

Simultaneous pH and EC control in hydroponics through real-time manipulation of the ammonium-to-nitrate ratio in the nutrient solution

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Abstract

A control algorithm was developed and tested on a hydroponic ebb-and-flow system to assess the efficacy of the proposed control scheme. Three experimental runs were conducted with the purpose of testing the proposed control system on *Brassica oleracea* var. *acephala*. The first was an ideal case under sterile conditions, the second was under non-sterile conditions where bacteria were allowed to colonise the plant roots, and the last was a baseline run where nitrate was applied as the only nitrogen source. The system was able to control pH to within 0.5 of the set point (in this case 6.1) while EC control was sufficient to ensure that a steady stream of nutrients were available to the plants at all times. Relative growth rates were fast at maximum average values of between 0.20 day^{-1} and 0.21 day^{-1} for all of the runs and the yield of organic leaf matter was essentially the same across all the runs at 83 % to 86 % of total plant mass. Finally, the plants grown under the proposed control system were observed to exhibit some improvement in protein and chlorophyll content while the other nutritional characteristics considered were essentially unchanged between treatments. This was all accomplished without having to add any additional toxic ions like Cl^- and Na^+ as is the case in conventionally controlled systems.

Keywords: nitrate, ammonia, pH, hydroponics, control

1 Introduction

For much of the last century, nitrogen nutrition in hydroponics has been dominated by nitrates (Bugbee, 2004; Hoagland & Arnon, 1938; Savvas *et al*, 2013). However, research has highlighted that many potential benefits can be had by adding ammonium into the mix with nitrate (Falovo *et al*, 2006; Song, G Li, *et al*, 2016; Song, L Li, *et al*, 2017; Wang *et al*, 2022). These include potentially increased growth rates, greater concentrations of some minerals and phytochemicals, increased protein concentrations, and the reduction in carcinogenic nitrate within the edible organs of the plant (Song, Yi, *et al*, 2012; Song, L Li, *et al*, 2017; Tabatabaei, Yusefi & Hajiloo, 2008; Zhu *et al*, 2018). Despite these benefits, the spectre of ammonium toxicity – the wilting and yellowing of leaves, reduction of growth rate, and sometimes even the death of the plant, when ammonium concentrations grow too large or the ratio of ammonium to nitrate grows too high (Assimakopoulou *et al*, 2019; Falovo *et al*, 2006; Wang *et al*, 2022; Zhang *et al*, 2007) – has served to constrain the use of ammonium more liberally in hydroponics (Bugbee, 2004). Although most common nutrient formulations add ammonium to the mix with the intention of moderating the pH change of the system, the amounts are paltry when compared to the amount of nitrate supplied and ultimately necessitate direct pH control anyway (Hoagland & Arnon, 1938; Savvas *et al*, 2013). The pH response of a plant in hydroponics is a function of the different ways in which molecules and ions are absorbed by plant roots. Many nutrients, in particular N containing molecules, are absorbed through active transport (Hopmans & Bristow, 2002; Petoussi & Kalogerakis, 2023). This involves the pumping of protons between the roots and the nutrient medium in order to create the necessary electrochemical gradient for anions and cations to flow from the nutrient medium into the roots (Petoussi & Kalogerakis, 2023; Mengel *et al*, 2001: 118, 120, 128). Broadly speaking, in order to absorb cations, protons are pumped out of the root into the nutrient solution. This creates a net positive charge in the nutrient solution that encourage cations like K^+ , Ca^{2+} , and NH_4^+ to move from the nutrient solution into the roots. Conversely, when anions are absorbed, they often enter the cell via cotransport,

whereby each anion is complexed with one or more protons. This complex carries a net positive charge, allowing it to flow down the same electrochemical gradient that the cations do. As a result, protons travel into the root from the nutrient solution. Naturally, both of these processes change the concentration of protons in the nutrient solution, necessitating pH control. Of particular interest is the ratio of protons released/absorbed per mole of ammonium/nitrate because nitrogenous ions like these represent the bulk of ions absorbed by plants. Theoretically, the absorption of 1 mol of nitrate should result in the absorption of $\frac{2}{3}$ mol protons while the absorption of 1 mol of ammonium should release $\frac{4}{3}$ mol of protons (Raven, 1985). As a result, ammonium heavy solutions tend to see a pH decrease while nitrate rich solutions tend to see a pH increase. van Rooyen & Nicol (2022) measured that the absorption of 1 mol of ammonium results in the release of 1 mol of proton while the absorption of 1 mol of nitrate results in the absorption of 0.5 mol of protons (and therefore the release of 0.5 mol of hydroxide ions). Both of these ratios were calculated in the presence of Hoagland solution, accounting for the difference from the theoretical values that would be caused by the other anions and cations that the plant needs.

As stated earlier, ammonium is often added to temper the pH response, though this is seldom relied on as a dedicated pH control strategy. Table 1 considers different studies and their control strategies in order to illustrate how rare this type of control is, especially in actively controlled systems.

Table 1: Summary of different control system types.

Type of system	References	Controlled variables	Manipulated variables
Acid/base dosing	Velazquez-Gonzalez <i>et al</i> (2022)*	pH, EC	Acid/base dosing,
	Dunn & Singh (2016)		nutrient solution
	Savvas <i>et al</i> (2013)		dosing
Ammonium/nitrate manipulation	Scherholz & Curtis (2013)	pH	Ammonium addition
	Pitts & Stutte (1999)	pH	Ammonium addition
Ammonium addition	Ismande (1986)***	pH	Ammonium addition
	Hoagland & Arnon (1938)	pH	Ammonium addition
Proton ratios	van Rooyen & Nicol (2021b)	pH, EC**	Nitric and nitrate addition
	van Rooyen & Nicol, 2022	pH, EC**	Ammonium, nutrient solution addition
Miscellaneous	van Rooyen & Nicol (2021a)	pH, EC**	Phosphate addition

*Presents a review of many articles on hydroponic control in a table. All the articles that addressed pH control used acid/base dosing to control pH in parallel with nutrient dosing to control EC (Atmadja et al, 2017; Chang, Hong & Fu, 2018; Kaewwiset & Yooyativong, 2017; Ruengittinun, Phongsamsuan & Sureeratanakorn, 2017; Saputra, Irawan & Nugraha, 2017).

**EC was not measured explicitly. The amounts of protons added in the dosing solution was calculated to replace those lost due

to nitrate absorption, hence maintaining a relatively constant nitrate concentration (a loose analogue for EC).

87 ***Involved adding ammonium and nitrate in a stoichiometric amount, such that when the ammonium and nitrate were depleted,
88 the pH returned to the starting value; the pH was not actively controlled.

Table 1 gives the impression that a plethora of novel ammonium based systems are in use, however, this is not the case. As the addendum to the table shows, simple acid/base addition dominates the reference list. Moreover, handbooks on the subject tend to counsel the use of acid and base dosing. While it is common for hydroponic systems to add ammonium to moderate the pH response (Hoagland & Arnon, 1938; Savvas *et al*, 2013), active pH control systems that leverage the pH relationship between ammonium and nitrate are decidedly rarer, accounting for only two of the studies found (Scherholz & Curtis, 2013; Pitts & Stutte, 1999). Of these, one was a computer simulation (Pitts & Stutte, 1999). The best example that could be found of a sophisticated use of ammonium to control pH is the study by Scherholz & Curtis (2013), in which an attempt was made to control pH through the addition of ammonium and nitrate to an algae culture in the hope that the simultaneous consumption of both would maintain a constant pH. Although failing when conducted as a batch experiment, the system was able to control pH when a fed batch system was implemented whereby ammonium nitrate was dosed to a nitrate medium containing the algae. Algae have the tendency to consume solely ammonium if given the opportunity (Fernández & Cárdenas, 1982; Florencio & Vega, 1983). As such, adding ammonium in the presence of nitrate has an immediate pH effect as the algae switch to ammonium nutrition before going back to consuming nitrate after the ammonium is depleted. Unlike algae, terrestrial plants tend to consume ammonium and nitrate in ratios corresponding to their concentration in the root zone (Song, G Li, *et al*, 2016), making pH control through nutrient manipulation somewhat more nuanced. Moreover, if too much ammonium is present, this can have potentially lethal implications for the plants in the system as they will start suffering from ammonium toxicity (Assimakopoulou *et al*, 2019; Britto & Kronzucker, 2002; Cramer & Lewis, 1993; Wang *et al*, 2022). This point alone discourages people from using ammonium in hydroponics, foregoing the possible benefits of operating the system at an appropriate ammonium-to-nitrate ratio.

