



Article

# Application of an Alternative Nutrient Replenishment Method to Electrical Conductivity-Based Closed-Loop Soilless Cultures of Sweet Peppers

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Abstract: The nutrient replenishment method primarily impacts the nutrient variations in a closedloop soilless culture system. However, there is still a lack of systematic approaches for the effective way of nutrient replenishment. Our previous study theoretically derived and experimentally validated an alternative nutrient replenishment method expecting synchronized total fertilizer supply to total nutrient absorption by crops and lower concentration fluctuations than conventional methods. However, no individual nutrient management has been performed. The objective of this study was to apply individual nutrient management to the alternative nutrient replenishment technique under experimental- and commercial-scale electrical conductivity (EC)-based closed-loop soilless cultures. Automated nutrient solution mixing modules and sweet peppers grown on rockwool slabs were used. Nutrient concentrations and crop productivity were compared between the closed-loop system using the alternative nutrient replenishment and the conventional open-loop systems. During early treatment, rapid decreases in  $K^+$  and  $H_2PO_4^-$  were observed in the closed-loop system. However, after the stock solution nutrient adjustment, the decreasing trend was stabilized and returned close to initial concentrations. No significant differences in sugar content, incidence of blossom-end rot, and productivity of sweet peppers were observed between the closed- and open-loop soilless cultures. We confirmed that the nutrient variation stabilizing effect of the alternative nutrient replenishment method was valid under nutrient adjustment conditions and had comparable nutrient management performance with the open-loop system.

**Keywords:** closed-loop soilless culture; crop productivity; electrical conductivity; nutrient management; nutrient replenishments



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# 1. Introduction

In general, sustainable management of resource use in a system can be ensured through collecting and reusing outflow products, which requires closed or semi-closed-loop structures [1,2]. However, soilless cultures have been regarded as a source of nutrient effluent at high concentrations due to standard open-loop supply practices, which routinely produce drainage [3–6]. While soilless culture has advantages regarding precise control, root zone nutrients are more sensitive in soilless systems than in soil systems. Thus, nutrient imbalance may occur when nutrient fluctuations are not properly managed due to these systems constraints. The problem of nutrient emission can be seen as a result of the conventional use of relatively simple nutrient management techniques that control root zone nutrient fluctuations by releasing a certain percentage of the drainage outside the system [4,5,7].

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A closed-loop soilless culture system that reuses discharged nutrients and water can minimize resource loss. Still, the closed-loop nutrient solution management is often accompanied by nutrient variations that result in a nutrient imbalance and instability in crop production [8–11]. Because of the complications caused by nutrient variation in the root zone, an ideal management technique is to measure and calibrate nutrient components in real-time [12]. Previous studies have focused on on-line measurements, and several applicable technologies were provided. However, it is still challenging to replace conventional open-loop nutrient management in technical stability or essential ion measurements [13,14]. Conventionally, one method that adjusts the total ion concentration by measuring electric conductivity (EC) has been applied, but problems related to managing individual nutrient variations have not been resolved [11,15,16].

Nutrient uptakes in plants mostly follow the Michaelis–Menten equation [17,18]. This means that plant systems can have steady-state solutions depending on input variables and parameters [19] and that the nutrient concentration in the root zone can converge to a specific value after a certain period. Thus, an appropriate nutrient input to the soilless culture system may lead to a steady state. In an EC-based closed-loop soilless culture system, nutrients are introduced to the system through the replenishment of standard stock solutions. However, considerable EC fluctuations are observed under actual cultivation conditions [8,20,21], indicating that the conventional nutrient replenishment method is unfavorable for the steady-state control of nutrients.

Ahn et al. [22] have analyzed the nutrient variation in closed-loop systems, leading to a new problem definition for nutrient variation in the system and supporting the formulation of an alternative nutrient replenishment method. This experimentally confirmed that maintaining the total nutrient concentration approximately at an initial concentration is associated with relatively stable changes in individual nutrient concentrations [23]. In addition to this, it is also important to control individual nutrient concentrations within a target range or at a target value. The objectives of this study were to apply the alternative nutrient replenishment method and the individual nutrient control to experimental- and commercial-scale EC-based closed-loop soilless cultures and verify its effect on crop productivity.

