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The Sustainability of Basil Production in Hydroponics Can Be Increased Using Low-Nutrient Regimes Incorporating Biofertiliser from Microbial Fuel Cells

Segun Ogunmefun *, Mukesh Sharma, Robin Thorn and Neil Willey

Centre for Sustainable Agri-Food & Environment, School of Applied Sciences, University of the West of England, Bristol BS16 1QY, UK
* Correspondence: segun.ogunmefun@uwe.ac.uk

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Abstract: Optimizing nutrient solution (NS) management in hydroponic systems is crucial to promoting sustainable food production whilst maintaining crop productivity and quality. Sweet basil was used to investigate how nutrient reduction and wastewater-fed MFC catholyte (WW) used as a source of Nutrient, with/without gypsum supplementation, affected growth and yield. Three experiments were conducted: (1) stepwise NS reduction (100, 75, 50, 25%); (2) stepwise NS reduction (80, 60, 40, 20%); and (3) NS substitution (100% NS; 50% NS + 50% WW; and 50% NS + 50% WW + 0.46 mM gypsum. In general, across the NS dilution experiments, basil response to treatments was nonlinear and trait specific—leaf canopy and biomass accumulation traits were sensitive to treatment, with a breakpoint in response at low NS, while moderate NS reductions produced growth similar to controls. In experiment 1, shoot biomass accumulation and leaf area were indistinguishable from control when NS was diluted from 100% to 50% but declined at 25% NS, indicating a potential breakpoint. In experiment 2, most measured growth traits peaked at 60% NS. In experiment 3, the WW-NS substitution resulted in a biomass penalty, with total phenolics lowest under 100% NS, intermediate under 50% NS + 50% WW, and highest during supplementation with gypsum. Overall, the outcome of our study suggests a promising window for making hydroponically grown basil more sustainable, based on a reduced total NS supply plus a substitution of 50% of this reduced NS with WW, and possible methods for controlling biomass quality.

Keywords: hydroponics; nutrient management; microbial fuel cell catholyte; wastewater nutrient recovery

1. Introduction

By 2050 global food demand is expected to rise sharply as human population approaches 9.7 billion, necessitating a significant increase in food production [1–4]. Traditional agriculture currently faces mounting challenges due to diminishing arable land, water scarcity, and the worsening impacts of climate change. Current soil-based systems consume more than 70% of global freshwater and contribute significantly to environmental degradation through nutrient runoff, greenhouse gas emissions, and land use conversion [3,5]. Controlled Environment Agriculture (CEA), particularly using hydroponic systems, is a promising approach for increasing food production. These systems enable year-round cultivation and higher crop yield per unit area. Furthermore, they have been claimed to minimize water and fertilizer use by 90% and 60%, respectively, compared to traditional



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farming, by allowing precise control of environmental parameters such as light, temperature, humidity, and nutrient supply [4,6].

Ocimum basilicum L. (Basil) is a high value crop that is cultivated, frequently in CEA, for both its biomass and functional quality (phenolics, flavonoids, volatile organic compounds) [7,8]. Despite established production techniques, the challenge of predicting precise outcomes of biomass and quality when nutrient solution strength (NS) and/or electrical conductivity (EC) are manipulated persists. Several studies have noted that plant responses (biomass yield and quality) to the manipulation of NS/EC are nonlinear and could result in growth-yield-quality trade-offs, instead of uniform reductions/increments [9–11]. This trade-off suggests that, reflecting differences in nutrient uptake and utilization, basil could maintain primary metabolism and physiological functions (e.g., building proteins, pigment production) without any visual symptoms but with a significant yield penalty.

NS reduction does not necessarily equate to less growth, although it can lead to multi-nutrient co-limitation, resulting in a reduced nutrient uptake and efficiency over time with changes in biomass content. In some cases, plants perceive nutrient reduction as stress, which signals a metabolic shift (from primary to secondary metabolism) to maintain survival [12,13]. Recent research in stress biology emphasises that when plants experience stress, chloroplasts and mitochondria can independently generate reactive oxygen species (ROS), which function as signalling molecules specifically between organelles and nucleus (retrograde communication) and then a broad stress response [14,15]. These ROS can trigger downstream processes that increases cytosolic concentration of calcium by activating Ca^{2+} channels. The increased calcium concentration interacts with ROS to activate protein kinase signalling cascades that ensure that specific transcription factors are switched on, thereby initiating nuclear gene expression that produces secondary metabolic products (phenolics, flavonoids etc.) that enhance plant stress tolerance [15–19]. This metabolic shift may persist and remain undetectable to non-destructive chlorophyll fluorescence measurement; hence chlorophyll fluorescence metrics might not be a perfect proxy for measuring a plant's physiological response to treatment effects [20].

Opportunities for more sustainable plant production are emerging from integrated bioelectric hydroponics systems, which are increasingly being explored as recycling technologies for food production. In such systems, waste streams are fed to biofilms in Microbial Fuel Cells that both produce bioelectricity and generate catholyte that can be used as nutrient rich biofertilizer—both useful for hydroponics. Nam et al. [21] confirmed that adequately treated UV/H_2O_2 -wastewater nutrient solution can grow vegetables without any phytotoxic effect and Nath et al. [22] have shown that MFC catholyte can be mixed with synthetic nutrient solution for the growth of basil in a closed loop hydroponic system. Recently, Gadja et al. [23] confirmed that catholyte derived from MFCs is rich in nitrogen, phosphorus and potassium with low Chemical Oxygen Demand (COD), which makes it a good source of biofertilizer, but that it is saline with a high electrical conductivity (EC). Calcium supplementation, such as gypsum, is notable for its effect in maintaining ionic balance under saline-sodic or sodic soil conditions and may also modulate ROS detoxification by improving the antioxidant defence system [24]. There is, however, no information regarding plant metabolic response to NS management-catholyte-gypsum combinations including on some key biochemical traits such total phenolic content (TPC), protein concentration, nitrate reductase activity (NR), pigment concentration.

Previous research has confirmed that basil growth does not always correlate with its leaf chemistry. For example, plant leaf chlorophyll might remain unchanged despite biomass changes [10,25]. We predicted that reducing NS would not necessarily show a significant change in key parameters when measured at harvest. Instead, the difference in biomass response is expected to reflect time integrated nutrient uptake and efficiency, canopy development and sink development under altered nutrient solution. Additionally, NS composition/EC are known to influence cellular redox balance and initiate key cell signals [26–29], thus, we predicted that NS substitution with wastewater-catholyte (WW) would initiate the production of secondary metabolites, thereby producing phenolic/flavonoid accumulation and thus affect quality-related traits in basil. Finally, calcium is widely known for its role in maintaining ion homeostasis under ionic loaded solution [24]—we therefore also hypothesized that the addition of a calcium source (gypsum) in NS-WW substitution would buffer the ionic imbalance and stabilize PSII (photosystem II) photochemical performance of the plant, while also improving the quality output under reduced nutrient solution strength without any significant growth penalty. We treated yield as a time integrated property that culminates in resource allocation over time in a basil plant, while secondary metabolism was conceived as a regulated physiological outcome. The objective of this study was, therefore, to determine how NS and wastewater-catholyte (WW) ± gypsum supplementation interact in hydroponic basil grown in a controlled environment to regulate growth, nitrogen metabolism, chlorophyll fluorescence and secondary metabolism. This research aimed to address two major questions:(i) Do biomass accumulation and biochemical traits increase linearly with NS concentration in hydroponic grown basil? (ii) How do NS-WW ± gypsum combinations alter metabolic processes in basil (i.e., primary to secondary metabolism)?

2. Methods

2.1. Growth Conditions

Three controlled-environment hydroponic experiments were conducted at the Envirotron facility of the University of the West of England (UWE), located at the Frenchay Campus in Bristol, England (51.5002° N, 2.5486° W). The experiments were sequential, used 16/8 h photoperiod with 400 ± 40 µmol m^{-2} s^{-1} at canopy level using LED panel (Phytolux LED growth lighting, model name: Attis-7) and 50–60% RH. Each treatment across the trials had 10 pseudo-replicates of hydroponic baskets fitted in troughs (Figure 1). Treatment positions in tanks were randomized across the experiments to minimize spatial effects.