The aim of this study is to develop a control scheme whereby pH and electrical conductivity (EC) are controlled simultaneously via the tactical addition of ammonium and

nitrate. In principle, this should allow the system to harness the benefits of ammonium nutrition while avoiding the pitfalls of ammonium toxicity (Song, L Li, *et al*, 2017). This aim is supported by two objectives. Firstly, the preference kale for ammonium and nitrate was assessed, and used to calculate the pH homeostasis point – the ammonium to nitrate ratio where the pH rise due to the absorption of nitrate is cancelled out by the pH drop from the absorption of ammonium. Secondly, having proven that the pH homeostasis point is not in the ammonium concentration range where ammonium toxicity begins to manifest, a control algorithm was developed to maintain this ratio without the need to explicitly measure the concentration of either ion.

Having identified the various mechanisms used by plants to absorb different ions, and in doing so confirmed that plants are selective of the ions they absorb, the uptake kinetics for kale were found from literature. These kinetics, in conjunction with knowledge regarding the pH effect on the root zone of the absorption and assimilation of nitrate and ammonium, were used to develop a dynamic computer model of the plant. This model was then used to develop and tune a simultaneous control algorithm making use of proportional-integral-differential (PID) control algorithm for the pH and proportional-integral (PI) control for the EC. Finally, this controller was trialled on an ebb-and-flow hydroponic system to confirm its efficacy. The results of this run were then compared to the results of a control experiment using nitrate as the only source of nitrogen to demonstrate that the inclusion of ammonium does not induce worse yields or generate less nutritious leaves than the traditional nitrate-only approach.

2 Experimental

2.1 Overview

All the plants used in this study were grown from kale seeds (*B. oleracea* var. *acephala* or Vate's Blue Curled Kale), purchased from Raw™. All nutrient ions were provided by stock solutions that were made from analytical standard chemicals. Deionised water was used exclusively in all experiments for all applications that needed water.

2.2 Analytical instruments

A UV-vis spectrophotometer (Agilent Technologies™, Cary 60 UV-Vis, G6860 A) was the only analytical instrument used in this study. It was used in conjunction with Merck/Supelco photometric tests to measure the concentration of ammonium, nitrate, and phosphate in the nutrient solution at wavelengths of 690 nm, 340 nm, and 690 nm, respectively.

2.3 Planning, methods, and apparatus

Three experimental runs were conducted in order to assess the efficacy of the system. The first experimental run, the sterile run, was a 21 day run in which the proposed control algorithm was used to maintain pH after the plants and apparatus had been sterilised with hydrogen peroxide. The sterile run served to show that the system worked according to the conditions upon which the underlying assumptions were based. The second was a non-sterile run. This was a 10 day run in which the proposed control algorithm was used to maintain pH where the plants and apparatus were unsterilised. The purpose of this run was to assess the robustness of the control system should bacteria – particularly nitrifying bacteria – colonise the system (Cytryn *et al*, 2012). In the third run, the system was fed with only nitrate using the direct dosing of nutrients in the acid reservoir employed

by van Rooyen & Nicol (2021b). This was necessary to establish a baseline to which the proposed system could be compared, hence it shall be known as the baseline run.

All of the experimental runs were carried out using an ebb and flow hydroponic apparatus consisting of four independent vessels. Each vessel had a volume of 1.64 L. An Arduino Mega 2560™ board was used to control all four vessels simultaneously. Haoshi™ pH probes (“pH meter Pro”) were used to measure pH. These were calibrated using two point calibration where solutions of di-sodium hydrogen-phosphate and citric acid/sodium hydroxide/hydrogen chloride from Merck provided mV readings at 7 and 4, respectively. Gravity™ analogue electrical conductivity sensors V2 were used for the online measurement of EC. These, too, were calibrated using two point calibration with calibration standards included with the probes by the supplier. Kamoer © peristaltic pumps “Precision Peristaltic Pump + Intelligent Stepper Controller” were used for the dosing of nutrient solution – the exact composition of which depended upon the experimental run. DFrobot™ peristaltic pumps (“digital peristaltic pumps”) were used for the addition of water to counteract the activity of transpiration. Xylem™ (“Flojet Diaphragm Electric Operated Positive Displacement Pumps, 3.8 L min⁻¹, 2.5 bar, 12 V DC”) were used to circulate nutrient solution between the plant and the reservoir. The plants were cultivated beneath Mars Hydro™ 400 W blue/red LED lights (Mars II 400 LED Grow Light ©), producing 10 000 Lux at the canopy. A schematic diagram of one vessel in the the system is presented in Figure 1. The system consisted of four of these in parallel.

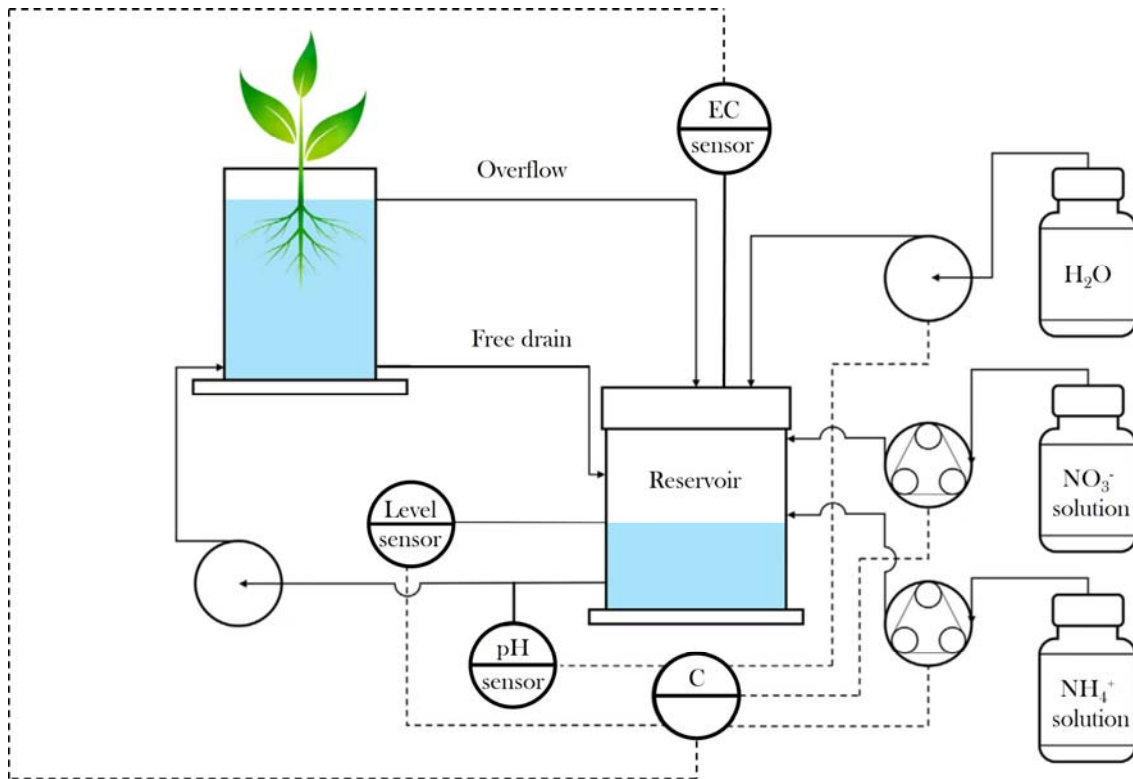


Figure 1: Schematic diagram of the ebb-and-flow hydroponic system.

Under normal operation, water is circulated between the reservoir and the plant continuously for 20 min. At this point the pump cuts out, allowing the system to drain freely back into the reservoir and measurements to be taken. This lasts roughly 10 min, after which the pumps resume circulating water between the reservoir and the plant. While the system drains, pH measurements are taken. Once the system has drained, the EC measurements are taken. The pH measurements have to be taken before the water level in the reservoir reaches the EC probes because the EC probes interfere significantly with the pH readings. Finally, the level of the system is measured with a float switch. If the level is too low, the float switch actuates an on/off controller that adds sufficient water to restore the water level, thereby keeping the volume of nutrient solution in the system constant.