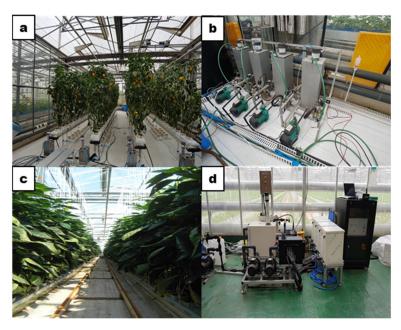
# 2. Materials and Methods

# 2.1. Cultivation Conditions for Experimental-Scale System

A Venlo-type greenhouse at the experimental farm of Seoul National University, Suwon, Korea (37.3° N 127.0° E) was used (Figure 1a). Three sweet pepper (Capsicum annuum L. 'Derby') plants were grown in a rockwool slab, and seven slabs were used per row. Four cultivation lines were installed in the greenhouse, each consisting of an independent closed-loop soilless culture system with a mixing tank, drainage tank, and stock solutions. In the greenhouse, daytime temperatures of 25 to 35 °C and nighttime temperatures of 15 to 22 °C were maintained. Experiments were carried out between April 2016 and July 2016. Open- and closed-loop soilless culture systems were applied for nutrient control. Target EC for the open-loop system was 2.6 dS·m<sup>-1</sup>. An integrated solar radiation method was used for irrigation control. The initial composition of the stock solution was prepared based on the PBG nutrient solution of the Netherlands. The composition of the prepared standard nutrient solution composition was 14.17 meq·L<sup>-1</sup> of  $NO_3$ <sup>-</sup>,  $1.14 \text{ meq} \cdot L^{-1} \text{ of } H_2PO^-, 5.92 \text{ meq} \cdot L^{-1} \text{ of } K^+, 8.85 \text{ meq} \cdot L^{-1} \text{ of } Ca^{2+}, 3.17 \text{ meq} \cdot L^{-1} \text{ of } Mg$ and 3.20 meq·L $^{-1}$  of SO $_4$ <sup>2 $^-$ </sup> as macro elements; and 0.038 meq·L $^{-1}$  of Fe $^2$ +, 0.020 meq·L $^{-1}$ of  $Zn^{2+}$ , 0.003 meq· $L^{-1}$  of  $Cu^{2+}$ , 0.021 meq· $L^{-1}$  of  $Mn^{2+}$ , and 0.001 meq· $L^{-1}$  of  $MoO_4^{2-}$ as microelements. The same nutrient solution mixing module from the previous study was used in this experiment (Figure 1b) [23]. The effect of tap water was minimized by using four reverse osmosis filters (150GPD, Waterpia, Suwon, Korea). Detectable nutrient concentrations in tap water were 0.001 meq·L<sup>-1</sup> of Na<sup>+</sup> at the initial installation. A solar radiation-based irrigation control was applied. When the cumulative radiation reached 100 J⋅cm<sup>-2</sup>, 150 mL of the nutrient solution was supplied to each plant. A drainage ratio

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of approximately 30% was maintained by setting the irrigation amounts and cumulative radiation values on meteorological conditions.



**Figure 1.** Experimental views of sweet pepper cultivation in greenhouses: an experimental greenhouse at Seoul National University Farm, Suwon, Korea (a) with irrigation systems (b), and a commercial greenhouse at Hwasung 21, Hwasung, Korea (c) with irrigation systems (d).

The experimental unit for open- or closed-loop soilless culture treatment was a nutrient solution mixing module. Two replications of the nutrient solution mixing system were assigned to each treatment. The sampling unit for collecting nutrient solution samples was a rockwool substrate. Three nutrient solution sample units were randomly collected from 14 substrates of each treatment every week. Among 42 plants in each treatment, randomly selected 28 plants were used to measure fruit yields per plant, and randomly selected 120 normal fruits were used to compare sugar content over the cultivation period.