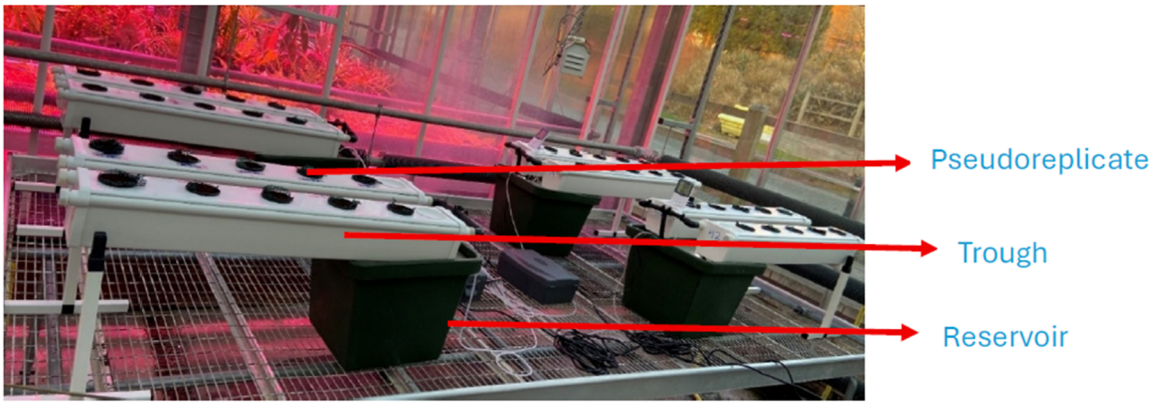


Figure 1. Terra Aquatica GrowStream system at UWE Envirotron. Sonneveld's solution was supplied using an N demand-led model. Nutrient solution was circulated from the reservoir (green tank) up into the troughs to a depth of 15 cm and then returned to the reservoir. Aeration was supplied in the reservoir tank.

2.2. Plant Material and Treatments

Sweet basil (*Ocimum basilicum* L., cv. Genovese 'Aroma 2' (F1)) seeds were sourced from a certified supplier (Royal Horticultural Society, Newmarket, UK). The hydroponic nutrient solution used was Sonneveld's solution [30]. Treatments used in experiments were as follows, all calculated using an N demand-led model (Supplementary Figures S1 and S2):

Experiment 1: 100% nutrient solution (NS), 75% NS, 50% NS, 25% NS, all basil plants were germinated in a thin layer of peat (Seedland Modular Low Nutrients; AICI Professional Horticulture, Israel) on top of a perlite filled basket (Vitax Ltd., Leicestershire, UK) and then grown in hydroponics for 39 days. The aim of the first experiment is to measure plant response to different nutrient solution (NS) dilutions. Experiment 2: 80% NS, 60% NS, 40% NS, 20% NS, all basil plant were germinated in a thin layer of vermiculite (PLANT!T, Hydrogarden Wholesale Supplies Ltd., Coventry, UK) on top of a perlite filled basket (Vitax Ltd.) and then grown in hydroponics for 40 days. In Experiment 2, different dilutions were investigated to provide a better resolution of the response of basil to nutrient solution dilutions. The germination substrate was changed between experiment 1 and 2, to check if peat affected long-term growth. Experiment 3: 100% NS + 0% Catholyte (WW), 50% NS + 50% WW (Table 1), 50% NS + 50% WW + 0.46 mM of gypsum. All basil plants were germinated in Perlite (Vitax limited) and peat (Seedland Modular Low Nutrients; AICI Professional Horticulture, Israel) and grown in hydroponics for 40 days. The WW used for the cultivation of basil in experiment 3 contains elevated level of Na^+ and Cl^- (Table 1), hence gypsum was added as a source of Ca^{2+} to moderate potential salt induced ionic imbalance and negative plant response to treatment. This inclusion was based on the established role of Ca^{2+} in moderating detrimental effects of salt stress [31]. Upon harvest, plant samples were frozen at −20 °C before further analysis.

2.3. Hydroponic System and Nutrient Solution Management

Basil seedlings were grown in the Terra Aquatica Growstream® system (Terra Aquatica, Fleurance, France) in which solution is circulated from a reservoir tank (Figure 1) up into two troughs in which solution is 15cm deep and flows back into the reservoir. Aeration pumps (AQQA Aquarium Air Pump, Shenzhen Aiku Pet Technology Co., Ltd., Shenzhen, China) and stones were submerged in reservoirs in all experiments. The nutrient solution used was Sonneveld's solution, the concentration of which was based on a growth driven model that calculated N supply based on N demand (Supplementary Figures S1 and S2). Assuming 3% N in final biomass, a standard sigmoidal growth curve and a necessary supply of 1.5x demand (Supplementary Figure S2), weekly additions were calculated

to meet demand whilst minimising excessive use of nutrients. This regime, which supplied 437.24 mg N in total per plant over the growth period in concentrations adjusted to meet demand, was defined as the 100% nutrient supply (NS). This regime matched demand with supply more closely than supplying Sonneveld's solution at, for example, 10 mM N throughout the growth period. The different % of NS were produced by scaling the N concentration. Solution pH was controlled and maintained to between 5.5 to 6.5 using a few drops of 1M NaOH and HCl solution, while deionized water was added to maintain 40L water volume as needed throughout the experiment. In experiment 3 gypsum ($CaSO_4 \cdot 2H_2O$; 99%; Sigma-Aldrich, St. Louis, MO, USA) was added to the third treatment to a final concentration of 0.46 mM (3.14g per 40 L). Basil samples from the two troughs fed from each reservoir were considered as pseudoreplicates across the three experiments.

Microbial Fuels Cells were set up, fed and run as previously reported with a raw sewage inoculum and feedstock [22]. Catholyte derived from this process was collected from the University of Southampton and transported to UWE, Bristol for experiment 3 (Table 1) [22]. The nitrogen contribution of the catholyte was calculated from the addition of its NH_4^+, NO_2^-, and NO_3^- concentrations (Table 1), and incorporated into the N-demand -led model to determine the volume of catholyte required to fulfil the targeted amount of plant nitrogen demand at different growth stages.

Table 1. Physicochemical and Ionic characterization of Catholyte. (1a) Characterization of catholyte samples. (1b) Cation composition and (1c) Anion composition [22].

1a: Characterization of Catholyte Samples from MFCs with Raw Sewage Feedstock				
Sample	**Color**	**pH**	**Electrical Conductivity (mS cm^{-1})**	**COD (mg/L)**
Raw Sewage	Dark grey/Black	7.5	1.89	372 ± 12
Catholyte-sewage fed MFC Tray	colorless	10.3	5. 69	--

1b: Cations Present in the Catholyte Samples					
Sample	**Na^+ [mg/kg]**	**NH_4^+ [mg/kg]**	**K^+ [mg/kg]**	**Ca^{2+} [mg/kg]**	**Mg^{2+} [mg/kg]**
Raw Sewage	314 ± 3	<0.5	18 ± 15	85 ± 4	28 ± 12
Catholyte sewage-fed MFC	1759 ± 3	<0.5	95 ± 14	23 ± 4	<0.2

1c: Anions Present in the Catholyte Samples					
Sample	**F^- [mg/kg]**	**Cl^- [mg/kg]**	**NO_2^- [mg/kg]**	**NO_3^- [mg/kg]**	**PO_4^{2-} [mg/kg]**	**SO_4^{2-} [mg/kg]**
Raw sewage	<0.08	480.5 ± 0.3	<0.8	<0.3	<0.3	77 ± 1
Catholyte sewage- fed MFC	<0.08	618.5 ± 0.3	<0.8	25 ± 1	9 ± 3	101 ± 1

2.4. Measurement of Growth and Physiological measurements

Non-destructive repeated plant trait measurement was conducted all through the experiments. Briefly, shoot height was recorded with a ruler, shoot fresh weight with a balance, leaf number by manual counting, and leaf area by measuring leaf length and width multiplying them and applying a correction factor of 0.68 [32]. Total biomass of plant parts was then recorded at harvest. Leaf NR (nitrate reductase) activity was quantified using a commercial colorimetric Nitrate Reductase/NR Activity Assay Kit (NBP3-25889, Novus Biologicals, Centennial, CO, USA) following the manufacturer's protocol. Total soluble protein was quantified using the Quantipro Bicinchoninic acid (BCA) Assay kit, following the manufacturers guide (Sigma-Aldrich, Cat. No. QPBCA). Total phenolic content (TPC) of basil was determined using the Folin-Ciocalteu colorimetric method as described by Mahmoud et al. (2022) [33]. Briefly, plant fresh leaves were extracted using 96% ethanol, 0.25 mL of 20 mg/mL of extract was then added to 0.25mL Folin-Ciocateu reagent (Sigma-Aldrich); (Folin was initially diluted using 1:1, *v*/*v* deionized water), then 0.5 mL of 7.5% Na_2CO_3 **(**Sigma-Aldrich), and 4.0 mL distilled water was added to the mixture. The resulting mixture was then incubated at 23 degrees for thirty minutes (30 min), then centrifuged for 10 min and absorbance was measured at 725 nm. TPC was quantified as gallic acid equivalents (GAE). Plant pigment was quantified using 80% acetone as an extractant, which was then centrifuged at 12,000 rpm for 15 min. The absorbance was measured using a microplate reader at 663, 645, and 470 nm. Pigment content was calculated using the following absorbance (A) calculations [34]

$$C_a = (12.25A_{663.2}) - (2.79A_{646.8}),$$

$$C_b = (21.5A_{646.8}) - (5.1A_{663.2}),$$

$$Car = (1000A_{470} - 1.82C_a - 85.02C_b)/(198);$$

where C_a, C_b and Car are chlorophyll a, chlorophyll b, and carotenoids respectively.

