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Research Paper

Exploring nutrient reduction strategies without yield losses in hydroponic lettuce production

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ABSTRACT

This study investigated the impact of reduced nutrient input in closed-loop hydroponic systems on yield, leaf nitrate (NO_3^-) content, and nutrient uptake in three lettuce cultivars: butterhead, lollo bionda, and lollo rossa. Two greenhouse experiments were conducted using four nutrient solutions (NS) treatments combining two electrical conductivity (EC) levels (2.5 and 2.0 dS m^{-1}) with two nitrogen (N) to potassium(K) ratios (3.28 and 2.28). In the lower N:K treatment, NO_3^- was partially substituted by Cl^- to maintain ionic balance. Fresh weight (FW) was not significantly affected by either EC or N:K ratio, indicating that yield can be preserved despite a reduction in nutrient input. In contrast, leaf NO_3^- content was significantly influenced by both factors EC and N:K ratio, with reductions of up to 33 % observed in the low EC/low N:K treatment. Leaf NO_3^- content ranged from 1431 to 1642 mg NO_3^- kg^{-1} of FW across cultivars, which is well below European Union's safety limit of 5000 mg kg^{-1} FW. Nutrient uptake concentrations (UCs) were estimated using both mass-balance and plant tissue analysis methods. Significant discrepancies were found between the two approaches for N, K, Ca, and Fe, in the mass-balance method yielding higher values, suggesting losses of these nutrients from the system. The Cl^- UC increased significantly under low N:K conditions. These findings demonstrate that reduced EC and a lower N:K ratio than standard recommended values can result in lower NO_3^- accumulation and nutrient input without compromising yield, supporting the development of more sustainable fertigation protocols for hydroponic lettuce production.

1. Introduction

Soilless cultivation surpasses soil-growing crops in terms of productivity, environmental footprint, and water and nutrient use efficiency (Fussy and Papenbrock, 2022; Singh et al., 2024). However, significant nutrient losses, particularly of nitrogen (N) can occur through leaching, run-off and gaseous emissions, especially in intensive production systems with free drainage of the fertigation effluents (Libutti and Monteleone, 2017; Qasim et al., 2021; Zhang et al., 2024). Sanjuan-Delmás et al. (2020) found that nutrient losses in free drainage soilless systems can account for up to 51 % of total nutrient inputs, a problem that can be mitigated by recycling the drainage solution (DS) in closed-loop systems. Hydroponic culture systems, where plant roots are grown directly into the nutrient solution (NS), are a prominent example of closed-loop cultivation. In such systems, the root solution (RS), i.e. the NS in the root zone, is replenished with water and nutrients to compensate for plant uptake (Savvas et al., 2024). Leafy vegetables, such as lettuce, are well suited to hydroponic cultivation given their short growing period, which allows multiple harvests per year. In such cultivation cycles, the oxygenation issues in the NS that arise from the extensive root systems of long-cycle fruiting crops can be avoided (Mattson and Lieth, 2019).

At commercial scale, different cultivars of lettuce are cultivated within the same NS for operational efficiency. Therefore, it is crucial to develop a NS that fulfils the requirements of different lettuce cultivars to ensure optimal growth and product quality (Ropokis et al., 2018). In closed-loop systems, nutrient and water supply must closely match plant uptake expressed as 'uptake concentrations,' (UCs) to prevent nutrient depletion or accumulation in the root zone (Thompson et al., 2013). For a given species and growth stage, UC remain stable under similar climatic conditions. Therefore, UCs can be used as a good basis to establish optimal nutrient solution (NS) compositions in closed-loop soilless

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Table 1
Impact of EC and N:K ratio on leaf fresh and dry weight of the three different cultivars (BH, LB, LR) (data pooled over the two experiments).