### 2.2. Cultivation Conditions for Commercial-Scale System

A Venlo-type greenhouse at the commercial farm of 'Hwasung 21' (Hwasung, Korea, lat. 37.0° N, long. 126.8° E) was used (Figure 1c). Sweet pepper plants (*Capsicum annuum* L. 'Veyron') were arranged in two rows for each lane, and 126 plants were planted in each row and allocated for closed- and open-loop system treatments, respectively. The experiments were carried out between May 2016 and July 2016. The nutrient solution mixing module installed in the commercial farm (Figure 1d) has the same components as the small-scale experiment with the addition of a UV sterilizer (UV-C-lamp, 100 W) in the mixing tank circulation pipe. Thus, when nutrient solution mixing occurred in the mixing tank, the reused nutrient solution was sterilized. To assess the effect of sterilization in this experiment, the biological contamination level was evaluated using a portable ATP water test kit (3M Clean-Trace, 3M, St. Paul, MN, USA) at the end of each experiment. In order to confirm the control effect of nutrient input by stock solution, the effect from tap water was minimized by using eight reverse osmosis filters (150GPD, Waterpia, Suwon, Korea). The same standard nutrient solution and irrigation management methods were applied to the commercial system that was used in the experimental system.

The experimental unit for open- or closed-loop soilless culture treatment was a gutter lane consisting of 42 rockwool substrates. The sampling unit for collecting nutrient solution samples was a rockwool substrate. Three nutrient solution sample units were randomly collected from 42 rockwool substrates of each treatment gutter lane every week. Among 126 plants in each treatment, four randomly selected plants were used to measure fruit

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yield per plant and randomly selected 18 normal fruits were used to compare sugar content over the cultivation period.

# 2.3. Alternative Nutrient Replenishment Technique

The three phases in the process of nutrient balance control were applied in this experiment (Figure 2a): first, nutrient model calibration was performed (*P*-phase); then, a short-term increase process was performed for the nutrients exhibiting large decreases in concentration (*S*-phase); finally, the steady-state solution of each nutrient was calculated based on the calibrated model and used to adjust the stock solution composition (*A*-phase). The representative equations for processing the three phases are as follows (Equations (1)–(3)).

$$V_n \frac{dC_n^I}{dt} = Q_{n-1} C_{n-1}^I - Q_n C_n^I - P_{RSA} J_n^I$$
 (1)

 $V_n$  is the volume of water in a substrate layer n, C is the molar concentration of nutrient (mM), superscript I is the type of ions (K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>), n is the substrate layer,  $J_n^I$  is the uptake rate of nutrients (mmol m<sup>-2</sup> min<sup>-1</sup>), and  $P_{RSA}$  is the specific root surface area (m<sup>2</sup>), which is described as the root length density and specific root surface area.

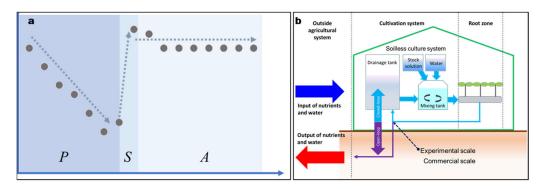
$$N_{sup} = N_{tar}W_{cont} - N_{cur}W_{cont}$$
 (2)

 $N_{sup}$  is the nutrient amount required for the short-term increase (mmol),  $N_{tar}$  is the target nutrient concentration (mM),  $W_{cont}$  is the total volume of water in the system corresponding to the field capacity of the substrate,  $N_{cur}$  is the current nutrient concentration.

$$V_{stk} = \frac{C_{init}W_{cont} - C_{drg}V_{drg} - C_{mix}V_{mix} - C_{drg}W_{cont}}{C_{stk}}$$
(3)

where  $V_{init}$  is the initial total volume of water in the system;  $C_{init}$  is the initial total concentration of the system;  $V_{drg}$ ,  $V_{mix}$ , and  $V_{stk}$  are the volumes of water stored in the drainage tank, mixing tank, and the input volume of stock solution, respectively; and  $C_{drg}$ ,  $C_{mix}$ , and  $C_{stk}$  are the total nutrient concentrations estimated by EC in the drainage tank, mixing tank, and the stock solution, respectively.