Each run used plants that were cultivated from seed for roughly 28 days in aeroponic cloners. The plants were cultivated under a 24 h light cycle. After this time, plants of

approximately the same weight (typically between 10 and 15 g) were transferred to the ebb and flow setup where the control system experiments were conducted. In order to eliminate the potential effect bacteria could have on the results of the sterile run and baseline, the seedlings for these experimental runs were dosed with 0.3 mL of 90 % hydrogen peroxide every 3 to 5 days for the 28 days of their cultivation in the aeroponic cloners. Their roots were also soaked in 1 % hydrogen peroxide solution for 10 min before they were transferred to the ebb and flow setup. Conversely, the plants for the non-sterile were not exposed to hydrogen peroxide so that bacteria could be given the opportunity to establish themselves on the roots and hence allow for the assessment of the proposed control system in a comparatively less sterile environment.

As this is essentially a control system design, it is necessary to consider the variables in the context of control system design where one defines controlled variables, manipulated variables, and disturbance variables (Seborg *et al*, 2017: 3). There are two controlled variables: pH and EC. As such, there are two manipulated variables: N dosing rate and ammonium-to-nitrate ratio in the dosed N. Finally, there are two (important) disturbance variables: the nutrient uptake ratio of the plant and the nutrient uptake rate of the plant.

Figure 2 illustrates how the proposed control system functioned.

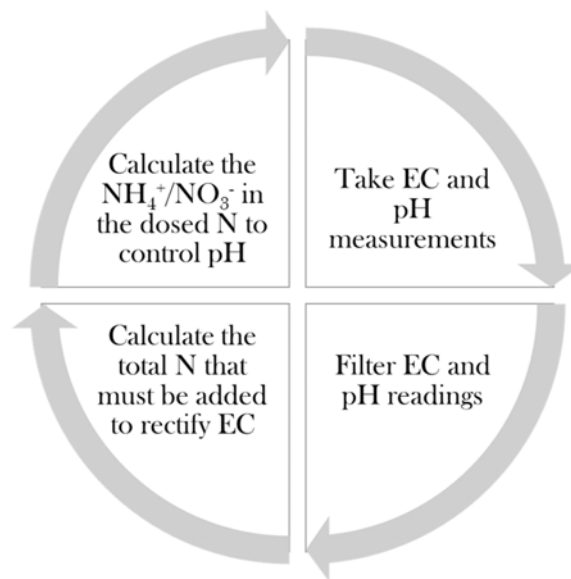


Figure 2: Schematic diagram illustrating the control action.

212 A PI control algorithm was used for EC control. Having determined the amount of total N
213 to be added to the system in order to maintain EC, the controller calculated the fraction
214 of this N that had to be dosed from the nitrate reservoir and the amount that had to
215 be dosed from the ammonium reservoir. This fraction was calculated by a PID control
216 algorithm. Figure 3 shows the block diagram of the closed loop control system. It is
217 presented in the Laplace domain, as is customary in controller design, though it should
218 be noted that all calculations for the simulation (not shown here) were made in the time
219 domain.

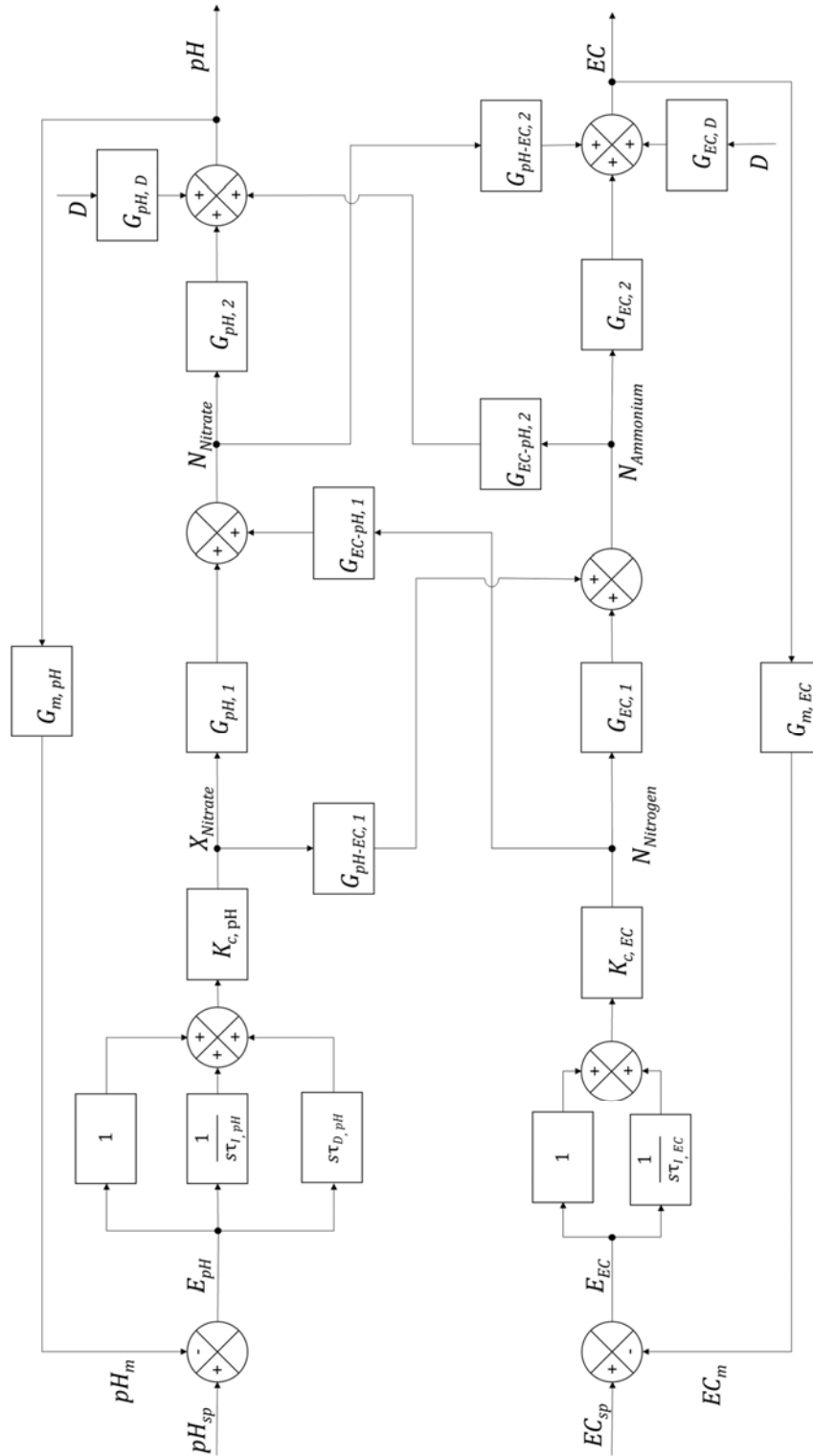


Figure 3: Block diagram of the control system depicting the closed loop control system. The transfer functions are depicted in the Laplace domain as per the style prescribed by Seborg *et al* (2017). D represents disturbance (this can be anything from plant exudates to temperature change), N_i represents the number of moles of i , X represents the mole fraction of i , and G_j is a transfer function.

The formulae for the transfer functions of the PI and PID controllers are depicted, while the transfer functions for the others are simply represented by G_j , where j is the identifying subscript. The reason for this is that the other transfer functions are either unknown (as is the case with $G_{pH,D}$, $G_{EC,D}$, $G_{pH,2}$, and $G_{EC,2}$), or unnecessarily complicated, threatening to clutter the diagram without lending any additional understanding to the process. This latter point is particularly the case with $G_{EC-pH,1}$ and $G_{pH,1}$, and $G_{EC-pH,2}$ and $G_{EC,1}$. In the time domain the mathematical operations are simple

$$n_{\text{nitrate}} = x_{\text{nitrate}} n_{\text{nitrogen}} \quad \text{and} \quad n_{\text{ammonium}} = (1 - x_{\text{nitrate}}) n_{\text{nitrogen}}$$

but in the Laplace domain, the expressions are decidedly more complex (owing to the fact that the product of two functions in the Laplace domain is not equivalent to the product of the functions in the time domain, but is instead their conjugate). The mathematical treatment necessary to get the Laplace expression can be found in Alahmad (2021), but it serves no purpose to delve into it in this paper, considering that all calculations were made in the time domain anyway.