Chlorophyll fluorescence parameters and Electron Transfer Rate (ETR) in photosynthesis was measured using a Plant Stress kit (PSK) fluorometer (Opti-Sciences/PSK, Hudson, NH, USA) according to manufacturer's instructions.

2.5. Statistical Analyses

The reservoir utilized throughout the three experiments were considered the experimental unit because all plants in a given treatment shared the same nutrient solution and treatment condition. Therefore, the individual plants within each of these reservoirs were pseudoreplicates (i.e one treatment reservoir was fed to 2 growth troughs containing 10 individual basil plants). Statistical analysis was done using the pseudo-replicates. All recorded data were analysed using R (v 4.5.1) software and means were compared with the Tukey and Sidak tests where applicable. For longitudinal variables (leaf area, leaf number, shoot height and shoot fresh weight), Linear Mixed-effects Models (LMM) were used to analyse the non-destructive repeatedly measured growth trait data over time (days after planting, DAP) because it could accommodate repeated measures and missing data points caused by some regular destructive harvesting. Repeated measurements (for which LMM is suitable) were analysed using a fixed effect of time (DAP_c), treatment and interaction, while plant identity was used as a random intercept to account for repeated observations. Models were fitted by REML (restricted maximum likelihood) using package 'lme4' [35]. Treatment-specific growth rates were then estimated as marginal time slopes with 95% confidence intervals (CI) and Tukey-adjusted pairwise comparison were used to differentiate treatment effects for individual traits. This posthoc test was chosen because it is appropriate for posthoc comparisons of model-estimated marginal means from mixed effect models and controls for family-wise Type I error rate. One way or two-way ANOVA with either Sidak or Tukey HSD was adopted where necessary, Briefly, endpoint data (pigments, biochemical data, NR, TPC) were analysed using one-way Anova (Tukey HSD; $\alpha = 0.05$), while chlorophyll fluorescence, growth and yield data was analysed using two-way ANOVA with and without pseudoreplicates respectively (Šidák; $\alpha = 0.05$). Tukey HSD posthoc test was adopted when all pairwise treatment means were compared, whereas Šidák adjustment was used for specified simple-effect comparisons where observation has a single observation per treatment x day combination (single harvest per day). All statistical analyses were done using pseudoreplicates (plants benefitting from same treatment reservoir), therefore, the inferential outcomes of the analysis should be considered as exploratory plant-level comparisons rather than independently replicated reservoir-level treatment effects.

3. Results

3.1. Rates of Increase in Shoot Growth

For plants germinated in perlite+peat traits differed in growth response to %NS (Figure 2). From 100% to 50% NS there was no significant effect on the average rate of increase in leaf area, leaf number, shoot fresh weight and shoot height across the growth period, while 25% NS had lower average rate across leaf area, leaf number and shoot fresh weight, with the exception of shoot height where similar response was observed across all treatments.

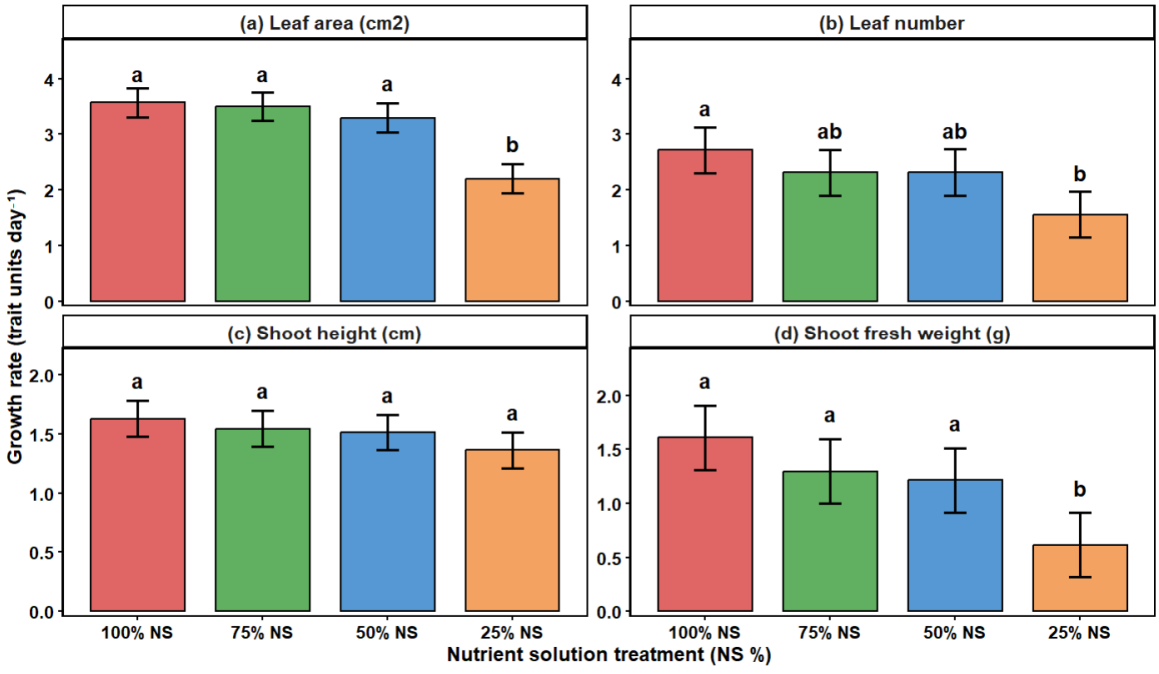


Figure 2. Growth-rate comparison for (**a**) leaf area, (**b**) leaf number, (**c**) shoot height, (**d**) shoot fresh weight at different % of Sonneveld's solution for *Ocimum basilicum* (Basil) cv. Genovese. Bars show mean slope estimates and error bars show 95% confidence intervals from a linear mixed model (LMM) fitted to repeated measurements

across 39 days. Traits a, b, and c were assessed on 10 plants, whereas (d) was assessed on 5 plants. Different letters indicate significant differences between treatments based on Tukey-adjusted pairwise comparisons (α = 0.05). Note: Analysis was conducted using pseudoreplicates; inferential results are exploratory plant-level comparisons, and not independently replicated reservoir-level treatment effects.

For plants germinated in perlite and vermiculite then grown with 80% NS, 60% NS, 40% NS, 20% NS (Figure 3), the average rate of increase in the leaf area and shoot fresh weight traits were lower overall compared to the outcome of experiment 1 (Figure 2), indicating that plants grew better over 39–40 days when germinated in peat. In experiment 2, 60% NS produced a pronounced leaf number response, which exceeds the highest leaf number response in Figure 2 (experiment 1). Shoot height growth rates were similar across both Figures, with a slightly higher maximum at 60% NS in Figure 3 (experiment 2). For the majority of the parameters in Figure 3, the rates were highest, often statistically significant so, at 60% NS.

