactions between biodiversity, agriculture, climate change, and international trade: research and policy priorities. *One Earth*, 4 (1), 88–101. https://doi.org/10.1016/j.oneear.2020.12.008

R Core Team (2024). *R: A Language and Environment for Statistical Computing* (4.3.3). R, R Foundation for Statistical Computing. https://www.R-project.org/

Rahimikhoob, H., Sohrabi, T. & Delshad, M. (2024). Variance-based global sensitivity analysis and uncertainty quantification of the AquaCrop model parameters for basil (*Ocimum basilicum* L.) under different nitrogen fertilizer rates. *Journal of Plant Nutrition*, 47 (4), 628–645. https://doi.org/10.1080/01904167.2023.2280142

Rakocy, J., Shultz, R.C., Bailey, D.S. & Thoman, E.S. (2004). Aquaponic production of tilapia and basil: Comparing a batch and staggered cropping system. *Acta Horticulturae*, (648), 63–69. https://doi.org/10.17660/ActaHortic.2004.648.8

Reidsma, P., Ewert, F., Boogaard, H. & Diepen, K.V. (2009). Regional crop modelling in Europe: The impact of climatic conditions and farm characteristics on maize yields. *Agricultural Systems*, 100 (1–3), 51–60. https://doi.org/10.1016/j.agsy.2008.12.009

Rigon, J.P.G., Capuani, S., Fernandes, D.M. & Guimarães, T.M. (2016). A novel method for the estimation of soybean chlorophyll content using a smartphone and image analysis. *Photosynthetica*, 54 (4), 559–566. https://doi.org/10.1007/s11099-016-0214-x

Robbins, N.E. & Dinneny, J.R. (2018). Growth is required for perception of water availability to pattern root branches in plants. *Proceedings of the National Academy of Sciences*, 115 (4). https://doi.org/10.1073/pnas.1710709115

Santosh, D.T. & Gaikwad, D. (2023). Advances in Hydroponic Systems: Types and Management. 16–28.

Scafaro, A.P., Posch, B.C., Evans, J.R., Farquhar, G.D. & Atkin, O.K. (2023). Rubisco deactivation and chloroplast electron transport rates co-limit photosynthesis above optimal leaf temperature in terrestrial plants. *Nature Communications*, 14 (1), 2820. https://doi.org/10.1038/s41467-023-38496-4

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P. & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9 (7), 676–682. https://doi.org/10.1038/nmeth.2019

Semida, W.M., Beheiry, H.R., Sétamou, M., Simpson, C.R., Abd El-Mageed, T.A., Rady, M.M. & Nelson, S.D. (2019). Biochar implications for sustainable agriculture and environment: A review. *South African Journal of Botany*, 127, 333–347. https://doi.org/10.1016/j.sajb.2019.11.015

Singh, V., Sharma, N. & Singh, S. (2020). A review of imaging techniques for plant disease detection. *Artificial Intelligence in Agriculture*, 4, 229–242. https://doi.org/10.1016/j.aiia.2020.10.002

Son, K.-H., Sim, H.-S., Lee, J.-K. & Lee, J. (2023). Precise sensing of leaf temperatures for smart farm applications. *Horticulturae,* 9 (4), 518. https://doi.org/10.3390/horticulturae9040518

Sonnentag, O., Hufkens, K., Teshera-Sterne, C., Young, A.M., Friedl, M., Braswell, B.H., Milliman, T., O'Keefe, J. & Richardson, A.D. (2012). Digital repeat photography for phenological research in forest ecosystems. *Agricultural and Forest Meteorology*, 152, 159–177. https://doi.org/10.1016/j.agrformet.2011.09.009

Specht, K., Siebert, R., Hartmann, I., Freisinger, U.B., Sawicka, M., Werner, A., Thomaier, S., Henckel, D., Walk, H. & Dierich, A. (2014). Urban agriculture of the future: an overview of sustainability aspects of food production in and on buildings. *Agriculture and Human Values*, 31 (1), 33–51. https://doi.org/10.1007/s10460-013-9448-4

Strock, C. (2021). Protocol for extracting basic color metrics from Images in ImageJ/Fiji. https://doi.org/10.5281/ZENODO.5595203

Tackenberg, O. (2006). A new method for non-destructive measurement of biomass, growth rates, vertical biomass distribution and dry matter content based on digital image analysis. *Annals of Botany*, 99 (4), 777–783. https://doi.org/10.1093/aob/mcm009

Taiz, L., Zeiger, E., Møller, I.M. & Murphy, A.S. (eds) (2015). *Plant physiology and development*. Sixth edition. Sinauer Associates, Inc., Publishers.