In the sterile and non-sterile runs, two dosing reservoirs were necessary: one to provide ammonium and the other to provide nitrate. When the controller demanded ammonium be dosed into the system, the system drew from the ammonium rich reservoir. When the controller demanded nitrate be dosed into the system, the system drew on the nitrate rich reservoir. These two solutions were composed so as to achieve high concentrations of N and a balance of elements similar to that of Hoaglands solution while eliminating precipitation. The composition of the dosing solutions are laid out in Table 2.

Nitrate dosing reservoir	
Salt	Concentration (mM)
KNO ₃	50
Ca(NO ₃) ₂	50
Cations	
Anions	
% of N as nitrate	100
% of N as ammonium	0
Ammonium dosing reservoir	
Salt	Concentration (mM)
NH ₄ NO ₃	37.5
(NH ₄) ₂ HPO ₄	4.5
NH ₄ H ₂ PO ₄	20.5
(NH ₄) ₂ SO ₄	22.7
% of N as nitrate	25
% of N as ammonium	75

Table 2: Compositions of the dosing solutions. Both solutions were made up to a concentration of 150 mM N. Anions and cations were included in the nitrate dosing reservoir at 10 times the strength of Hoagland solution.

Undesirable ions like Cl⁻ and Na⁺ were able to be excluded through the use of phosphate buffering in the ammonium reservoir (Miller, Adhikari & Nemali, 2020). In an attempt to minimise the build-up of inert ions like SO₄²⁻ (Maathuis, 2009), the ammonium dosing solution was adulterated with nitrate in the form of ammonium nitrate. The inclusion of 25 % nitrate reduced the need for phosphate and sulphate, while only slightly reducing the ability of the solution to alter the composition of the system (*i.e.* its potency). The composition is such that when fed in an ammonium:nitrate ratio of 33:67, the dosed ions will essentially mirror the elemental composition of Hoagland solution. This represents a significant benefit of this system over others that rely on a dedicated pH controller.

Direct pH control ultimately requires the use of acids and bases like HCl and NaOH in order to maintain the appropriate nutrient ratios, and as such, leads to an accumulation of undesirable ions in the nutrient medium (Miller *et al*, 2020; van Rooyen & Nicol, 2021b; van Rooyen, Brink & Nicol, 2021).

Because Mg^{2+} and Fe^{2+} are only sparingly soluble at the concentrations involved, they were added into the reservoir of the level control system at concentrations of 1 mM each. This meant they were added proportionally to the rate of transpiration of the plants, which was found to be more-or-less proportional to nutrient demand.

The baseline run needed only one dosing reservoir where the necessary nutrients were dissolved in an acid solution such that the ratio of nitrate to protons was 1:0.6. This hypothetically allows for maintenance of both pH and [N] in the growth medium through the use of only pH measurement and one dosing reservoir (van Rooyen & Nicol, 2021b). This composition of the dosing solution is the same as that used by van Rooyen & Nicol (2021a), with the addition of phosphate to balance the nutrient composition.

The nutrient solutions charged into the system at the beginning of each experimental run were variants of Hoagland solution where the ratio of ammonium to nitrate was varied while attempting to keep the ratio of elements relatively constant. These compositions are detailed in Table 3.

	Concentration (mM)		
	Sterile run	Non-sterile run	Baseline run
KH_2PO_4	0.5	0.832	0.5
KNO_3	1	3.324	2.5
$\text{Ca}(\text{NO}_3)_2$	2	3.324	2.5
MgSO_4	1	1.664	1
K_2SO_4	3	0	0
$(\text{NH}_4)_2\text{SO}_4$	1.25	1.664	0
Nitrate fraction	0.67	0.75	1.0

Table 3: The composition of the nutrient solution charged into the system at the beginning of each run. Micronutrients were included but are not shown here.

Daily measurements of the concentration of ammonium, nitrate, and phosphate, as well as plant mass, were made for each experimental run. The ammonium and nitrate measurements were to see if the system was actually operating at the anticipated ammonium-to-nitrate ratio suggested by literature, while the phosphate measurement was to gauge the extent of phosphate accumulation/depletion, serving as a bellwether for salt accumulation.

The dry mass of the plant was found by drying the plants at 60 °C in an oven for at least a week. The dry leaf matter was then milled in a Retsch ball mill with the resultant finely ground leaf material analysed for nitrate, ammonium, total nitrogen, chlorophyll, and ash.

Nitrate content was obtained by the method outlined by Zhao & Wang (2017) with the exception that the plant leaves were dried and crushed instead of frozen. Ammonium content was analysed for using an analogous method to that outlined by Zhao & Wang (2017) where the leaves were similarly dried and Merck-Spectroquant tests were used to measure ammonium content instead of salicylic acid. Both of these tests were conducted in triplicate. Elemental analysis was conducted by the Department of Chemical Engi-

neering of the University of Pretoria on the dried leaves of all of the plants in the sterile and baseline runs, and plant 2 and plant 3 of the non-sterile run. The protein content of the leaves was calculated by subtracting the amount of nitrogen in ammonium/nitrate from the total nitrogen. This new value was then multiplied by 5.77 to convert from the mass of nitrogen to the mass of protein. 5.77 is the mass of protein per mass of nitrogen contained in protein and is based on the general formula $C_nH_{1.58n}N_{0.28n}O_{0.30n}S_{0.01n}$ (Torabizadeh, 2011).

The ash content of the leaves was found by weighing known amounts of the dried, crushed plant leaves into crucibles and placing these in a furnace at 850 °C for 8 hours. This was done in triplicate.

The chlorophyll content of the leaves were assessed by soaking ± 0.02 g of dried and ground leaf matter in 8 mL of 80 % acetone – 20 % water (volume basis) for 24 hours. The supernatant was then centrifuged and the absorbance of the supernatant at 663 nm and 645 nm was measured and used in conjunction with the equations developed by Arnon (1949). The results were compared against those derived from the newer model detailed in Porra (2002). Little difference between the two was found, so it was decided that the results from the more widely used Arnon (1949) model would be the ones considered.

The experimental results were analysed using ANOVA (Diez, C, etinkaya-Rundel & Barr, 2019: 285) and t-testing (Diez *et al*, 2019: 267) where the Welch–Satterthwaite equation was used to calculate the degrees of freedom (NIST, 2023).

The success of the proposed control system is based on its performance as compared to the more traditional approach embodied by the baseline run. To be considered a success, it must at least achieve similar crop yields, with any nutritional benefits on top of this being an added bonus.