A model was generated according to the work of [23] and used for the model calibration alongside progress curve analysis (Equation (1)). The approximate amount of nutrients needed for a short-term increment for a largely decreased nutrient was calculated based on Yamazaki n/w method (Equation (2)) [24]. The alternative nutrient replenishment technique proposed by Ahn and Son [23] was used in the closed-loop soilless culture system (Equation (3)).



**Figure 2.** (a) Conceptual diagram of the nutrient balance control technique; *P*-phase: online-parameter estimation for nutrient uptake model (progress curve analysis); *S*-phase: short-term increment of largely decreased nutrient; *A*-phase: application of adjusted stock solution composition (indicates desired nutrient changes). (b) Schematic diagram of open- and closed-loop soilless culture systems in this experiment.

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# 2.4. Measurement of Fruit Yield and Analysis of Nutrient Content in Leaves and Fruits

Total fruit yield, fruit weight, sugar content, and the ratio of blossom-end rot (BER) to the total number of fruit yields per plant were measured during experimental periods. At the end of the experiment in the commercial-scale farm, three leaves (including petiole) at the fourth and fifth nodes from the top of the stem and at the second and third nodes from the bottom of the stem were collected respectively from each treatment. The sugar contents (%) of fruits were measured after harvest with a refractometer (PAL-1, Atago, Tokyo, Japan) to compare fruit quality. Graphs were expressed with Sigma Plot Ver. 13 (Systat Software, San Jose, CA, USA). To analyze fruit nutrient content, matured fruits (three fruits per treatment) were collected in the middle and last harvest of the experiment. The fruits were dried at 70 °C in an oven until they reached a constant weight. Leaves and fruits were washed in tap water, and leaves were dried for 48 h at 70 °C in the drying oven. The dried leaves and fruits sampled were ground, and 0.5 g of each sample was digested with concentrated nitric acid. Then, 1 mL of concentrated perchloric acid was added to maintain a set solution temperature of 180 °C and to accelerate the digestion process on a 90 °C hot plate for about one hour until a clear solution was obtained. After the digestion, the tube was cooled and topped up with 25 mL of deionized water. The total contents of K, Ca, Mg, P, and S from leaves were determined by the inductively coupled plasma optical emission spectrometer (ICP-730ES, Varian, Mulgrave, VIC, Australia). Total-N was measured by Kjeldahl (Kjeltec 8400, Foss, Hoganas, Sweden).

# 2.5. Nutrients Analyses and Statistics

To observe changes in nutrient concentrations and ratios in the root zone of the soilless culture system, samples of nutrient solution in the rockwool slabs were extracted using a syringe. The collection points of the nutrient solution in the rockwool slab were randomly selected to obtain representative samples of the overall concentration in the root zone. Five 10 mL samples of root zone nutrient solution were collected for each extraction, yielding a total of 50 mL of sample. Three samples per treatment were collected each week.  $K^+$ ,  $Ca^{2^+}$ ,  $Mg^{2^+}$ ,  $S(SO_4^{2^-})$ ,  $P(H_2PO_4^-)$  were analyzed using the inductively coupled plasma optical emission spectrometer. The concentration of  $NO_3^-$  was measured with a spectrophotometer (PhotoLab, 6100 (VIS), WTW, Weilheim, Germany). SAS software (version 9.2, SAS Institute, Cary, NC, USA) was used for statistical analysis. In the experimental-scale system, seven rockwool slabs were connected to a single automated module, and two modules were assigned to each treatment (closed- and open-loop system).