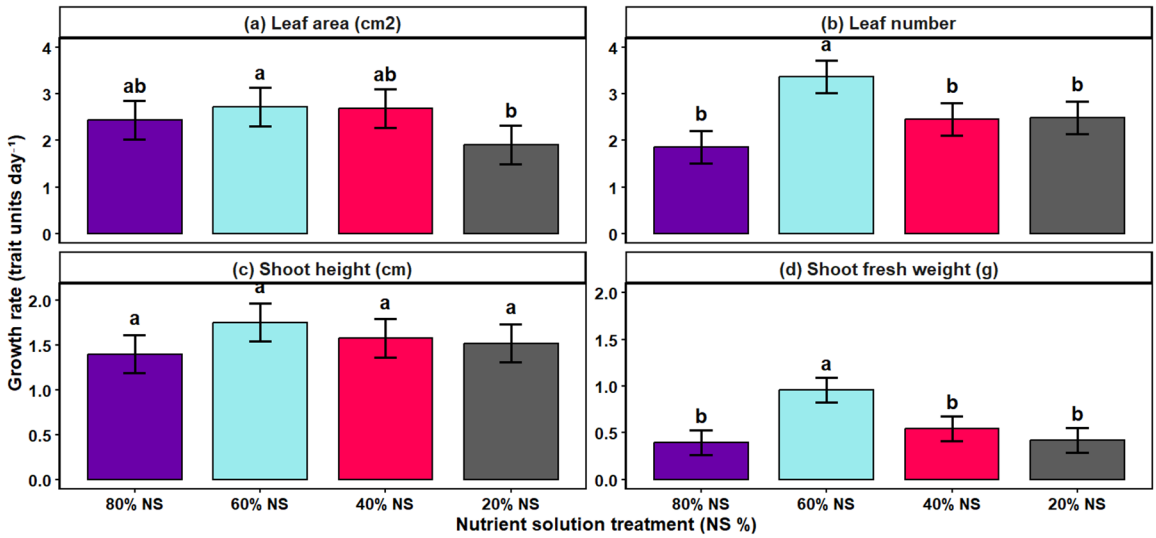
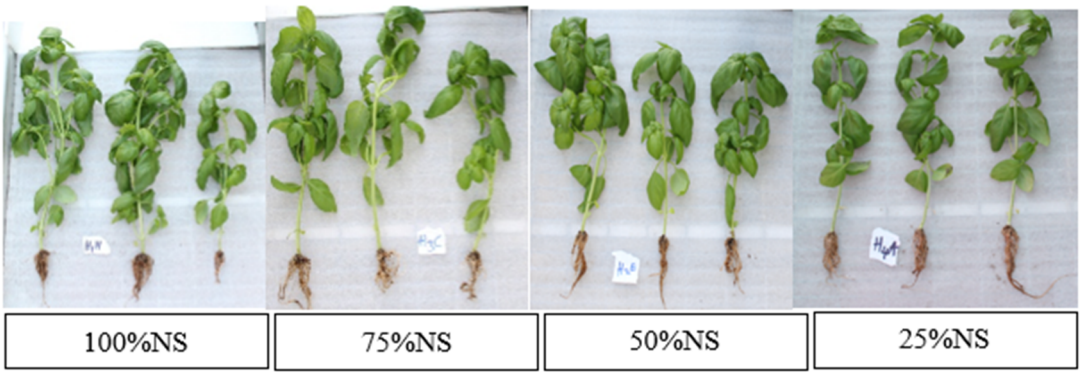


Figure 3. Growth-rate comparison for (**a**) leaf area; (**b**) leaf number; (**c**) shoot height; and (**d**) shoot fresh weight between treatments of % Sonneveld's solution for *Ocimum basilicum* (Basil) cv. Genovese. Bars show mean slope estimates and error bars show 95% confidence intervals from a linear mixed model (LMM) fitted to repeated measurements of each trait across 40 days. All traits were assessed on 10 plants. Different letters indicate significant differences between treatments based on Tukey-adjusted pairwise comparisons (α = 0.05). Note: Analysis was conducted using pseudoreplicates; inferential results are exploratory plant-level comparisons, and not independently replicated reservoir-level treatment effects.

3.2. Plant Morphology and Leaf Biochemistry at Harvest

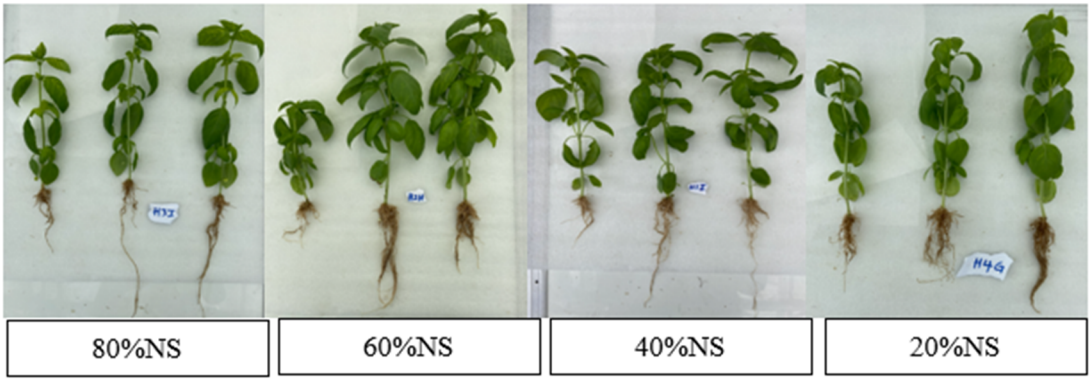
At harvest on day 39, for plants germinated in perlite+peat, in all treatments (100%, 75%, 50%, 25% NS), there were significant differences in biomass accumulation (shoot and root fresh weight), while all other measured traits were indistinguishable (Figure 4). Shoot and root fresh weight was similar from 100 to 50% NS, whereas there were differences at lower boundary (25% NS). For biochemical traits, there was no significant differences across NS strengths: nitrate reductase activity, protein, and total phenolic content (TPC) produced a similar response. Likewise, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids showed no treatment effects (Figure 4).

In plants germinated in perlite and vermiculite then grown in 80%, 60%, 40%, and 20% NS (experiment 2), several traits showed a statistically greater response at intermediate NS strength (Figure 5). Shoot height was highest at 60% NS, intermediate at 40% NS, and lower at 20% NS and 80% NS. Shoot fresh weight similarly peaked at 60% NS, whereas 80%, 40%, and 20% NS were comparable and lower. Root length was greatest at 40% NS, with 60% NS intermediate, and 80% and 20% NS lower. Root fresh weight was highest at 60% NS, intermediate at 40% NS, and reduced at 20% NS and 80% NS. In contrast, nitrate reductase activity (NR) and protein concentration did not differ among NS treatments. Total phenolic content (TPC) was lower at 80% NS but higher and comparable at 60% to 20% NS. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid concentration were similar across treatments.



Trait (unit)	100% NS	75% NS	50% NS	25% NS	N
Shoot height (cm)	33.19 ± 14.17 a	30.60 ± 11.01 a	30.26 ± 11.06 a	30.10 ± 8.59 a	10
Shoot fresh weight (g)	20.61 ± 13.44 a	15.81 ± 11.29 a	15.94 ± 10.07 a	8.16 ± 3.44 b	10
Root length (cm)	29.85 ± 8.08 a	31.50 ± 3.96 a	33.30 ± 4.54 a	31.73 ± 6.89 a	10
Root fresh weight (g)	6.03 ± 3.18 a	4.88 ± 3.46 ab	5.04 ± 3.14 ab	3.49 ± 1.47 b	10
Nitrate reductase activity (µmol NO_2^- g^{-1} FW h^{-1})	0.39 ± 0.28 a	0.24 ± 0.06 a	0.37 ± 0.19 a	0.25 ± 0.38 a	3
Protein (mg g^{-1} FW)	0.64 ± 0.18 a	0.57 ± 0.11 a	0.70 ± 0.16 a	0.61 ± 0.04 a	4
Total phenolic content, TPC (µg GAE g^{-1} FW)	184.60 ± 22.86 a	244.09 ± 236.12 a	229.14 ± 61.61 a	331.18 ± 201.30 a	3
Chlorophyll a (mg g^{-1} FW)	0.07 ± 0.01 a	0.05 ± 0.02 a	0.05 ± 0.01 a	0.06 ± 0.01 a	3
Chlorophyl b (mg g^{-1} FW)	0.03 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.00 a	0.03 ± 0.00a	3
Total Chlorophyll (mg g^{-1} FW)	0.09 ± 0.02 a	0.08 ± 0.03 a	0.07 ± 0.01 a	0.09 ± 0.01 a	3
Carotenoids (mg g^{-1} FW)	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	3

Figure 4. The table shows the effects of nutrient-solution (NS) strength on plant morphology, nitrate reductase activity (NR), protein, total phenolic content (TPC), and photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, carotenoids) after 39 days growth in different % of Sonneveld's solution. Values are mean ± SD (fresh-weight basis where applicable). Within each row, different letters indicate significant differences between NS strengths (Tukey HSD and Sidak, $\alpha = 0.05$ were used where applicable). N denotes the number of pseudoreplicates used for that trait. Germination medium was perlite and peat. Pictures at top are of plants from 100%, 75%, 50% and 25% NS. Different letters (a and b) in each row represent significant differences ($p < 0.05$). Note: Analysis was conducted using pseudoreplicates; inferential results are exploratory plant-level comparisons, and not independently replicated reservoir-level treatment effects.