		Leaf fresh weight (g)			Leaf dry weight (g)		
		BH	LB	LR	BH	LB	LR
EC	Standard	257.3	180.4	152.1	9.47	7.62	7.52
	Low	251.8	175.2	152.3	9.85	7.83	7.08
N:K	Standard	255.7	180.4	154.5	9.64	7.91	7.58
	Low	253.4	175.3	149.8	9.66	7.51	7.02
				Statistical significance			
EC		NS	NS	NS	NS	NS	NS
N:K		NS	NS	NS	NS	NS	NS
EC x N:K		NS	NS	NS	NS	NS	NS

In each column, means ($n = 4$) followed by different letters are significantly different according to the ANOVA test and the Tukey Honest Significant Difference test ($p \leq 0.05$). Regarding the variance components, according to associated P values, *** ($p \leq 0.001$), ** ($p \leq 0.01$), and * ($p \leq 0.05$) represent statistical significance, while NS signifies non-significance. BH: butterhead (cv. Fairly), LB: lollo bionda (cv. Jokary) and LR: lollo rossa (cv. Lea).

cropping systems to balance plant uptake (Neocleous et al., 2024; Ropokis et al., 2018).

N plays a key role in the main cell functions such as protein synthesis, nucleic acid synthesis and chlorophyll synthesis (Hawkesford et al., 2012). In NS, N is supplied mainly as nitrate N (NO_3^-), while a small fraction of about 0.5 to 0.15 of total N is supplied in ammonium (NH_4^+) form to maintain the pH in root solution (RS) within the optimal range and to avoid the risk of ammonium toxicity (Voogt and Bar-Yosef, 2019). The availability and concentration of N in the NS directly influence plant uptake rates (Marschner, 2012). Hydroponic lettuce exhibits a high demand for N, thus for hydroponic cultivation the standard N supply (NO_3^-+NH_4^+) is about 19 mM (Sonneveld and Voogt, 2009). However, these concentrations may exceed the actual plant requirements. Higher NO_3^- concentrations in the root-zone can substantially raise the amount of N absorbed by the plant, which may result in excessive nitrate accumulation in leaf tissues (White, 2012). Understanding the relationship between N supply, uptake and impact on yield and product quality is crucial for developing more efficient and sustainable fertigation strategies.

NO_3^- accumulation in the edible parts of leafy vegetables is a critical food safety parameter for human health with strictly regulated thresholds established by the European Union (Regulation No1258/2011). The permissible limits range from 3000 to 5000 mg NO_3^- kg^{-1} of fresh weight (FW), depending on the growing season (summer or winter) and cropping system (open field or under cover). These distinctions reflect the influence of environmental factors, particularly light intensity, on N assimilation processes. Under higher light intensity, increased photosynthetically active radiation (PAR) enhances the availability of carbon skeletons, promoting the assimilation of inorganic N to amino acids, thereby reducing the NO_3^- concentration in the vacuole (Taiz and Zeiger, 2002), and thus in the plant tidssues.

Partial substitution of NO_3^- by chloride (Cl^-) has been proposed as a strategy to reduce N input, improve agronomic efficiency of N (AE_N) and enhance product safety for consumers by decreasing leaf NO_3^- accumulation in lettuce (Neocleous et al., 2024) and tomato (Neocleous et al., 2021; Voogt and Sonneveld, 2004) grown in soilless culture systems. Another strategy that has proven effective in lowering leaf NO_3^- content and increasing NUE in lettuce was the reduction of the electrical conductivity (EC) in the root environment for the whole or part of the growing period (Martínez-Moreno et al., 2024).

Considering this background, the objective of the current study is to take the next step towards developing complete and adapted fertigation recommendations for hydroponic lettuce production. The overarching aim is to reduce nutrient inputs without compromising yield, while enhancing both environmental sustainability and food safety. Within this framework, the combined effect of two key NS composition modifications: a) a reduced EC level in the RS and b) a reduced N:K ratio compared to standard current recommendations was investigated, focusing on their impact on lettuce fresh yield and leaf NO_3^- content. The reduction of NO_3^- concentration in the NS is compensated for by equivalent Cl^- increase using calcium chloride for part of Ca input instead of calcium nitrate, thereby not altering the Ca supply. Furthermore, nutrient uptake concentrations, i.e. the nutrient to water uptake ratios, were calculated across all treatments, to estimate the necessary adaptations in the composition of nutrient solutions for lettuce grown in closed-loop hydroponic systems.