Ting, K.C., Lin, T. & Davidson, P.C. (2016). Integrated urban controlled environment agriculture systems. In: Kozai, T., Fujiwara, K., & Runkle, E.S. (eds) *LED Lighting for Urban Agriculture*. Springer Singapore. 19–36. https://doi.org/10.1007/978-981-10-1848-0_2

Van Delden, S.H., SharathKumar, M., Butturini, M., Graamans, L.J.A., Heuvelink, E., Kacira, M., Kaiser, E., Klamer, R.S., Klerkx, L., Kootstra, G., Loeber, A., Schouten, R.E., Stanghellini, C., Van Ieperen, W., Verdonk, J.C., Vialet-Chabrand, S., Woltering, E.J., Van De Zedde, R., Zhang, Y. & Marcelis, L.F.M. (2021). Current status and future challenges in implementing and upscaling vertical farming systems. *Nature Food*, 2 (12), 944–956. https://doi.org/10.1038/s43016-021-00402-w

Van Gerrewey, T., Boon, N. & Geelen, D. (2021). Vertical Farming: The Only Way Is Up? *Agronomy*, 12 (1), 2. https://doi.org/10.3390/agronomy12010002

Violle, C., Navas, M., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, 116 (5), 882–892. https://doi.org/10.1111/j.0030-1299.2007.15559.x

Voesenek, L.A.C.J. & Bailey-Serres, J. (2015). Flood adaptive traits and processes: an overview. *New Phytologist*, 206 (1), 57–73. https://doi.org/10.1111/nph.13209

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Supplements

Supplementary S1: Additional explanation on photosynthesis (retrieved from Taiz et al. 2015)

Photosynthesis (PhS) as one of the major biochemical pathways in the plant was assessed using the physiological parameters. To understand the choice of selected physiological parameters, the PhS and what happens during this energy acquisition pathway will be described in the following:
The light-dependent reactions (**Figure S1,** light-dependent reactions) take place in the chloroplast that contains chlorophyll and is filled with the thylakoids that are stacked in grana surrounded by the stroma. More precisely, the light reaction takes place in the thylakoid membrane of the chloroplast where photoexcitation of electrons takes place in photosystem II and I (PSII and PSI) simultaneously but spatially separated. The excited electron splits the water molecule and then is fed in the electron transport chain via cytochrome to PSI where NADP+ is reduced to NADPH (nicotinamide adenine dinucleotide phosphate) and eventually in the ATP (adenosine triphosphate) synthase where the energy is generated by the chemoelectrical gradient.
The light-independent reactions (**Figure S1**, light-independent reactions), also referred to as the carbon reaction, of PS in the Calvin Cycle are happening in the stroma of the chloroplast and are characterized by 3 major processes: (1) the CO_2 fixation to 3-ribulose bisphosphate (RuBP) catalyzed by ribulose-1,5-bisphosphate carboxylase (RuBisCo) and breaking down into 3-phosphate-glycerate molecules, (2) the reduction to glyceraldehyde 3-phosphate (G3P) that is high in energy and can be converted to sugars, cellulose, or starch, and (3) the regeneration of RuBP as the last process of the Calvin Cycle which uses a total of 9 ATP and 6 NADPH.
The key points for this project are the role of chlorophyll and its relationship to leaf chlorophyll content as well as the relationship of chlorophyll to leaf color for the overall plant performance. Chlorophyll is the pigment in the chloroplast that absorbs energy-rich red and blue light and reflects green light; the reason why it appears green. Nitrogen is a key component for chlorophyll molecule synthesis and consequently for photosynthetic efficiency. Nitrogen deficiency for instance becomes apparent because of the yellow-greenish leaves but also in reduced or slow growth, respectively (Mu & Chen 2021).
With photospectrometrical measurements, the absorbance of chlorophyll and the leaf nitrogen content can be determined non-destructively by e.g. a SPAD meter. With advancements in imaging technologies, image analysis became a powerful tool also in plant science. Advancements in imaging techniques allow us to assess plants also from a technological aspect. This is done for satellite images

already and the greenness index (GNI) that is applied in phenological monitoring of forests was utilized for this purpose in the project (Sonnentag et al. 2012).