3 Results and discussion

Successful simultaneous control of pH and EC using ammonium and nitrate

310 The results of the sterile and non-sterile runs are shown in Figure 4.

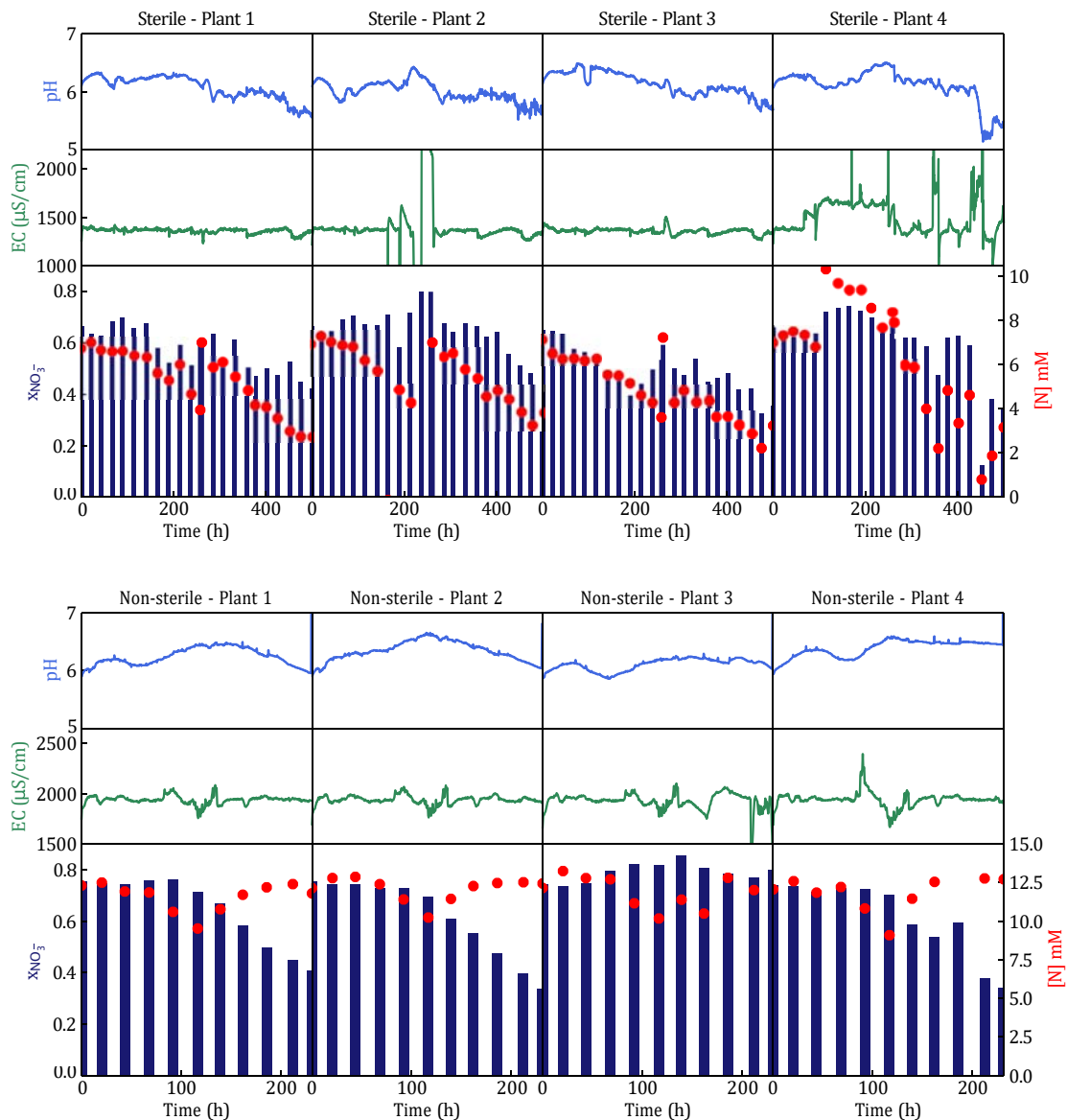


Figure 4: Online measurements of EC and pH compared to concentration and dosing data.

311 It is clear that in both the sterile and non-sterile runs that the system successfully con-

trolled pH within the bounds of 5 and 7. pH control was actually much tighter than the required range, never deviating more than 0.5 from the set point of 6.1 except in cases of component failures. Both the sterile and non-sterile runs witnessed gradual pH oscillations around the set point with the pH getting “stuck” either above or below the set point before corrective action caused it to swap sides. Similarly to the results of the computer model of this system (unpublished), these sustained periods of operation just above or below the set point appear to be an artefact of the heavy differential action of the controller that tended to suppress the movement of the controlled variable away from its current value. The gradual downward trend in pH seen in the sterile and non-sterile runs is also a result of this.

While pH was relatively smooth, the EC readings for the sterile run proved to be much bumpier. This can be explained largely as the result of a few minor component failures. Firstly, the level control for Plant 2 failed during day 10. This meant the probe was not submerged, leading the system to dose excessively and causing the EC spikes visible at this time. In addition to this, the EC probe for Plant 4 was repeatedly poisoned (something seemed to adsorb to the surface), causing it to give abnormally large readings. When this was detected, the probe was washed in deionised water, after which the readings normalised. These abnormal readings never lasted more than a day and hence are not believed to have significantly affected the results for Plant 4. The solution was replaced once on day 11 for all the plants, hence why the big jumps in [N] are observed at this time. The non-sterile run suffered no major disruptions.

Conversely, these breakdowns serve to further illustrate the effectiveness of the control system. It can be seen that the pH of Plant 4 in the sterile run collapsed at about 400 hours due to the momentary suspension of dosing owing to the poisoning of the EC probe surface. The fact that pH went haywire when control action was disabled shows that the control system was what was keeping pH stable.

Although [N] declines during the course of the sterile run, it never drops to the point where it would stymie growth. The fact that it drops in the first place while EC remains

constant suggests that inert ions are building up and replacing the N containing species. This is partly due to metal ions, but the most prominent ion that builds up is likely sulphate. This is because sulphate is supplied in excess of the ratio recommended by Hoagland & Arnon (1938). Considering that sulphate is non-toxic (Maathuis, 2009), this is a fair trade-off as it allows for the omission of more harmful ions like chloride that would have been used in its place.

The drop in [N] is also likely a result of operating at a lower nutrient concentration. The non-sterile run witnessed very little deterioration in the relationship between EC and [N] while operating at a higher N concentration compared to the sterile run. Higher N concentrations will have the effect that nutrient uptake rates will be higher, particularly in the case of ions that are predominantly taken up passively like Ca^{2+} . This, in turn, reduces the accumulation of inert ions by encouraging their uptake by the plant. Moreover, the controller appeared to find it easier to control EC at higher EC and [N] values. This can be seen in the slight downward trend in EC observed in the sterile run. Although small, this mild downward drift in EC corresponds to a very significant decrease for [N]. This is because [N] appears to be very sensitive to EC, highlighted by the small dip in EC at 125 hrs in the non-sterile run causing a major drop in [N] before recovering. This problem can be easily addressed by using a more aggressive EC control algorithm. Despite this, it is clear that the control system continued to supply the plants with a sufficient supply of nutrients so as to avoid inhibiting growth.

3.2 Absence of ammonium toxicity

Ammonium toxicity was successfully avoided by the control system. The signs of ammonium toxicity are weak growth, chlorosis, wilting, and high shoot-to-root ratios (Cramer & Lewis, 1993). Figure 5 shows the growth characteristics of the three treatments.

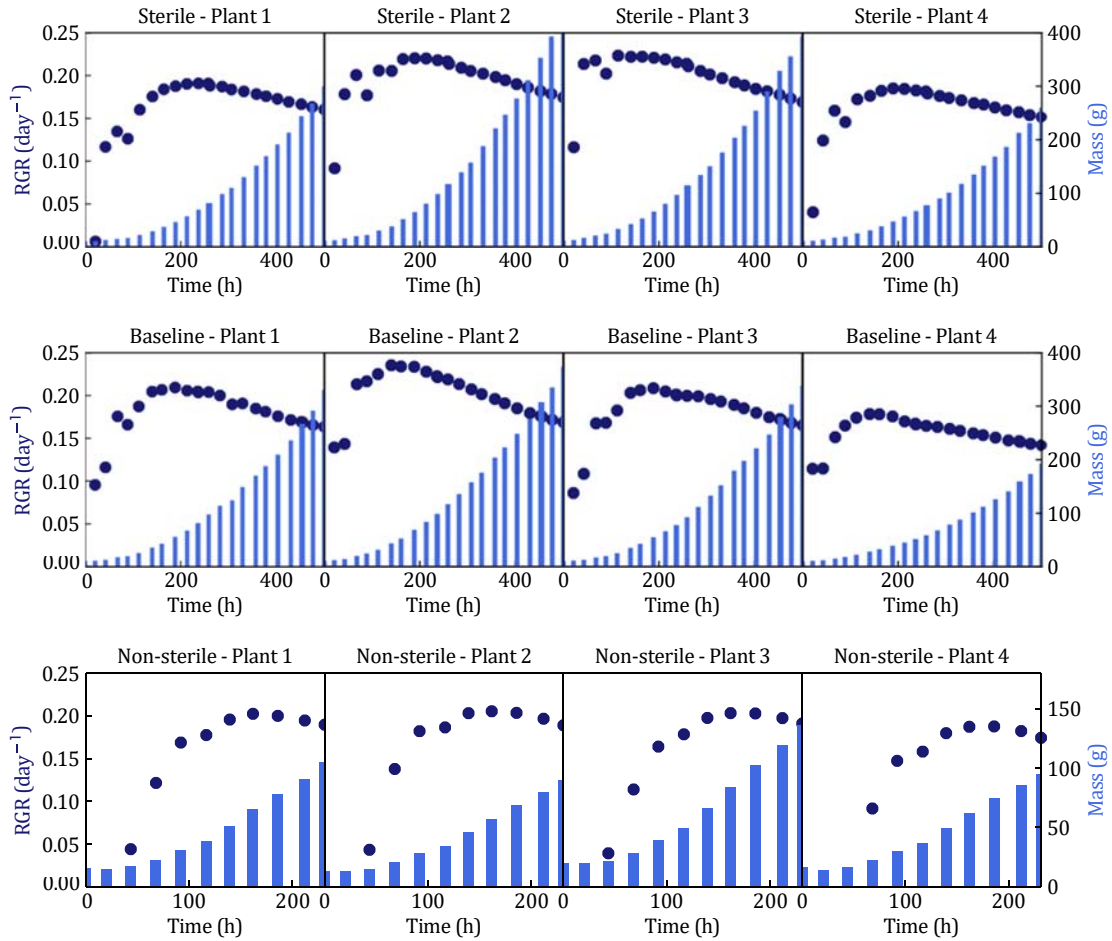


Figure 5: The daily measures of plant mass and the subsequent RGR. The largest RGR was used as the maximum, or "peak", RGR.