2. Materials and methods

2.1. Experimental design and growing conditions

Two sequential experiments were conducted in a greenhouse using a floating hydroponic system at the Agricultural University of Athens (AUA: 37°58′54.2″N 23°42′22.0″E, altitude 35 m). The experimental installation included sixteen independent floating systems each consisting of a main cultivation tank (1.50 × 0.60 × 0.30 m) filled with 180 L of NS at a depth of 0.20 m. A replenishment tank connected via a floating valve maintained stable NS levels in the system. Three lettuce cultivars representing three different types commonly cultivated together in hydroponic systems at commercial scale, were selected and used for the experiment, namely a butterhead type (cv. Fairly, Enza Zaden) (BH), a lollo bionda type (cv. Jokary, Enza Zaden) (LB) and a lollo rossa type (cv. Lea, Enza Zaden) (LR). In each tank, 15 lettuce plants were grown, 5 from each cultivar to reflect typical commercial hydroponic production conditions and allow accurate estimations of nutrient uptake concentrations. Each cropping cycle lasted 25 days. On 03/12/2023 the seedlings were transplanted on the floating systems for the first experiment and the lettuces were harvested on 04/07/2023, while the second experiment started on 11/01/2023 and was terminated on 27/11/2023. The greenhouse was heated and passively ventilated through roof and side windows, to maintain the temperature within the range 17 to 24 °C) and the relative humidity between 60 and 80 %. Furthermore, the plants were grown under natural light conditions. The same experimental design was used in both trials, which served as temporal replicates. Two EC levels were combined with two N:K ratios, i.e., a standard and a low, to create four NS treatments in a 2-factorial experimental design. The standard EC level was set at 2.5 dS m^{-1} and the low EC at 2.0 dS m^{-1}, with a K:Ca:Mg molar ratio of 0.413:0.484:0.103 at both EC levels. In the standard N:K treatment, the molar N:K ratio was 3.28, while in the low N:K treatment this ratio was reduced to 2.28. In the low N:K treatment, 30 % of NO_3^- supply was substituted for by Cl^-, using calcium chloride ($CaCl_2$) as source. Consequently, the four different treatments were as follows: T1: Standard EC – Standard N:K, T2: Standard EC – Low N:K, T3: Low EC – Standard N:K, T4: Low EC – Low N:K. These treatments were applied in the added solution (AS), i.e. the NS supplied to the plants during the crop to compensate for plant uptake. The composition of the irrigation water and the NS in the main cultivation tank are presented in Table 1 of Supplementary materials. Continuous addition of AS was implemented through a floating valve to compensate for the plant water and nutrient uptake, aiming to maintain EC and pH in the main cultivation tank.

2.2. Determination of nutrient uptake concentrations

The UCs of the macronutrients N, P, K, Ca and Mg (mM) and of the micronutrients Fe, Mn, Zn, Cu, B (μM) and Cl^- (μM), were calculated using two different methods. The mass-balance method was based on the calculation of the removed nutrients mass, and thus the nutrient consumptions, by subtracting the remaining nutrient mass from the supplied nutrient mass at the end of the experiment. To apply this method, the concentrations of these nutrients were measured in the NS immediately after transplanting and at crop termination, considering the water and nutrient addition during the crop through the added solution (AS). For the calculation of the UC, the following mass balance equation was used (Neocleous et al., 2024; Ropokis et al., 2018):

$$C_{iu} = \frac{V_r(C_{ib} - C_{ie}) + V_w C_{ia}}{V_w} \quad (1)$$

where, C_{iu} is the UC of the i nutrient, V_r is the volume of the NS in the main cultivation tank in each system (L), V_w is the cumulative water consumption of plants (L), C_{ib} and C_{ie} (mM or μM) are the concentrations of the i nutrient at the beginning and at the end of the crop, and C_{ia} denotes the concentration of the i nutrient in the AS (mM or μM).

The second method was based on nutrient recovery in plant tissues. According to this method, the UCs are calculated as the ratio between the quantity of nutrients in the dry plant biomass, which corresponds to the total absorbed nutrient mass by the plants, and the total water consumption of the plants. The UCs estimated according to the nutrient recovery method were calculated using the following equation (Neocleous et al., 2024; Ropokis et al., 2018):

$$C_{iu} = \frac{C_{il}B_{il} + C_{ir}B_{ir}}{V_w} \quad (2)$$

where, C_{iu} is the UC of the i nutrient, V_w is the cumulative water consumption of plants (L), C_{il} and C_{ir} denote the leaf and root i nutrient concentration (mmol or μmol g^{-1} of dry weight), respectively and B_{il} and B_{ir} denote the leaf and root dry weight (g), respectively.