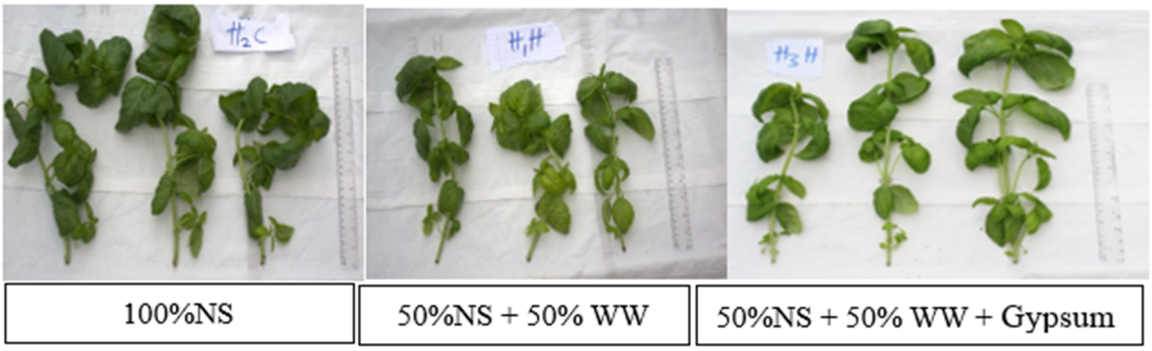


Trait (unit)	80% NS	60% NS	40% NS	20% NS	N
Shoot height (cm)	26.97 ± 3.11 c	32.64 ± 2.38 a	31.16 ± 4.47 ab	28.22 ± 2.81 bc	5
Shoot fresh weight (g)	7.07 ± 1.36 b	14.06 ± 4.21 a	9.36 ± 2.91 b	6.79 ± 1.48 b	5
Root length (cm)	23.47 ± 2.65 b	27.40 ± 4.18 ab	33.19 ± 4.48 a	22.14 ± 4.12 b	5
Root fresh weight (g)	1.50 ± 0.24 c	3.91 ± 1.25 a	2.85 ± 0.81 ab	2.56 ± 0.09 bc	5
Nitrate reductase activity (µmol NO_2^- g^{-1} FW h^{-1})	0.09 ± 0.08 a	0.42 ± 0.47 a	0.19 ± 0.33 a	0.11 ± 0.20 a	3
Protein (mg g^{-1} FW)	0.63 ± 0.18 a	0.68 ± 0.01 a	0.54 ± 0.13 a	0.94 ± 0.40 a	4
Total phenolic content, TPC (µg GAE g^{-1} FW)	128.92 ± 42.12 b	296.04 ± 100.50 a	266.92 ± 25.33 a	267.60 ± 42.48 a	4
Chlorophyll a (mg g^{-1} FW)	0.05 ± 0.02 a	0.05 ± 0.00 a	0.07 ± 0.00 a	0.05 ± 0.01 a	4
Chlorophyl b (mg g^{-1} FW)	0.02 ± 0.01 a	0.02 ± 0.00 a	0.03 ± 0.00 a	0.02 ± 0.01 a	4
Total Chlorophyll (mg g^{-1} FW)	0.08 ± 0.03 a	0.07 ± 0.00 a	0.09 ± 0.00 a	0.07 ± 0.02 a	4
Carotenoids (mg g^{-1} FW)	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	4

Figure 5. Table shows the effects of % of Sonneveld's solution on growth, nitrate reductase activity (NR), protein, total phenolic content (TPC), and photosynthetic pigments (Chlorophyll a, chlorophyll b, Total chlorophyll, carotenoids) of *Ocimum basilicum* cv. Genovese (basil). Values are mean ± SD (fresh-weight basis where

applicable). Within each row, different letters indicate significant differences between NS strengths (Tukey HSD and Sidak, α = 0.05 were used where applicable). N denotes the number of pseudoreplicates used for that trait. Growth media was Perlite and vermiculite. Pictures at top show plants grown at different % NS. Different letters (a, b and c) in each row represent significant differences ($p < 0.05$). Note: Analysis was conducted using pseudoreplicates; inferential results are exploratory plant-level comparisons, and not independently replicated reservoir-level treatment effects.

At harvest plants germinated in experiment 3 (perlite + peat and grown in 100% NS, 50% NS + 50% WW, and 50% WW + 50% NS + 0.46 mM gypsum) responded differently to the dilutions (Figure 6). Shoot height was highest under 100% NS, intermediate under 50% NS + 50% catholyte N and lowest when gypsum was added to the WW + NS substitution. In contrast, basil shoot fresh weight declined significantly under both 50% WW + 50% NS ± 0.46 mM gypsum, 100% NS produced the greatest biomass, whereas 50% NS + 50% WW, and 50% WW + 50% NS + 0.46 mM gypsum produced a similar and significant low response. The same trajectory was observed in basil leaf number response to treatment application, where 100% NS had a significantly higher response compared to both 50%WW + 50% NS ± 0.46 mM gypsum, which were similar in response. By contrast, protein concentration did not differ between treatments, indicating stability in this trait across nutrient regimes. Total phenolic content (TPC) showed the opposite trend to biomass: it was lowest under 100% NS, intermediate under 50% NS + 50% WW, and highest with gypsum supplementation.



Trait (Unit)	100% NS	50% NS + 50% WW	50% WW + 50% NS + 0.46mM Gypsum	N
Shoot height (cm)	24.21 ± 10.76 a	21.71 ± 10.04 ab	20.54 ± 11.00 b	8
Shoot fresh weight (g)	15.46 ± 11.31 a	8.18 ± 5.79 b	8.34 ± 6.83 b	8
Leaf number	33.42 ± 16.24 a	26.17 ± 12.85 b	24.79 ± 11.48 b	8
Protein (mg g^{-1} FW)	1.39 ± 0.51 a	1.89 ± 0.56 a	1.29 ± 0.36 a	7
Total phenolic content, TPC (µg GAE g^{-1} FW)	244.29 ± 107.99 b	444.29 ± 347.27 ab	612.86 ± 226.99 a	7

Figure 6. Table shows the effects of wastewater catholyte N (WW) and gypsum supplementation on *Ocimum basilicum* cv. Genovese (basil) growth and biochemical traits. Treatments were 100% nutrient solution (NS), 50% NS + 50% WW (wastewater catholyte), and 50% WW + 50% NS supplemented with 0.46 mM gypsum. Values are means ± SD (fresh-weight basis where applicable). Within each row different letters indicate significant differences between treatments (Tukey HSD and Sidak, α = 0.05). *N* indicates the number of pseudoreplicates for each trait. Germination medium was perlite and peat. Pictures at the top show three plants from different treatments. Different letters (a and b) in each row represent significant differences ($p < 0.05$). Note: Analysis was conducted using pseudoreplicates; inferential results are exploratory plant-level comparisons, and not independently replicated reservoir-level treatment effects.

3.3. Photosynthetic Function and Pigment Responses

As shown in Figure 7, in plants germinated in perlite+peat in experiment 1 there were some statistical differences in chlorophyll florescence between treatments through the growth period. DAP had a significant effect on Y(Fm), as compared to treatment and treatment-DAP interaction with a peak response around 31 DAP, followed by a marked depression at 38 DAP, and a minor gain at 39 DAP (Supplementary Table S1). In contrast, treatment had a significant effect on α (Fv/Fo) as compared to the day and treatment-DAP interaction, where 100% NS and 50% NS had similar and significant effect from 25% NS which had the lowest and 75% NS had an intermediate response (Supplementary Table S1). ETR exhibited a significant treatment-day interaction effect on basil plants, with diverse effect of treatment at different sampling dates, most notably at around 33DAP where 100% NS had a significant effect compared to other treatments (75% NS, 50% NS and 25% NS) while ETR response were minimal at other dates (Supplementary Table S1).

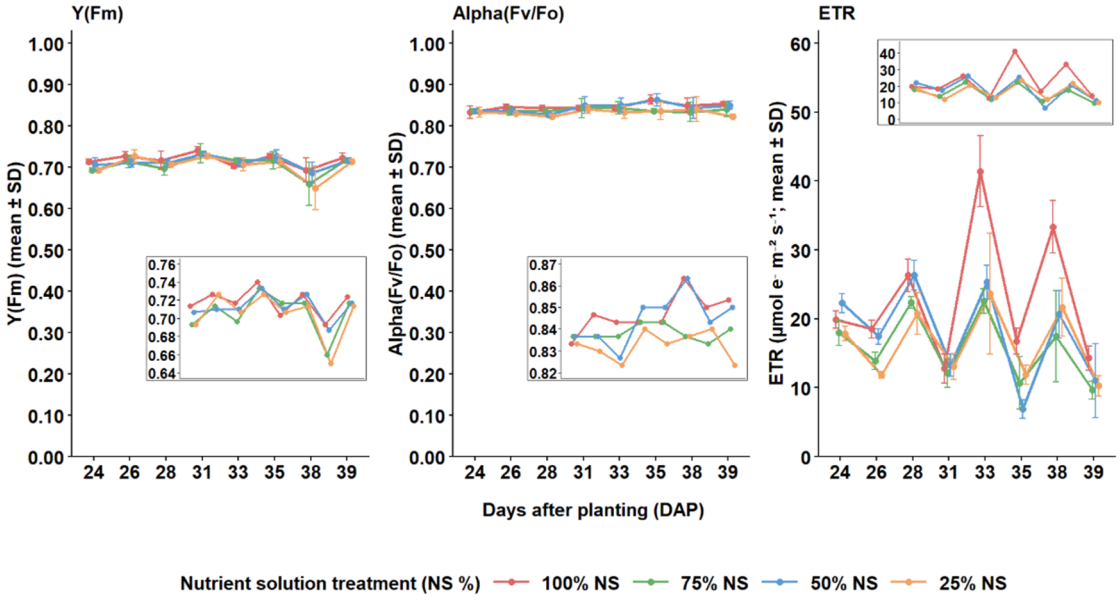


Figure 7. Chlorophyll fluorescence parameters of basil (*Ocimum basilicum* cv. Genovese) were measured over 39 days under different % of full Sonneveld's nutrient solution (NS) (n = 3): effective PSII quantum yield (Y(Fm)), a proxy for PSII efficiency **(**Alpha (Fv/Fo), and electron transport rate (ETR, µmol e^- m^{-2} s^{-1}). Insert boxes show curtailed Y axes. See Supplementary Table S1 document for mean separation. Note: Analysis was conducted using pseudoreplicates; inferential results are exploratory plant-level comparisons, and not independently replicated reservoir-level treatment effects.