Ammonium fractions beyond 50 % of total nitrogen in the nutrient solution have generally been found to inhibit growth in *B. oleracea* (the most systematic sign of ammonium toxicity) with the best growth rates in literature being at around 25 % NH_4^+ as a fraction of total N (Wang *et al*, 2022; Assimakopoulou *et al*, 2019). In principle, the system should have operated at about 70 % nitrate as a fraction of total N (corresponding to 30 % ammonium) as a fraction of total nitrogen, putting it safely out of the range where ammonium toxicity manifests. In reality, the system tended to operate more in the region of 50 % nitrate, skirting the danger zone. Despite this, no symptoms of ammonium toxicity were observed. Chlorosis was absent in virtually all of the plants (and where it was present it could be linked to nutrient deficiencies when the plant was in the nursery)

and any wilting was transient. More importantly, the growth rates of all the plants involved were very healthy. Because of the variety of starting sizes of the plants and the vast difference in length between the runs, growth was compared by calculating daily relative growth rates (RGRs) for each plant, with the maximum representing the “true” value of the RGR. These RGR curves are shown in Figure 5.

RGR can be seen to rise rapidly and then steadily decreases. The rapid rise can be attributed to the plant recovering from the shock of transplantation as well as a positive response to being moved to a location more conducive to growth. Conversely, the decline is a result of increasing plant size. As the plants get bigger, they would have to divert a greater share of energy to maintenance as opposed to growth. This would be exacerbated by the leaves exploiting the maximum available light once they achieve full coverage of the available area (i.e. leaves growing on top of each other will not absorb additional light). It is apparent from Figure 5 and Figure 6d that there is no significant difference between growth rates across the the different treatments.

However, RGR is only half the story when considering the yield of crops. The most commercially valuable part of the plant is the leaves, which represent the edible part of the plant. Because of this it is important to know the effect of the different treatments on the leaf fraction of the plant. The relevant growth metrics are presented in Figure 6.

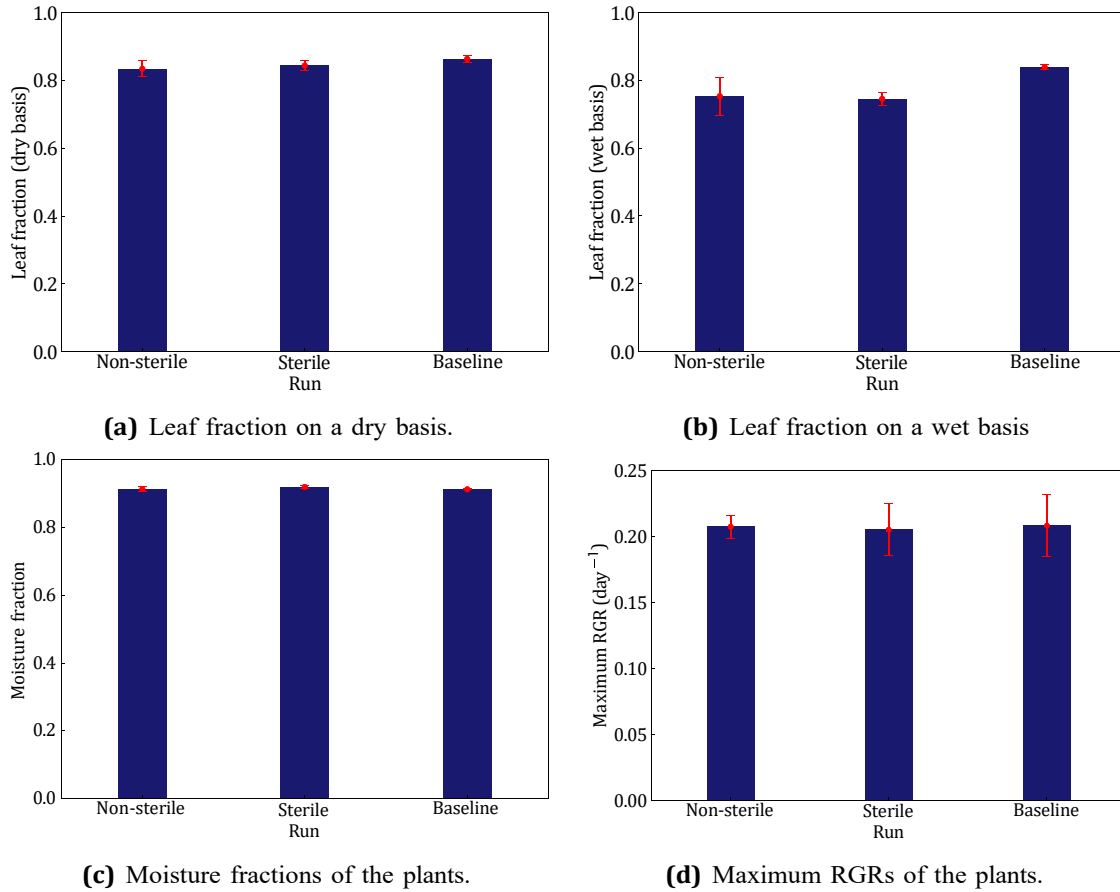


Figure 6: Growth metrics of the three experiments. Error bars represent one standard deviation where $n = 4$.

At first glance, the mass of leaves as a fraction of the total plant mass is appreciably higher in the nitrate treatment than in the ammonium treatments. However, when considering the dry mass fraction, the gap narrows considerably. Coupled with the fact that the growth rates and moisture fractions are essentially the same across all of the treatments, the biomass yield of the three treatments is very close. This conclusion is further supported by ANOVA conducted on the maximum RGR data, leaf fraction (dry basis), and moisture fraction that found there to be no significant difference between the different runs on a 5 % confidence interval. Interestingly, while ANOVA found no significant difference in moisture fraction, t-testing found a statistically significant difference to exist between the sterile and baseline run. However, this amounted to a fraction of a percent (92.0 % versus 91.3 %) and thus hardly serves as an indictment on the performance of

403 the sterile run.

404 This indicates conclusively that ammonium toxicity has not manifested as the growth
405 rates are the same and the leaf yields are similar.

406 **3.3 Significant contribution of bacteria to nitrogen uptake characteristics**

408 Figure 7 compares the cumulative fractions of nitrate dosed and absorbed in the non-
409 sterile and sterile runs. Figure 8 shows the dosing of ammonium and nitrate in twelve-hour
410 time intervals and how the cumulative amount dosed varied with time.