2.3. Sampling and laboratory analysis

Samples of NS from the main cultivation tank from each experimental unit, were collected immediately after transplanting and at harvest to determine the [K^+], [Ca^{2+}], [Mg^{2+}], [Na^+], [NO_3^-], [P], [Cl^-], [Fe], [Mn], [Zn], [Cu], and [B] concentrations. The data from these analyses were used to calculate the UC using the mass-balance method. At crop termination three plants, one from each cultivar, were randomly selected from each tank and used to determine the leaf and root concentrations of the above referenced nutrients. Leaves and roots were sampled separately, cleaned using distilled water, dried at 65 °C to constant weight and powdered using a blade mill. The powdered samples were then subjected to the dry ashing procedure to extract K, Ca, Mg, Na, Fe, Mn, Zn, Cu, and B (Campbell and Plank, 1997). The Cl was extracted from the dry tissues using hot water, at 40 °C for 60 min. Organic-N concentration in the plant tissues was measured according to the Kjeldahl method, using a Labtec DT 220 and a Tecator Kjeltec 8200 (FOSS A/S, Hillerod, Denmark). Total-N concentration was calculated by adding the oganic-N and the inorganic-N (NO_3) concentration. The NS samples and the plant tissues extracts were analysed for the [K^+], [Ca^{2+}], [Mg^{2+}], [Na^+], [Fe], [Mn], [Zn] and [Cu] using an atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan). Nitrates in the NS samples were determined photometrically at 540 nm (Schnetger and Lehners, 2014) and in the dried leaf tissues according to the salicylic acid method at 410 nm (Cataldo et al., 1975). Phosphorus was measured according the Murphy and Riley (1962) method at 880 nm, while [Cl^-] was determined photometrically at 460 nm (Iwasaki et al., 1952; Zall et al., 1956). Agronomic efficiency of N (AE_N) was calculated a kg of fresh yield produced per kg of supplied N (kg^{-1}) (Ladha et al., 2005).

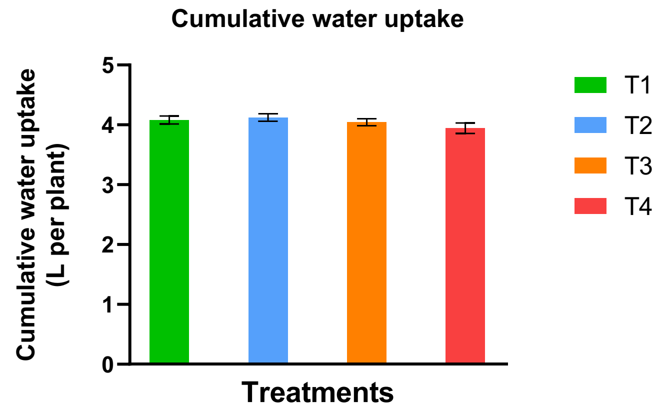

Fig. 1. Cumulative water uptake by the plants in each treatment (T1-Standard EC- Standard N:K, T2-Standard EC-Low N:K, T3-Low EC- Standard N:K, T4-Low EC- Low N:K) (data pooled over the two experiments). Vertical bars indicate ± standard errors of means.

Table 2
Impact of EC and N:K ratio on i) total-N (organic + inorganic N) content in lettuce leaves expressed as mg per g of dry matter (DM) and ii) leaf nitrate (NO_3^-) content as mg of NO_3^- per kg of fresh weight (FW) of the three different cultivars (BH, LB, LR) (data pooled over the two experiments).

EC	N:K	Total-N (mg g^{-1})			Leaf NO_3^- (mg kg^{-1} FW)		
		BH	LB	LR	BH	LB	LR
Standard	Standard	45.29	44.50	43.90	2360 a	2103 a	2421 a
Standard	Low	41.27	40.84	40.46	2000 b	1804 b	1985 c
Low	Standard	39.29	37.61	38.36	2264 a	1793 b	2145 b
Low	Low	34.05	34.35	32.60	1642 c	1431 c	1614 d
	Main effects						
EC	Standard	43.28	42.67	42.18	2180	1953	2203
	Low	36.84	36.08	35.67	1953	1612	1880
N:K	Standard	42.29	41.05	41.13	2312	1948	2283
	Low	37.90	37.81	36.79	1821	1617	1800
	Statistical significance						
EC		***	***	***	***	***	***
N:K		***	**	**	***	***	***
EC x N:K		NS	NS	NS	*	*	*