As shown in Figure 8, for plants germinated in perlite+vermiculite (experiment 2) chlorophyll florescence varied significantly with treatment, DAP (days after planting) and treatment-DAP interaction. Y(Fm) exhibited a significant treatment-DAP interaction effect on basil plants, with varying significance at different dates, notably, at 33 DAP, where 60% NS treatment produced a significantly lower response than other treatments (20, 40, and 80% NS) (Supplementary Table S2). Similarly, at 35 DAP 60% NS had the lowest response as compared to other treatments. Whereas the responses towards the end of the experiment were not significant and similar across treatments (38 and 40 DAP). However, treatment effect was significant for alpha (α (Fv/Fo)), while DAP and the treatment-DAP interaction were not significant. 60% NS had the lowest alpha value while other treatments (80%, 40% and 20% NS) were similar and not significantly different. ETR response was significantly affected by treatment and day, whereas the treatment-day interaction was not significant. Notably, 60% NS was significantly different from 80% NS, whereas 40% and 20% NS were similar and intermediate.

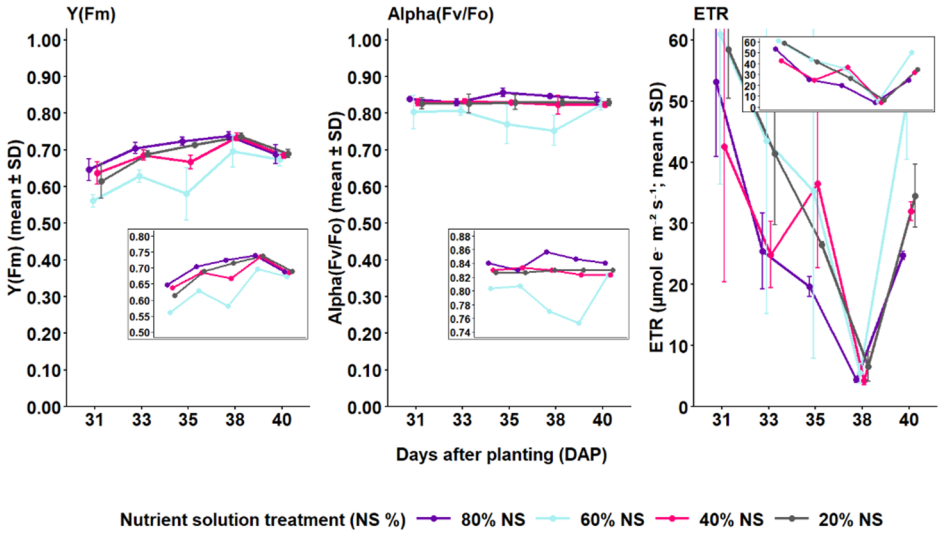


Figure 8. Chlorophyll fluorescence parameters of basil (*Ocimum basilicum*) measured under different nutrient solution (NS) strengths (n = 3): effective PSII quantum yield (Y(Fm))**,** electron transport rate (ETR, µmol e^- m^{-2} s^{-1}),

and PSII efficiency proxy (Alpha (Fv/Fo). NS treatments were 80%, 60%, 40%, 20% NS. Bars show means ± SD. See Supplementary Table S2 for mean separation. Note: Analysis was conducted using pseudoreplicates; inferential results are exploratory plant-level comparisons, and not independently replicated reservoir-level treatment effects.

For plants germinated on perlite+peat and treated with wastewater NS via MFC catholyte there were some statistically significant effects of DAP and treatment on chlorophyll fluorescence parameters (Supplementary Table S3). In particular, for Y(Fm) at some DAPs plants with WW were different from those in 100% treatment but gypsum moderated this effect at the end. Contrary to this, alpha (Fv/Fo) response was significant in control treatment as compared to both WW treatments (Figure 9). For ETR, the WW treatment was sometimes lower across the days than the 100% treatment whereas with gypsum were sometimes higher than the 100% treatment and were significantly higher at the last measurement.

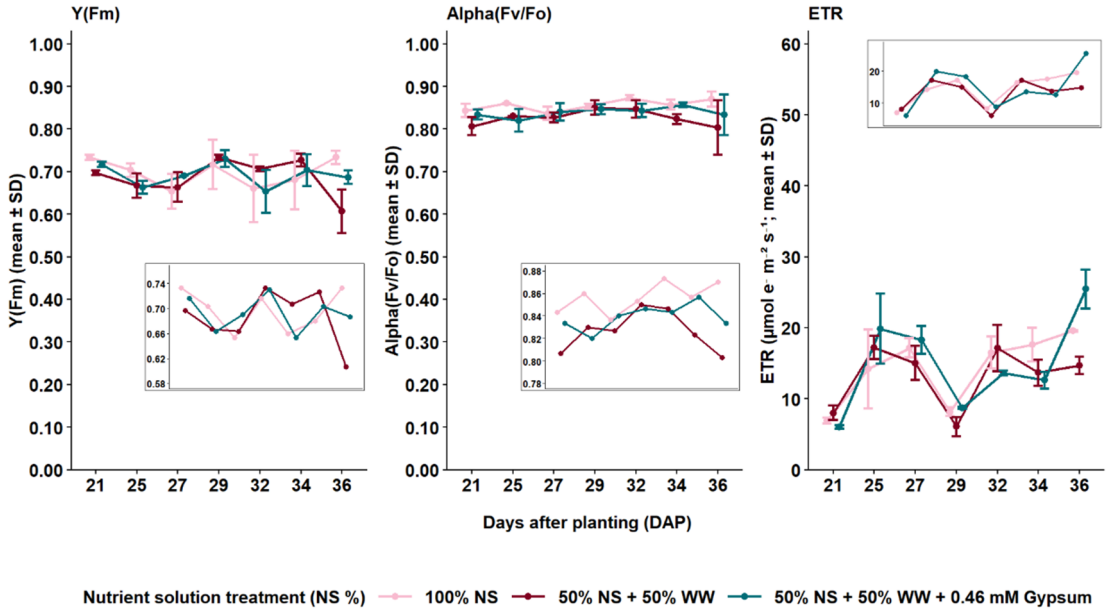


Figure 9. Chlorophyll fluorescence parameters of basil (*Ocimum basilicum* cv. Genovese) measured under different % Sonneveld's solution (n = 3): effective PSII quantum yield (Y(Fm)), a proxy for PSII efficiency (Alpha (Fv/Fo), and electron transport rate (ETR, µmol e^- m^{-2} s^{-1}). NS treatments were 100% nutrient solution (NS), 50% NS + 50% WW, and 50% WW + 50% NS supplemented with 0.46 mM gypsum. See Supplementary Table S3 for statistical details. Note: Analysis was conducted using pseudoreplicates; inferential results are exploratory plant-level comparisons, and not independently replicated reservoir-lvel treatment effects.