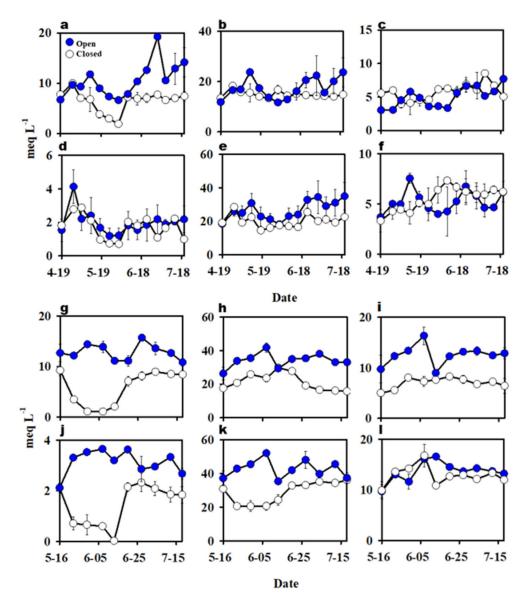
The limited replication of the installed modules in this study may limit the reproducibility of the performance of the drainage recycling module. However, the substrate cultures have isolated system boundaries of the root zone. Thus, even in the single system (i.e., a single greenhouse with a single irrigation system), each slab condition could display significant variations in root zone nutrients and water depending on the microclimate fluctuation of each slab. Therefore, it can be expected that the rockwool slab could provide a replicate of the independent root zone boundary samples for assessing nutrient concentration and yield components.

### 3. Results and Discussion

### 3.1. Nutrient Concentration Changes

In the experimental-scale closed-loop soilless culture system, an initial rapid decrease was observed in  $K^+$  before nutrient adjustment (P-phase) (Figure 3a). After the rapid decline in the P-phase and subsequent short-term increment in the S-phase, such decreasing trend disappeared at A-phase. The stabilization of  $K^+$  can be interpreted as an indication that the addition of stock solution according to progress curve analysis helps stabilize the system.

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**Figure 3.** Changes in ion concentration under the nutrient balance control in the experimental-  $(\mathbf{a}-\mathbf{f})$  and commercial-  $(\mathbf{g}-\mathbf{l})$  scale experiments:  $K^+$   $(\mathbf{a},\mathbf{g})$ ,  $Ca^{2+}$   $(\mathbf{b},\mathbf{h})$ ,  $Mg^{2+}$   $(\mathbf{c},\mathbf{i})$ ,  $H_2PO_4^ (\mathbf{d},\mathbf{j})$ ,  $NO_3^ (\mathbf{e},\mathbf{k})$ , and  $SO_4^{2-}$   $(\mathbf{f},\mathbf{l})$ . P-phase: 19 April-1 June  $(\mathbf{a}-\mathbf{f})$ , 16 May-13 June  $(\mathbf{g}-\mathbf{l})$ , S-phase: 8 June  $(\mathbf{a}-\mathbf{f})$ , 20 June  $(\mathbf{g}-\mathbf{l})$ , A-phase: 8 June-18 July  $(\mathbf{a}-\mathbf{f})$ , 20 June-15 July  $(\mathbf{g}-\mathbf{l})$ .

In anions,  $H_2PO_4^-$  tended to decrease rapidly following an initial increase in both the open- and closed-loop systems (Figure 3d). However, no decrease was observed below the initial value in the open-loop system, while the decline continued until the adjustment point in the closed-loop system. The same correction procedure was used for the stock solution and short-term recovery for  $H_2PO_4^-$  as used for  $K^+$ . In the case of  $SO_4^{2-}$ , a gradual increase was observed during the P-phase. After the A-phase, the increasing trend was reversed and gently changed to a continuous decreasing trend. This suggests that the control for the decline in nutrients in the closed-loop system should depend on the absorption rate of plants, unlike the nutrient replenishment control.  $Ca^{2+}$ ,  $Mg^{2+}$ , and  $NO_3^-$  did not exhibit recognizable trends, and instead, those ions displayed fluctuating behaviors around the initial concentration in the experiment (Figure 3b,c,e).