In each column, means (n = 4) followed by different letters are significantly different according to the ANOVA test and the Tukey Honest Significant Difference test ($p \leq 0.05$). Regarding the variance components, according to associated P values, *** ($p \leq 0.001$), ** ($p \leq 0.01$), and * ($p \leq 0.05$) represent statistical significance, while NS signifies non-significance. BH: butterhead (cv. Fairly), LB: lollo bionda (cv. Jokary) and LR: lollo rossa (cv. Lea).

2.4. Statistical analyses

The experiment was set up as a 2 × 2 factorial completely randomised design with four replications per treatment. The significance of the differences between treatments was assessed by applying factorial ANOVA and Duncan's multiple range test. The statistical analysis of the data, ANOVA and Duncan's multiple range test were performed using STATISTICA 12.5 for Windows (StatSoft Inc., Tulsa, USA).

3. Results

3.1. Fresh yield, dry matter, and water consumption

Table 1 summarizes the effects of EC and N:K ratio on the leaf fresh weight for the three lettuce cultivars (BH, LB, and LR) across the two experimental cycles. No significant differences in above-ground fresh

Table 3
Impact of EC and N:K ratio on leaf K, Ca, Mg and P concentrations expressed as mg per g of dry matter (DM) in three different cultivars (BH, LB, LR) (data pooled over the two experiments).

		K (mg g^{-1})			Ca (mg g^{-1})		
		BH	LB	LR	BH	LB	LR
EC	Standard	70.53	69.80	78.65	18.00	19.70	19.24
	Low	59.77	59.51	66.21	15.85	17.40	16.96
N:K	Standard	64.33	63.77	71.62	16.79	18.41	17.97
	Low	65.96	65.54	73.24	17.06	18.69	18.23
		Statistical significance					
EC		***	***	***	***	***	***
N:K		NS	NS	NS	NS	NS	NS
EC x N:K		NS	NS	NS	NS	NS	NS
		Mg (mg g^{-1})			P (mg g^{-1})		
EC	Standard	3.32	3.53	3.37	9.33	8.97	10.49
	Low	3.20	3.28	3.16	9.12	8.77	10.35
N:K	Standard	3.26	3.45	3.30	9.15	8.79	10.35
	Low	3.27	3.36	3.22	9.31	8.95	10.48
		Statistical significance					
EC		NS	NS	NS	NS	NS	NS
N:K		NS	NS	NS	NS	NS	NS
EC x N:K		NS	NS	NS	NS	NS	NS

In each column, means (n = 4) followed by different letters are significantly different according to the ANOVA test and the Tukey Honest Significant Difference test ($p \leq 0.05$). Regarding the variance components, according to associated P values, *** ($p \leq 0.001$), ** ($p \leq 0.01$), and * ($p \leq 0.05$) represent statistical significance, while NS signifies non-significance. BH: butterhead (cv. Fairly), LB: lollo bionda (cv. Jokary) and LR: lollo rossa (cv. Lea).

biomass were observed among treatments, irrespective of the EC level or the N:K ratio. Similarly, the above-ground dry biomass remained unaffected by the treatments across cultivars (Table 1). The leaf dry matter content in BH ranged from 3.7 to 3.9 %, while for LB it was 4.2–4.5 % and for LR 4.7-it reached 4.9 %. Cumulative plant water uptake (Fig. 1) remained unaffected by the treatments ranging from 3.94 to 4.12 L per plant over the two experiments (data pooled).

3.2. Total nitrogen and leaf nitrate content

The effects of EC and N:K ratio levels on total-N concentration (mg g^{-1}) and leaf NO_3^- content (mg NO_3^- kg^{-1} fresh weight are presented in Table 2. Both parameters were significantly influenced by both the EC and the N:K ratio, indicating the crucial role of N supply in NO_3^- accumulation within plant tissues. Standard EC (2.5 dS m^{-1}) and N:K ratio (3.28) levels in the RS resulted in significantly higher total-N and NO_3^- concentrations. The highest NO_3^- accumulation occurred under standard EC and high N:K ratio, while it was also cultivar-dependent, reaching 2360 mg kg^{-1} in BR, 2103 mg kg^{-1} in LB and 2421 mg kg^{-1} in LR. Lower EC (2.0 dS m^{-1}) and N:K ratio (2.28) ratios reduced nitrite levels by 30–33 % in all three lettuce cultivars. The statistically significant interaction between EC and N:K ratio concerning the NO_3^- concentration in lettuce leaves indicates that this was affected mainly by the NO_3^- concentration in the NS rather than the molar N:K ratio.