An integrative plot (Figure 10) shows non-linear trait dependent responses to NS strength across the dilution trials (experiment 1 and 2). In experiment 1, biomass growth rate (leafarea_slope, shootheight_slope, leafnumber_slope and shootfreshweight_slope) responded similarly across different dilutions with similar response between 50–100% NS but there is clear decline at 25% NS. Similarly, the harvest metrics, showed root and shoot fresh weight response were fairly similar for dilutions between 50% to 100% NS, but lower for 25% NS, while shoot height and root length were comparable. The protein and TPC response followed a similar pattern across dilutions, while the ETR_slope response showed a U-shaped response, which was high at the extremes (100% and 25% NS), α_slope and Y(Fm)_slope were similar across different NS dilutions. On the other hand, experiment 2 had most of the measured trait peaked at 60% NS with other dilutions having a similar response, which is notable at LeafNumber_slope, ShootHeight_slope, and ShootfreshWeight_slope, while leafarea_slope was highest at 40%NS and lowest at 20% NS. At harvest metrics of root and shoot fresh weight was peaked at 60% NS, while root length was peaked at 40% NS (other dilutions remained fairly similar) and shoot height was similar across dilutions. Protein response to dilutions in experiment 2 was similar with 20% NS and 40% NS having the highest and lowest response respectively, TPC followed a different pattern with 60% NS having the highest response, while other dilutions were similar. Also, α_slope in experiment 2 also assumed a U-shaped response to treatment (peaked at the lowest and highest treatment), while ETR_slope and Y(Fm)_slope response to NS dilutions were comparable. In experiment 3, chlorophyll fluorescence response to treatment were trait specific. Y(Fm) response was comparable between 100% NS and 50% NS + 50% WW, whereas 50% NS + 50%

WW + gypsum was higher. ETR response was comparable across treatments including control. In contrast, α_slope response for the WW-based treatments was lower than the 100% NS control. Also, Leaf number, shoot height and shoot fresh weight response were similar between 100% NS and 50% NS + 50% WW, while 50% NS + 50% WW + gypsum resulted in a lower metrics. Protein and TPC response were higher in WW-based treatments than the 100% NS control, with the strongest increase observed under the WW + gypsum treatment.

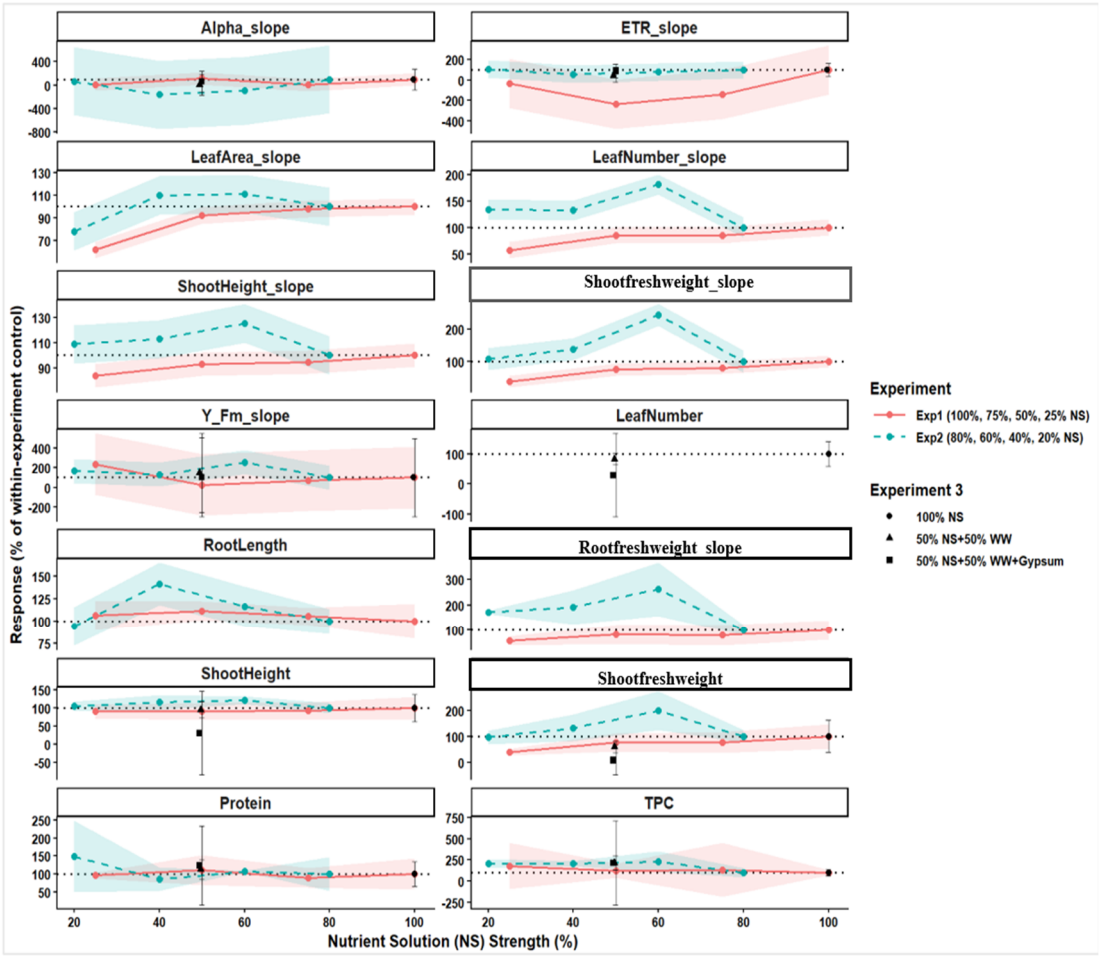


Figure 10. Cross-experiment integrative response plot showing trait responses to nutrient-solution strength and substitution treatments. Responses were expressed as percentage of the within-experiment control (100% NS for experiment 1 and 3, 80% NS for experiment 2) across nutrient solution (NS). Experiment 1 (solid red line) and 2 (dashed green line) shown with shaded bands to indicate 95% uncertainty around the mean response (as plotted). Points denote Experiment 3 substitution treatments (100% NS control; 50%NS+50% wastewater; and 50%NS+50% wastewater + gypsum) with 95% CIs. Panels show growth-rate slopes, photosynthetic slopes, harvest traits, and quality-related traits (protein and total phenolic content, TPC). The horizontal dotted black line marks the control (100%). Note: Analysis was conducted using pseudoreplicates; inferential results are exploratory plant-level comparisons, and not independently replicated reservoir-lvel treatment effects.

4. Discussion

4.1. Potential Nutrient-Sparing Nutrient Solution (NS) Range for Basil Production

The combined results of the three experiments suggest an optimization guide for increasing the sustainability of basil production, based on a reduction of total nutrient supplied and the use of recycled biofertilizer from MFC catholyte, while maintaining good growth and controlling biomass quality. Providing nutrients at low strength (20–25% NS) resulted in a reduced growth outcome but the results showed that a reduced strength of 50–75% NS maintained comparative growth response across different traits measured and that gypsum could help sustain quality traits at 50% NS. This suggests that good quality basil could be produced with the addition of just 200–300 mg N per plant (436/2 = 218 mg) alongside other NS using demand-led N supply and WW supplementation—a very significant reduction in NS and N utilisation compared to 10mM N of full strength Sonneveld's solution.

There are some important limitations which should be considered when interpreting the above conclusions. First, the hydroponic system lacks a true replicate—the ten plants in each treatment benefit from a single nutrient reservoir across the experiments (Figure 1), hence they are regarded as pseudoreplicates and not independent experimental units/treatment level replicates. These pseudoreplicates were used to make exploratory plant-level comparisons. Secondly, it is important to note that experiments conducted in this study had different germination substrate. Therefore, comparison between these experiments does fully reflect the effects of NS effect alone, as the germination substrate might be a contributing factor to the observations recorded. Additionally, the experiment was conducted using a single basil cultivar under a single growth system (trough hydroponics), this may limit generalization of the outcome to other basil cultivars and cultivation systems. Finally, it is important to note that the content and composition of MFC derived catholyte could vary a lot as a result of different feedstocks, environment or membrane properties, potential affecting the reproducibility of the results.

Overall, the results reported here suggests that a demand-led graduated addition of NS (Supplementary Figures S1 and S2) can significantly increase the efficiency of nutrient use by basil and significantly decrease nutrient losses, particularly nitrogen, which is a key plant nutrient and pollutant, and is energy expensive to produce. This reinforces the claim that invariant full-strength nutrient formulations are not required for good crop performance in hydroponics, (i.e., crop performance can be based on efficiencies in plant uptake and demand-led supply of nutrient). In line with this pattern, other studies have indicated that moderate reductions in EC/NS can improve phytochemical quality (e.g., TPC and antioxidant capacity) [11,36]. Importantly, evidence also suggests a trade-off, whereby the NS level that optimises biochemical content may not coincide with the level that maximises biomass yield [9]. In addition to this, the response of basil may also depend on basil genotype, timing and environment. Furthermore, it should also be noted that once nutrient supply exceeds the requirement for plant growth, further addition of ions could result not only in waste but also in diminishing growth due to increasing ionic load—especially in the case of a recirculating hydroponic system in which ions can easily buildup [37].

The outcome of this experiment also suggests that basil plants can be cultivated in a hydroponic system without using peat as a germination substrate. Plants not germinated in peat and grown with 60% NS were sometimes morphologically distinguishable from plants germinated in peat and grown at 100% but for traits related to biomass quality were not. We therefore suggest that the growth protocol reported here can produce basil with less NS waste and no use of peat. The results reported here also suggest that basil plants with half of the NS supplied from MFC-catholyte would grow less big but are likely to have higher concentrations of key phytochemicals. Such plants, and in contrast to standard hydroponics, could be grown with minimal use of synthetic NS (including nitrogen).