In the commercial-scale closed-loop soilless culture system, a similar result was seen to that reported for the experimental-scale experiment: a rapid decrease was observed in  $K^+$  before the S-phase (Figure 3g). However,  $K^+$  did not decrease after the nutrient adjustment point (S-phase) and instead stayed relatively stable during the A-phase. Ca<sup>2+</sup> and Mg<sup>2+</sup>

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exhibited slightly increasing trends; however, after A-phase,  $Ca^{2+}$  was gradually decreased and remained stable, and  $Mg^{2+}$  showed a gradual descending (Figure 3h,i). For anion,  $H_2PO_4^-$  rapidly reduced during the P-phase, and after a short-term increase in the S-phase, the nutrient was stabilized similarly to  $K^+$ . In addition,  $NO_3^-$  displayed a slight decline and gently recovered around to the initial level after the A-phase. As in the experimental-scale system,  $SO_4^{2-}$  concentration increased from the initiation of the experiment, and a sudden decrease was observed before reaching the adjustment phases (A-phase). However, during the A-phase, the concentrations of  $SO_4^{2-}$  displayed stabilized behaviors.

An increase in divalent ions such as  $Ca^{2+}$ ,  $Mg^{2+}$ , and  $SO_4^{2-}$  in closed-loop soilless cultures are commonly observed trends [11,22,25]. However, we did not distinctively observe such trends in this experiment except  $SO_4^{2-}$ . In our previous work for the alternative nutrient replenishment method in the EC-based closed-loop system, which is also applied to this study, we reported similar trends in  $Ca^{2+}$  and  $Mg^{2+}$  [23]. Similar to our study,  $Ca^{2+}$  and  $Mg^{2+}$  did not exhibit clear directional trends; instead, those ions fluctuated within a certain range around the initial concentrations. In the previous work, we assessed this as the effects originating from average steady-state control of the system EC by the alternative nutrient replenishment method.

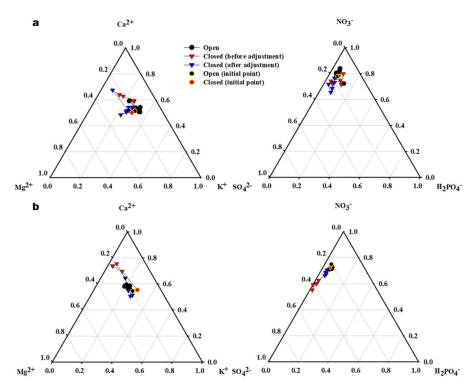
Furthermore, we reported that the average steady-state control of the system EC by the alternative method allowed the individual ions to approach the average steady-state. The alternative nutrient replenishment method is also featured by lower variabilities than the conventional nutrient replenishment method. That is, the changing trends were featured by explicit and more deterministic behaviors. We note that in this study, the alternative nutrient replenishment method yielded more clear trends of ion concentration changes than the open-loop system. Overall behaviors of the ion concentration observed in the open-loop system were fluctuating with larger variability than the closed-loop systems. Open-loop systems manage nutrient variations in the root zone by modulating leaching fractions with normally constant standard concentrations, and the systems tolerate specific ranges of root zone nutrient variations [4,26]. Instead, the closed-loop system with the alternative replenishment method limits the total nutrient concentration of the system to the average steady state. This validates again the theoretical predictions of our previous studies that the steady-state control of EC in the closed-loop system also limits degrees of variabilities of individual ions and allows more stabilized systems ion behaviors than the open-loop system [22,23].

So far, standard nutrient solutions have been developed to consider each nutrient requirement ratio, chemical stability, and yield of plants through plant nutritional studies [27–30]. Even though previous standard nutrient solutions were developed with information about nutrient requirement ratios, the input nutrient ratios for a steady state could be different from predictions because of nonlinear ratio change in nutrient uptake [31]. While previous studies have observed nutrient changes that could be interpreted as a steady state [32–34], they have not discussed results in terms of a steady-state control technique.