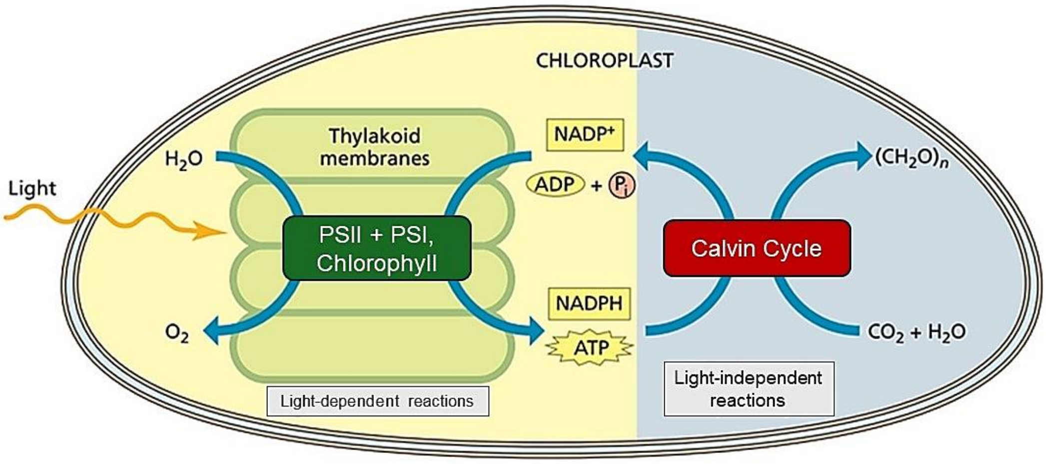


Figure S1: Schematic overview of the light-dependent and -independent reaction in the chloroplast. *Retrieved and slightly modified for better understanding from Taiz et al. (2015) Plant Physiology and Development, Chapter 8, p.204, Figure 8.1. PSII + PSI, photosystem II and photosystem I, ADP, adenosine diphosphate; NADP+, nicotinamide adenine dinucleotide phosphate (oxidized); ATP, adenosine triphosphate; NADPH, nicotinamide adenine dinucleotide phosphate (reduced),* H_20*, water molecules;* O_2 *oxygen molecules,* CO_2*, carbon dioxide molecules;* $(CH_2O)n$*, sugar molecules.*

Supplement S2:

Table S2: Results of the Student's t-test showing t-value (t), p-value (p) with standard deviation in parentheses (STD), and degrees of freedom (df) for BM and recovery differences (day 00 to day 07) between ebb and flow and nutrient film technique irrigation systems.
Plants growing in EF and NFT irrigation systems were compared concerning the vegetative biomass (determined using three representatives) for each time point. OAK, oakleaf lettuce, ROM, romaine lettuce; BAS, basil.

Species	Time [days]	t	p (STD)	df
OAK	00	0.492	0.649 (0.529)	4
OAK	03	0.983	0.381 (2.380)	4
OAK	07	0.129	0.904 (3.262)	4
OAK	11	2.571	0.062** (4.702)	4
OAK	14	12.180	0.0003*** (4.622)	4
ROM	00	0.127	0.9054 (0.632)	4
ROM	03	2.609	0.056** (0.510)	4
ROM	07	0.194	0.856 (1.291)	4
ROM	11	2.168	0.096* (7.501)	4
ROM	14	1.457	0.219 (13.822)	4
BAS	00	0	1 (0.037)	4
BAS	03	0.531	0.6233 (0.075)	4
BAS	07	0.762	0.488 (0.112)	4
BAS	11	0.415	0.700 (0.253)	4
BAS	14	0.085	0.936 (0.470)	4

Statistical significance is marked with * $p < 0.1$; ** $p < 0.05$; and *** $p < 0.01$.

Supplement S3:

Formula and parameters used for logistic fit in Figure 6.

Parameters are listed in the table below for each species and irrigation system. BAS, basil; ROM, romaine lettuce; OAK, oakleaf lettuce; EF, ebb and flow; NFT, nutrient film technique.

$$BM(t) = \frac{a}{1 + e^{\left(-\frac{t-b}{c}\right)}}$$

Species	Irrigation system	a	b	c
BAS	EF	3.243	13.772	5.541
	NFT	2.301	10.183	4.271
ROM	EF	3.796e+03	3.724e+01	5.172
	NFT	12.752e+01	14.072	3.421
OAK	EF	35.582	5.118	2.420
	NFT	6.218e+03	3.568e+01	5.115

Supplement S4:

Table S4: Results of the Pearson correlation test of greenness index and chlorophyll content for basil, romaine, and oakleaf lettuce.