4.2. Non-Linear Growth Responses to Nutrient-Solution Dilution

Across both NS series (experiment 1 and 2), basil growth response was not directly related to NS strength, instead, the observed responses suggested a threshold between 50% and 75% NS. In line with this, other studies have also shown that basil response to NS/EC treatments are non-linear, where specific NS/EC treatments could optimize yield and the treatment with the best response may differ according to production system, environment or cultivar [9,11,38]. The combination of results from this study and the literature demonstrates that effective management of NS can significantly affect growth and biomass allocation. This reinforces the suggestion that the NS strength effects are nonlinear to plant response and biomass outcome is a culmination of nutrient uptake-allocation in plants [39]. It also suggests significant potential to optimise the use of key nutrients in hydroponic systems for basil, and likely other species too, in order to minimise losses that may ultimately result in environmental contamination.

4.3. Leaf Canopy and Biomass Traits Are Sensitive Indicators of Optimum Treatment

A consistent pattern across the experiments (Figures 2 and 3) is that leaf canopy/leaf area and biomass linked traits, including shoot and root fresh weight (Figures 4–6), are sensitive and consistent indicators of a suboptimal/optimal/excess NS strength as compared to shoot height, which often showed a similar or weak response to different dilutions. Therefore, the minimum NS threshold for optimum performance can be determined from accumulated biomass and leaf area instead of shoot elongation (shoot height).

This outcome is consistent with previous reports that plant height is relatively insensitive to nutritional effects, even when canopy and biomass accumulation traits show significant responses. An example of this is the report of a Japanese mint experiment, where it was demonstrated that mint height is insensitive to nitrogen limitation but has a negative impact on reduced leaf area, total dry weight and shoot-to-root ratio when compared to control treatments, meaning that nutritional constraint may preferentially affect biomass accumulation and

canopy development rather than stem elongation [40,41]. Similarly, the addition of silicon to nutrient solution, which made no significant difference to lettuce height as compared to the control but had a significant effect on shoot fresh weight, further supports the view that yield and canopy related attributes of plants are more sensitive indicators of treatment effects as compared to shoot height [42]. This is likely because they increase as a direct response to sink activities, primarily nutrient driven leaf development, which influences photosynthetic activities of plant (i.e., higher leaf area captures more carbon during photosynthesis for plant use). These processes can be easily influenced by nutrient limitation as compared to height which can be more related to CO_2 supply and the production of structural carbohydrate molecules.

In addition, across the experiments, chlorophyll fluorescence data indicated that PSII was comparatively buffered as compared to biomass and canopy traits, which were more sensitive to treatment effect (Supplementary Tables S1–S3). This outcome indicates that growth and yield penalty observed across the three experiments (experiment 1, 2, 3) were not driven by Photosystem II damage (PS II), but rather by nutrient uptake, assimilation or utilization differences. A caveat is that previous studies have shown that chlorophyll fluorescence parameters are powerful stress diagnostics but might not always reflect stress in plants [20]. Hence, the use of chlorophyll fluorescence data to assess optimum NS input should be treated with caution, and it might not correlate with growth/biomass response driven by nutrient supply that adjusts in order to optimise the photosynthesis that can occur. Collectively, the repeated observation that fluorescence remains near-optimal while biomass declines signify that plants under different treatments can alter sink activities, nutrient acquisition, and allocation, while fluorescence measurement can be used as a timing/diagnostic tool rather than a stand-alone yield predictor [20,39,43].

4.4. Phenolics Indicate a Context-Dependent Growth-Quality Trade-Off

The TPC outcome showed a regime dependent response across treatment series (Figures 4–6). In experiment 1 (Figure 4), the treatment effect on TPC was similar across all NS. Contrary to this response, in experiment 2 (Figure 5), treatment effect on TPC outcome was statistically similar from 20% to 60% NS which was significantly higher as compared to 80% NS. In addition to this, experiment 3 revealed a further increase in TPC, with the combination of NS-WW-Gypsum having the highest response which was significantly different from full strength/ 100% NS (Figure 6). This outcome agrees with the findings from literature in which it has also been suggested that NS/EC manipulation can alter phenolics and antioxidant capacity of basil. This response has been confirmed to be dependent on cultivar/genotype, time of NS application, NS/EC strength/stress [9,11,44] but indicates that in addition to optimising the use of key nutrients such as N, the management of nutrient regimes might also be used to optimise biomass quality.

4.5. Photosynthetic Pigments and Nitrate Reductase Were Buffered across Treatments

Despite the biomass accumulation and leaf expansion response across the treatment series, plant pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) remained largely stable (Figures 4 and 5), which suggests that these pigment pools were adjusted in response to biomass production differences induced by the applied treatments. Similarly, nitrate reductase activity (NR) was comparable across all treatment series, which could be because NR is strongly regulated by light and post-translational regulators at specific timepoint, therefore, a single assay time (at harvest) may underestimate treatment-driven differences in in vivo NR regulation [45,46]. The outcome of the chlorophyll a and carotenoid results is in alignment with a previous basil study, in which it was confirmed that treatments had no significant effect on chlorophyll a and carotenoids, but produced a significant result in terms of the response from chlorophyll b pigment [47].

4.6. Wastewater Substitution and Gypsum Supplementation: Sustainability with a Trade-Off Profile

The outcome of this experiment suggests a two-step sustainability strategy by showing that (i) hydroponic basil production can be carried out with 50–75% NS with no serious quality or growth penalty; and (ii) the NS could be substituted with WW-catholyte to not only reduce synthetic nutrient use but also to boost quality (TPC) of the biomass. It is evident from the outcome of the WW-NS treatment series (Figure 6) that replacing half of full-strength NS with wastewater led to a decline in biomass accumulation and leaf formation, while the supplementation with gypsum didn't produce any biomass gain—instead it led to the production of the highest TPC (Figure 6). Several studies have confirmed that MFC-treated wastewater could be a viable nutrient source for the production of vegetables hydroponically [22,23,48,49]. However, special care must be taken in the utilization of the catholyte for production, as it could be highly alkaline and contain high ionic load (i.e., pH 10.3, EC 5.69 mS/cm) [22]. This could affect nutrient availability, for instance, solubility of iron and basic anions decreases with high pH, which might result in reduced growth and development [46,50]. Hence nutrient solution pH should be maintained at optimum

range for hydroponic production (5.5 to 6.5 for optimum response and nutrient availability) [51]. Furthermore, WW-catholyte might have contributed to nutrient enrichment and high TPC, while also altering ionic balance in the solution simultaneously due to its robust elemental content [22,52,53].

5. Conclusions

Overall, this study demonstrates that the application of nutrient solution and resultant basil response is nonlinear, and that a promising nutrient reduction range for sole synthetic nutrient application is a demand-led supply between 50 to 75% of Sonneveld's solution, and that treatments <25% NS should be avoided due to growth and yield penalties. It also shows that the utilization of wastewater-catholyte and nutrient solution (1:1), plus gypsum (0.46 mM) can more sustainably produce basil with higher TPC concentration. The findings suggest a promising operating window for reducing synthetic fertilizer input (NS), particularly nitrogen which requires judicious management. Also, this study confirms that wastewater-catholyte-nutrient solution substitution is a feasible strategy to improving sustainability and biomass quality provided pH /ionic balance are properly managed. Our conclusion is based on pseudoreplicated basil samples used for the experiment and analysis, hence our findings should be regarded as exploratory and should be validated using independently replicated reservoir-level experiments to establish treatment effects.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2610131023222435/JPPM-26040164-SM.pdf. Figure S1. Modified Sonneveld solution dozing template. Figure S2. standard sigmoidal growth curve that assumes 3% N in final biomass used for nutrient dozing above (Table S1). Table S1. (A,B,C,D,E): Experiment 1 chlorophyll fluorescence statistical analysis. Table S2. (A,B,C,D,E,F): Experiment 2 chlorophyll fluorescence statistical analysis. Table S3. (A,B,C,D,E,F): Experiment 3 chlorophyll fluorescence statistical analysis.

Author Contributions

S.O.: conceptualization, methodology, data curation, original draft preparation. carried out the practical work and wrote the paper; M.S.: methodology, data curation, writing-reviewing and editing; R.T.: supervision, writing—reviewing and editing; N.W.: supervision, investigation, writing-reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of College of Health Science and Society of the University of the West of England, Bristol (protocol code 1164 and approval date 22 April 2024).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data will shortly be available at https://zenodo.org/ and https://www.uwe.ac.uk/study/library/research-support/research-data-repository.

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Conflicts of Interest

The authors declare no conflicts of interest

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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