## 3.2. Nutrient Ratio Changes

Changes in ion balance are shown in Figure 4. In the open-loop soilless culture systems of the experimental- and commercial-scale systems, nutrient concentrations exhibited relatively large fluctuations. However, the level of instability tended to decrease when data were converted to ternary nutrient ratios (Figure 4a,b). In contrast to the closed-loop system, nutrient ratio variations in the open-loop system did not largely deviate from the initial ratio points. Our previous study reported reduced variability from the mutual ion ratio-based data. In the root zone, water absorption by plants primarily impacts fluctuations in the root zone nutrient concentrations [4,35]. However, it was suggested that the nutrient ratio conversion might filter the fluctuations from the transpiration variations [22].

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**Figure 4.** Changes in ion balance under the nutrient balance control in the experimental- (a) and commercial- (b) scale experiments.

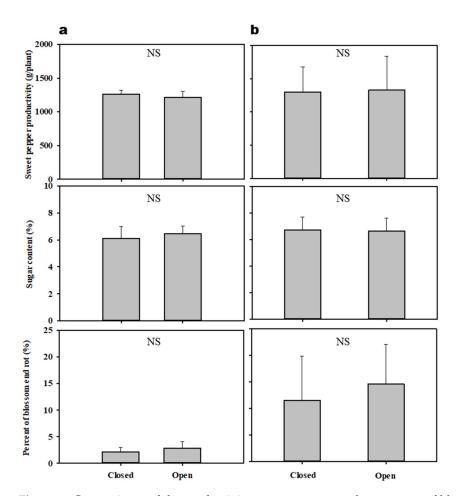
Contrastingly, in the closed-loop systems, steadily deviating nutrient ratios from the initial points were visualized on the ternary plots during the P-phases (Figure 4). However, after nutrient adjustment (A-phase), the ratios returned close to the initial points. Nutrient concentration ratios are reported in Figure 4. The ratios of cations and anions in the open-loop system were lower than those in the closed-loop system. However, in the case of the closed-loop system, the change in the direction of change for  $K^+$  (decrease) and  $Ca^{2+}$  (increase) occurred in the cation until the initial analysis but returned to the initial ratio after adjustment. A similar pattern was observed for anions:  $SO_4^{2-}$  and  $H_2PO_4^{-}$  showed reversed trends, and ratios were returned to their starting points after adjustment.

# 3.3. Fruit Yield Components

Changes in nutrient concentration and ratio observed in the closed-loop soilless culture system did not show statistically significant effects on sweet pepper fruit productivity, sugar content, or blossom rot incidence (Figure 5). No statistically significant differences were observed in the productivity, sugar content, and incidence of blossom-end rot of sweet peppers (Figure 5a,b). According to Ehret et al. [15] and Zekki et al. [11], it took a certain period until nutrient concentrations in the closed-loop system affected plant growth. So, it is unlikely that the exposure of plants to fluctuations and deviations in nutrient concentration and ratios from the desired level affect plants' yield components significantly before the *A*-phase.

Leaf analysis showed that there was a significant difference in total nitrogen (T-N) and lower leaf Mg content in the top leaves. Still, the values were not high enough to constitute a deficiency or excess, according to the sweet pepper growing manual [36]. The difference between T-N and Mg was 0.15%p and 0.11%p, respectively (Table 1). In the case of sweet pepper harvested at the first nutrient correction, there was a significant difference between Mg and S, about 0.02%p. There was no significant difference in ion content corresponding to late harvest after the nutrient correction.

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**Figure 5.** Comparisons of the productivity, sugar content, and percentage of blossom-end rot of sweet peppers between the closed-loop soilless culture under the nutrient balance control and the open-loop soilless culture in the experimental- (a) and commercial- (b) scale experiments (*t*-test). NS: not significant.

**Table 1.** Comparison of nutrient contents in leaves and fruits of sweet peppers between the open-and closed-loop soilless cultures under nutrient balance control in the commercial farm.