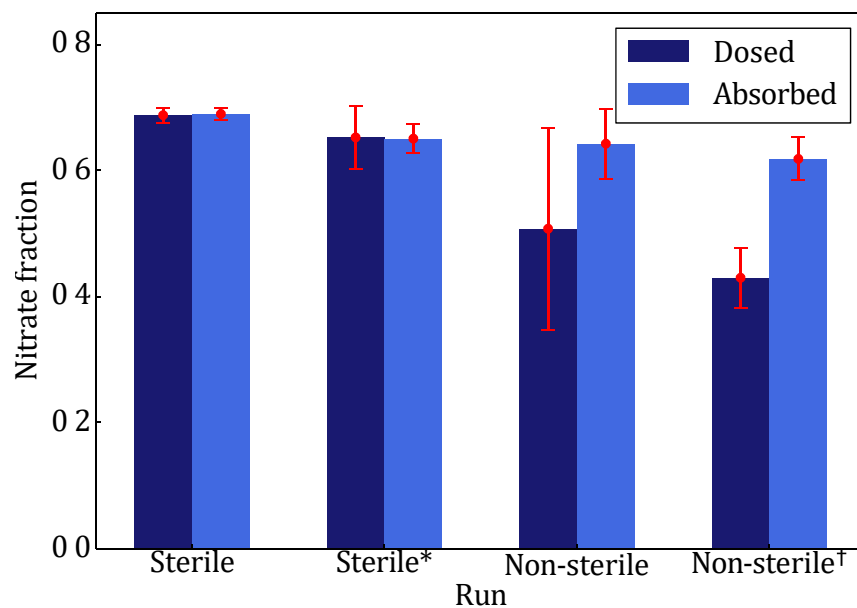


Figure 7: Nitrate as a fraction of the total amount of nitrogen dosed to the system and absorbed by the plant. Error bars represent a standard deviation where $n = 4$. Sterile* refers to the first 210 hours of the sterile run (this is equivalent to the length of the non-sterile run). Non-sterile† refers to the data for Plants 1, 2, and 4 (hence $n = 3$ for this data set).

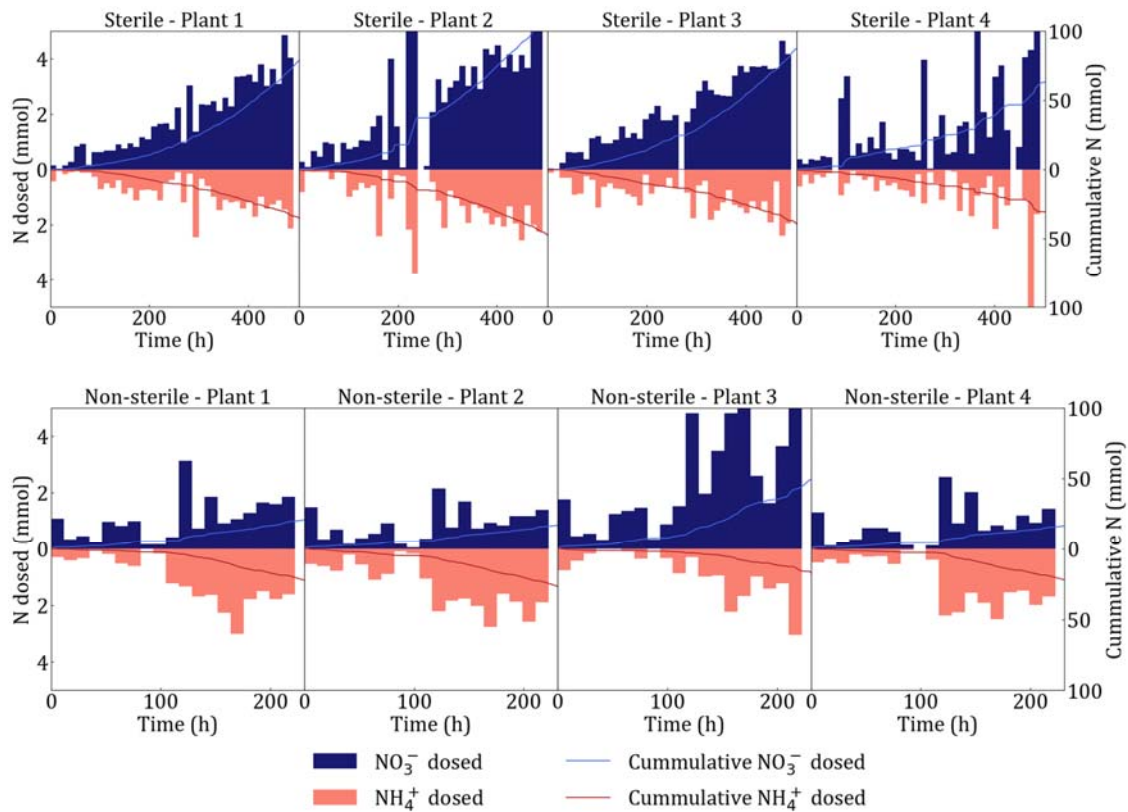


Figure 8: Dosing data for the experiments.

Although the ratio of nitrate to total nitrogen concentration in solution deviated quite significantly from the expected value of 70 % needed to maintain pH, the ratio of nitrate to total nitrogen supplied and consumed cumulatively during the sterile run was close to the 67 % calculated from literature (68.8 % and 69.0 %, respectively). This shows that the underlying assumption of 0.5 mol of protons being absorbed for every mole of nitrate absorbed (thereby releasing 0.5 mol of OH^{1-} into the growing medium) and 1 mol of protons being released for every mole of ammonium absorbed, is sound.

There are two big differences between the sterile and non-sterile runs that are apparent from Figure 7. The first is the higher variability of the non-sterile run as compared to the sterile run. Part of this can be attributed to the shorter length of the non-sterile run (as evidenced by how the error bars of the "Sterile*" data set are wider than those of the "Sterile" data set). However, even after this adjustment is taken into account, the error bars of the "Non-sterile" data set are still clearly much larger. Although there are

numerous reasons for why the two runs should differ, such as differing starting [N] and run-time, the most plausible explanation is the infiltration of the non-sterile run by bacteria (as was the intention). The presence of free ammonium and the absence of high carbon concentrations in the nutrient medium should prove especially attractive to nitrifying bacteria; a family of bacteria that respire by oxidising ammonium to nitrate (Madigan *et al*, 2022: 530). It is known that nitrifying bacteria rapidly colonise hydroponic systems (Cytryn *et al*, 2012), further supporting the hypothesis that nitrifying bacteria have taken up residence in the system. Moreover, nitrifying bacteria are known to live in discreet colonies (Silber & Bar-Tal, 2008: 300) – they do not propagate homogeneously throughout a medium – explaining the unpredictable behaviour among the four plants as a result of each hosting colonies of differing size (and likely differing consortia) on their roots.

The second major difference between the two runs is the tendency of the dosing fraction and absorption fraction to diverge in the non-sterile run but not the sterile run. ANOVA suggested that there was a difference in the dosing behaviour in the system at large and t-testing found statistically significant differences in the dosing behaviour of "Sterile"- "Non-sterile" and "Sterile*"-"Non-sterile*", yet found no such deviation between the sterile and non-sterile runs with regards to absorption. This can not simply be attributed to differing run-time as the absorbed and dosing fractions in "Sterile*" line up almost perfectly. What this implies is that there is a consistent oversupply of ammonium (causing the accumulation seen in Figure 4) to the non-sterile run by the control system. This, in turn, suggests that the controller is trying to suppress some kind of upwards pH pressure, which means that something unexpected is absorbing protons. Other than normal microbial respiration (which should be at a minimum owing to the lack of a significant carbon source within the medium), the only other confounding factor at work here is the activity of nitrifying bacteria. This is an acidic reaction commonly believed to emit two protons for every ammonium molecule nitrified to nitrate (van Rooyen & Nicol, 2022; Silber & Bar-Tal, 2008: 301), though some sources also suggest only one proton is released (van Rooyen, Brink, *et al*, 2021). If the actual proton ratio was less than 1.5, this would cause a basic response in the system at the high initial nitrate concentration of the

system. This would prompt the controller to start adding ammonium in an attempt to push pH lower. The nitrification of some or all of the ammonium in conjunction with the general tendency of plants to absorb nitrate preferentially would cause the ammonium accumulation observed in Figure 4. The virtue of this explanation is evidenced by the prominent peak and subsequent decline visible in the pH of Plant 1 and Plant 2 in Figure 4 for the non-sterile run, where this turning point likely indicates an overshoot of the ammonium needed to bring pH back to the set point.

Regardless of these deviations, which in the grander scheme of things seem only to represent a slightly more bumpy pH profile and high ammonium fractions, the non-sterile run shows that the control system continues to deliver satisfactory pH control in a non-sterile environment. Despite the high ammonium fractions, the system still delivered satisfactory yields of leaf material on par with the sterile and baseline runs, implying that ammonium toxicity was successfully avoided.