3.3. Nutrient concentrations in leaves

Leaf concentrations of K^+ and Ca^{2+}, were significantly influenced by the EC levels of the NS, as shown in Table 3. Both ions showed higher values under standard EC level (2.5 dS m^{-1}). Specifically, the K^+ concentration decreased by 15.35 % in BH, 14.7 % in LB and 15.8 % in LR under the low EC treatment, while the Ca^{2+} concentration decreased by 11.9 %, 11.7 % and 11.9 %, respectively. However, different N:K ratios did not impose any changes in K^+ and Ca^{2+} in leaf tissue. Furthermore, the Mg^{2+} leaf concentrations (Table 3) showed no significant differences between treatments, remaining stable with mean values ranging from 3.20 to 3.32 mg g^{-1} for BH, 3.28 to 3.53 mg g^{-1} for LB and 3.16 to 3.37 mg g^{-1} for LR. Furthermore, the leaf tissues P concentrations also were not affected by either treatment, with mean values ranging from 9.12 to 9.33 mg g^{-1} for BH, 8.77 to 8.97 mg g^{-1} for LB and 10.35 to 10.49 mg g^{-1} for LR.

3.4. Nitrogen uptake concentrations

The effects of EC, N:K ratio and calculation method on nitrogen UCs are presented in Fig. 2. The mass-balance method resulted in significantly higher N UC (17.74 mM) compared to the estimations from plant tissue analysis (17.05 mM), indicating methodological discrepancies. Significant differences were also observed for the main effects of EC and N:K ratio on N UC. Standard EC (2.5 dS m^{-1}) and N:K molar ratio (3.28) resulted in higher nitrogen UC (18.57 and 18.52 mM, respectively), compared to those measured at low EC (2.0 dS m^{-1}) and N:K (2.28) (16.22 and 16.28 mM, respectively).

3.5. Nutrient uptake concentrations

The UC calculated for the current study revealed significant differences for P, Ca and Fe between the two calculation methods. The UC of P calculated according to the mass balance method was 1.99 mM, while the respective value calculated based on plant tissue analysis yielded a value of only 1.65 mM (Fig. 3A). Similarly, the UC was 2.90 and 2.60 mM for Ca, and 58.6 and 48.9 mM for Fe, using the mass balance and the plant tissue methods, respectively (Fig. 3B-C). The impact of EC level on the UC of K and Ca is presented in Fig. 3D The UC of K was higher under standard EC treatment (11.78 mM), compared to the UC under low EC treatment (10.48 mM). Similarly, the Ca UC in the treatments with standard EC was 2.87 mM and with low EC 2.63 mM (Fig. 3E). Regarding the impact of N:K ratio on the UC of nutrients, only Cl uptake was affected by the replenishment of NO_3^- with Cl^-, with an uptake of 1.85 mM in low N:K treatment, while in the standard N:K treatments the UC remained significantly lower at 0.13 mM (Fig. 3F). In Table 4, the UCs of the other nutrients are presented, which were not significantly influenced by the treatments or the calculation methods. The Mg UC ranged from 0.85 to 0.95 μM, while the UCs for the micronutrients Mn, Zn, Cu and B were in the ranges of 4.89–5.28, 3.77–3.88, 0.55–0.64 and 31.97–33.80 μM, respectively.