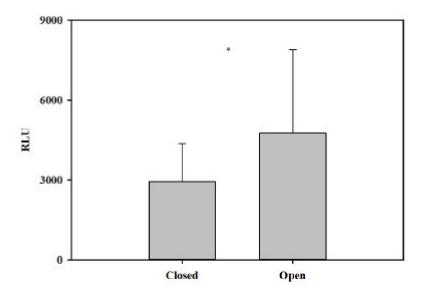
Position	Treatment	T-N	P	K	Ca	Mg	S
Тор	Open	$3.52 \pm 0.06$	$0.36 \pm 0.06$	$6.14 \pm 0.81$	$3.15\pm0.12$	$0.44 \pm 0.04$	$0.52 \pm 0.03$
-	Closed	$3.67 \pm 0.02$	$0.42\pm0.03$	$5.38 \pm 0.33$	$3.54 \pm 0.22$	$0.51\pm0.05$	$0.57 \pm 0.02$
	t-test	*	NS	NS	NS	NS	NS
Bottom	Open	$2.59 \pm 0.15$	$0.21\pm0.03$	$5.72 \pm 0.83$	$4.53 \pm 0.17$	$0.58\pm0.01$	$0.42\pm0.07$
	Closed	$2.65 \pm 0.26$	$0.25\pm0.05$	$6.61 \pm 0.65$	$4.46\pm0.27$	$0.69 \pm 0.04$	$0.52\pm0.05$
	t-test	NS	NS	NS	NS	*	NS
Fruit (6.14)	Open	$2.01\pm0.14$	$0.38 \pm 0.02$	$2.66 \pm 0.12$	$0.09 \pm 0.01$	$0.11 \pm 0.009$	$0.23 \pm 0.01$
	Closed	$1.85 \pm 0.09$	$0.33 \pm 0.02$	$2.36 \pm 0.34$	$0.08\pm0.01$	$0.09 \pm 0.003$	$0.21\pm0.01$
	<i>t</i> -test	NS	NS	NS	NS	*	*
Fruit (7.12)	Open	$2.16 \pm 0.18$	$0.38 \pm 0.03$	$2.62 \pm 0.13$	$0.07 \pm 0.01$	$0.11\pm0.011$	$0.22 \pm 0.03$
	Closed	$1.99 \pm 0.08$	$0.37 \pm 0.01$	$2.63 \pm 0.41$	$0.08 \pm 0.02$	$0.11 \pm 0.013$	$0.23 \pm 0.01$
	t-test	NS	NS	NS	NS	NS	NS

p < 0.05 by t-test. NS, not significant; \*, significant at p = 0.05. The numbers in parentheses indicate the date of harvest.

Indirect measurement of microbial concentration through an ATP activity test on the nutrient solution in the root zone showed a significantly lower value in the closed-loop soilless culture system than in the open-loop systems (Figure 6). In this study, the UV sterilizer was installed in the path of circulation pipes in the mixing tank. Therefore, reused

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nutrient solutions were sterilized whenever mixing occurred for the irrigation water. While closed-loop soilless culture systems can cause contamination of the reused nutrient solution, UV sterilization systems can act as inactivation kinetic constants for microorganisms within a system depending on their type and condition [37]. Thus, introducing proper sterilizing power to the system may provide more stable microbial risk management than the open soilless culture system [38].



**Figure 6.** Comparison of relative light unit (RLU) values in the closed- and open-loop soilless cultures at the end of the commercial farm. An asterisk indicates significant differences (\* p < 0.05 by t-test).

## 4. Conclusions

The purpose of our alternative nutrient replenishment method in the closed-loop soilless culture system was to control the average steady state in total nutrient concentrations. Therefore, the overall aspect of nutrient concentration changes was distinguished from the open-loop soilless culture system changes. The theoretically predicted ion behaviors by the alternative nutrient replenishment method were validated here again. Our previous study for the alternative nutrient replenishment method did not conduct individual ion control. Subsequently, in this study, we contributed to optimizing the closed-loop soilless culture system by adding individual ion managements to our alternative nutrient replenishment method. Consequently, these nutrient management methods operated without compromising the yield and quality while no nutrient and water discharge was permitted.

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