3.4 Nutritional value of the leaves

The results of both the ammonium and nitrate analyses showed no statistical difference on either ANOVA or t-testing. The ammonium content in all cases was almost negligible. The values of nitrate content, on the other hand, were generally on the higher side of what can be found for similar species in literature (Song, L Li, *et al*, 2017), but were not so high so as to seem unusual.

The results of the total nitrogen, protein, and chlorophyll analyses are shown in Figure 9.

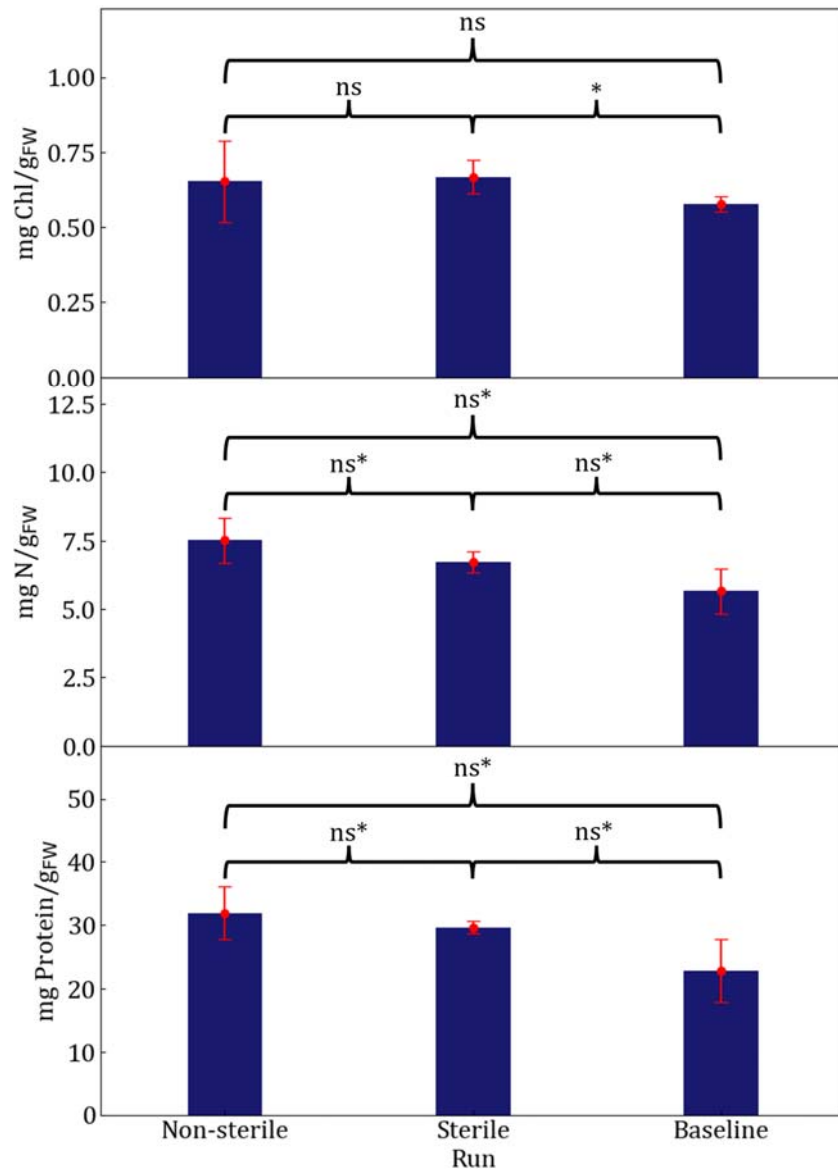


Figure 9: Content of nitrogenous species in leaves on a fresh mass basis. ns means no significant difference according to t-testing. ns* refers to the case where ANOVA suggested a significant difference but t-testing did not. * refers to the case where t-testing found a significant difference.

473 The results of the protein and total nitrogen analyses represent an interesting statistical
 474 dilemma. On one hand, ANOVA suggests in both cases that there is statistical significance
 475 between the sample populations. On the other, no individual pairing was found to be
 476 sufficiently distinct to fit within a 5 % confidence interval. This does not mean to say that
 477 there is no difference, it simply means the precise difference is ill defined. Considering

that literature has found that plants fed on ammonium and nitrate tend to generate more protein and accumulate more nitrogen than those fed purely on nitrate (Falovo *et al*, 2006; Song, L Li, *et al*, 2017; Zhang *et al*, 2007), this, in conjunction with the seemingly higher nitrogen and protein contents of the sterile and non-sterile run, can be taken as tentative proof that the proposed system, through its simultaneous supply of ammonium and nitrate, encourages both nitrogen accumulation and protein synthesis. In light of this it would seem that, not only does this system enhance the protein content of the produce, it also has the potential to increase the nitrogen use efficiency. This means that a higher fraction of the absorbed nitrogen is dedicated to protein synthesis. This is resultant from the ostensibly similar nitrate contents, but potentially higher protein and total nitrogen contents.

The chlorophyll contents appear reasonable when compared to literature (Yilmaz & Gökmen, 2016). While ANOVA failed to detect any significant difference, t-testing did. It is clear from Figure 9 that the sterile run showed a greater chlorophyll accumulation than the baseline run. This is desirable, not only because greater concentrations of chlorophyll should make the plant more productive, but also because of the possible health benefits of chlorophyll (Juber, 2022). This increased accumulation of chlorophyll is corroborated with other studies which have found an increase in chlorophyll content with the addition of ammonium to the growth medium (Hu *et al*, 2015).

The other nutritional aspect to consider is mineral content. The amount of mineral ions present in the leaves can be inferred from ashing analysis (*i.e.* by calculating the fraction of material left after ashing at high temperatures). The ash fractions are presented in Table 4.

		Non-sterile	Sterile	Baseline
Wet basis	Average (%)	1.59	1.53	1.66
	StDev (%)	0.105	0.124	0.074
Dry basis	Average (%)	16.6	16.9	18.5
	StDev (%)	1.1	1.5	0.9

Table 4: Results of the ash analysis

501 The marginally higher ash content in the baseline run agrees with literature as it stands
502 to reason that the increased competition from ammonium would suppress the movement
503 of other cations into the roots. However, ANOVA and t-testing revealed no statistical
504 significance between the runs on a wet basis. As it stands, the ammonium/nitrate system
505 is no better and no worse than the nitrate only system in this regard.

4 Conclusions and recommendations

As a means of controlling pH, the system is a success. pH was maintained close to the set point under both sterile and non-sterile conditions. EC was also maintained, though a more aggressive controller would probably be better, especially after the two week mark where the rate of nutrient absorption begins to outpace that of nutrient supply. Although the total nitrogen concentration was seen to decrease over time and the ammonium fraction in solution did on occasion breach the 50 % safety limit, there were no visible signs of ammonium toxicity. This is illustrated by the satisfactory growth rates achieved across all the runs. Moreover, despite the slightly depressed yield of leaves on a wet basis in the sterile and non-sterile runs, the dry leaf yield of both of these runs was very close to that of the baseline run, showing that the yield of edible plant matter is as good with the ammonium/nitrate system as with the conventional nitrate only system. While most literature on the subject of plant nutrition tends to find more convincing proof of statistically significant differences between ammonium/nitrate and nitrate only regimes, the sample size in this study was too small to attain statistical significance in many of the nutritional parameters measured. That being said, many of the findings mirrored that of literature and some statistically significant increases in protein and chlorophyll content were observed. Far from condemning this system, what the statistical analysis of the results revealed was that the nutritional parameters of the product plants were *at least* as good as those grown in pure nitrate, while avoiding any significant loss of yield.

The next logical step for a study into this type of control system would be to conduct experiments exceeding the three week period considered here so as to observe what effect the accumulation of the undesirable ions present in the baseline system would have. An alternative to this would be to reuse the nutrient solution in order to see if it will deleteriously affect new plants once their predecessors have been harvested. Moreover, the application of the proposed system in conjunction with organically sourced fertilisers would be an important step in creating a purely organic hydroponic system.

Possible improvements that can be made are further tuning of the pH control algorithm to try and reduce the tendency of the differential control to pin the pH and selecting more aggressive control parameters for the EC controller. It would also be informative to increase the operating time of the non-sterile run so as to see if the microbial interference would eventually be ironed out.

CRedit authorship

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