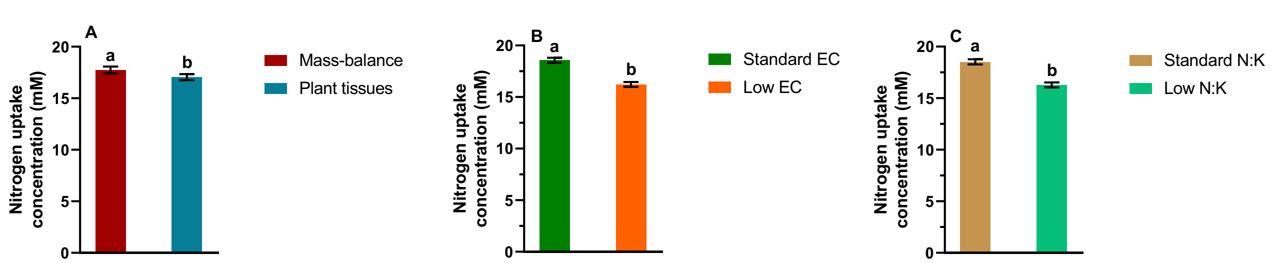

Fig. 2. Impact of EC and N:K ratio on nitrogen uptake concentrations (mM) as estimated using two calculation methods,. Different letters indicate significant differences between means according to the ANOVA test and the Tukey Honest Significant Difference test ($p \leq 0.05$). Vertical bars indicate ±standard errors of means (data pooled over the two experiments).