SPAD measurements and greenness index (GNI) calculated from RGB channel values of image analysis using the Pearson correlation test. Each species was compared separately. P-values and correlation values are indicated as well as the 95 % confidence interval. Scatterplots are shown in ***Figure 7***.

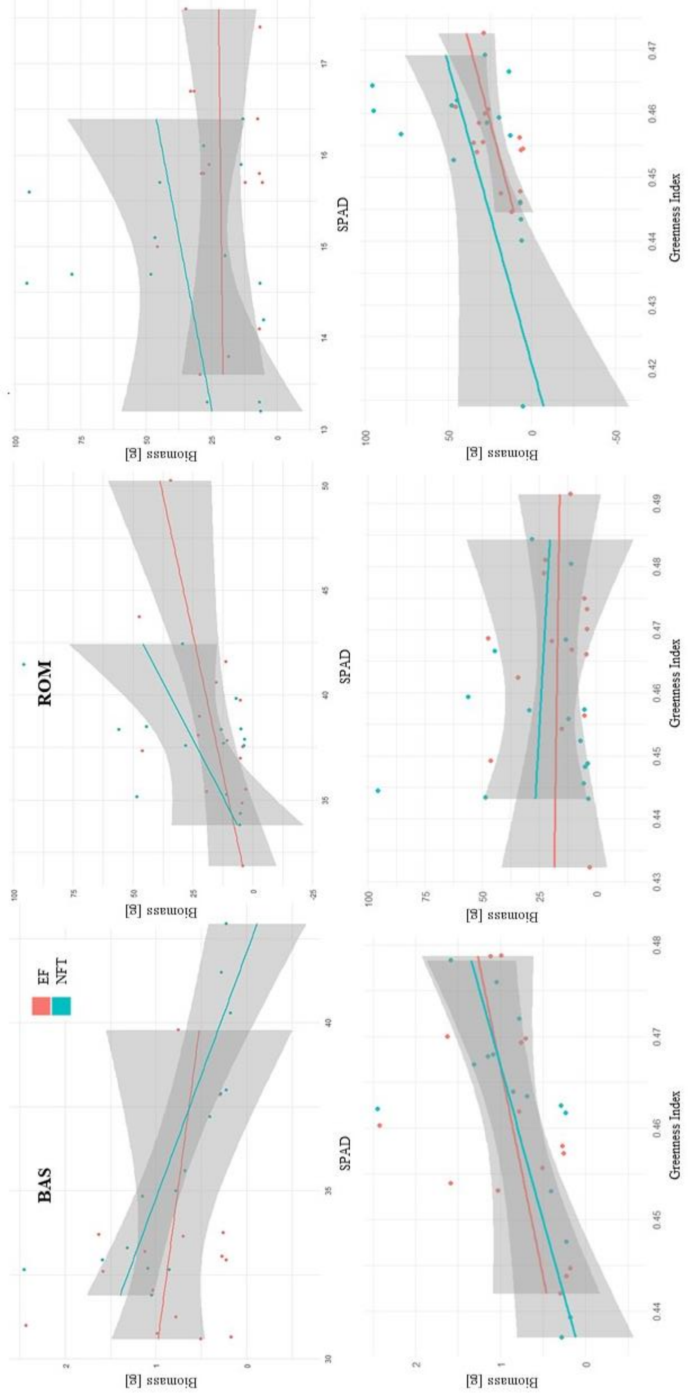
GNI x SPAD	p-value	95 % confidence interval	Correlation (cor)
total	0.037 **	0.014 0.409	0.220
BAS	0.023**	-0.673 -0.061	-0.4167
ROM	0.544	-0.2556 0.4566	0.115
OAK	0.051*	-0.001 0.637	0.359

Statistical significance is marked with * p < 0.1; ** p < 0.05; and *** p < 0.01.

Supplement S5:

Figure S5: Scatterplots of chlorophyll content and greenness index compared to vegetative biomass for basil, romaine, and oakleaf lettuce in ebb and flow and nutrient film technique irrigation system.

*The correlation of leaf chlorophyll values and the greenness index (GNI) was calculated from RGB channel values and correlated to vegetative biomass for BAS, ROM, and OAK and the irrigation system, ebb and flow (EF) and nutrient film technique (NFT). Trend lines are included and data points are distinguished for EF (red) and NFT (blue). SPAD, leaf chlorophyll content measured with SPAD meter; BAS, basil; ROM, romaine lettuce; OAK, oakleaf lettuce. Statistical significance was tested using the Pearson correlation test and is shown in **Table 1**.*



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