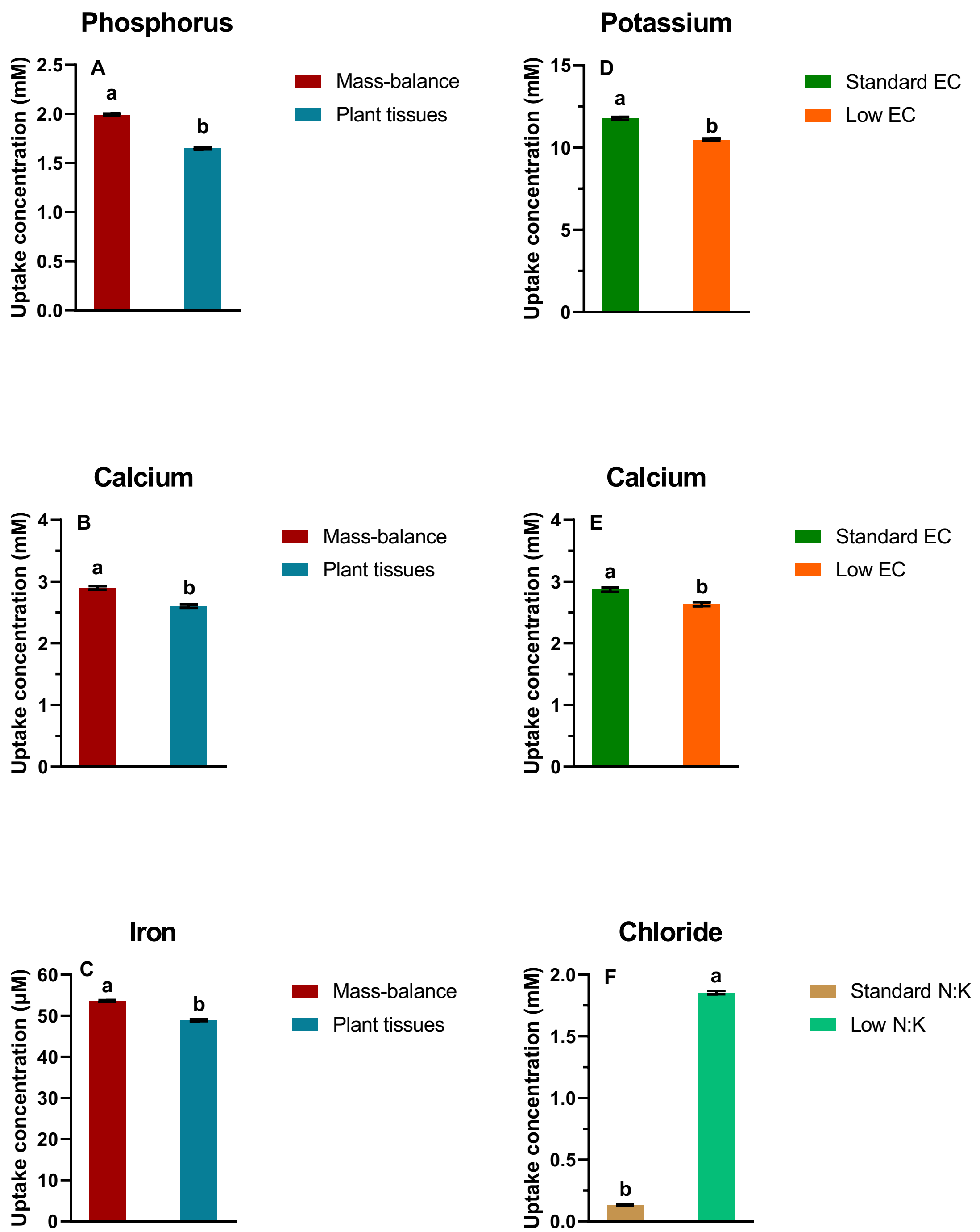

Fig. 3. Impact of the i) calculation method on uptake concentrations of P, Ca (mM) and Fe (μM), ii) EC level on uptake concentrations of K and Ca (mM) and iii) N:K ratio on uptake concentration of Cl. Different letters indicate significant differences between means according to the ANOVA test and the Tukey Honest Significant Difference test ($p \leq 0.05$). Vertical bars indicate ± standard errors of means (data pooled over the two experiments).

3.6. *Agronomic efficiency of nitrogen*

The AE_N, expressed as kg of fresh yield per kg of used N, was significantly influenced by both the EC and the N:K ratio in the NS, in BH and LB lettuces (Fig. 4). In BH, the AE_N efficiency increased under low EC by 15 % (287.1 vs 249.3 kg^{-1}) and under low N:K ratio by 18 % (290.3 vs 246.1 kg^{-1}). Similarly, in LB the AE_N increased by 17 % in the low EC treatments reaching a level of 201.7 kg^{-1}, and by 18 % at the low N:K ratio reaching a similar level (202.4 kg^{-1}). In LR, the low N:K ratio significantly increased the AE_N by 21 % (175.9 kg^{-1}) compared to the high N:K ratio, while the EC had no significant impact on the AE_N.

4. Discussion

This study investigated the potential of reducing N input and EC in closed-loop hydroponic lettuce production without compromising yield. Previous studies have successfully tested similar approaches involving reduced nutrient levels, particularly N, in the root zone solution for

Table 4
Impact of EC and N:K ratio on plant nutrient uptake concentrations as estimated using two alternative calculation methods (data pooled over the two experiments).

Method	EC	N:K	P	K	Ca	Mg	Cl	Fe	Mn	Zn	Cu	B
Mass balance	Standard	Standard	1.99	12.01	3.04	0.95	0.11	53.39	5.28	3.88	0.58	33.62
	Standard	Low	1.96	11.88	3.01	0.87	1.87	53.80	5.20	3.88	0.55	33.49
	Low	Standard	2.01	10.44	2.79	0.88	0.14	53.79	5.19	3.88	0.55	33.80
	Low	Low	2.00	10.50	2.75	0.88	1.87	53.39	5.05	3.88	0.59	33.49
Plant tissue	Standard	Standard	1.66	11.74	2.75	0.89	0.14	48.83	5.14	3.77	0.60	31.97
	Standard	Low	1.63	11.50	2.68	0.90	1.85	48.92	5.05	3.77	0.60	32.99
	Low	Standard	1.67	10.40	2.49	0.85	0.15	49.11	5.06	3.78	0.64	33.60
	Low	Low	1.65	10.57	2.50	0.86	1.83	48.93	4.89	3.77	0.57	32.42
Statistical significance												
Method			***	NS	***	NS	NS	***	NS	NS	NS	NS
EC			NS	***	***	NS	NS	NS	NS	NS	NS	NS
N:K			NS	NS	NS	NS	***	NS	NS	NS	NS	NS
Method X EC			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Method X N:K			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
EC X N:K			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Method X EC X N:K			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

In each column, means ($n = 4$) followed by different letters are significantly different according to the ANOVA test and the Tukey Honest Significant Difference test ($p \leq 0.05$). Regarding the variance components, according to associated P values, *** ($p \leq 0.001$), ** ($p \leq 0.01$), and * ($p \leq 0.05$) represent statistical significance, while NS signifies non-significance.

vegetable crops (Cedeño et al., 2023; Medrano et al., 2019). Our results demonstrated that the tested lettuce cultivars were able to grow effectively in NS with reduced N, K, Ca, and Mg concentrations, corresponding to reduced root-zone EC but similar mutual molar ratios, without compromising yield. Sonneveld and Van Der Burg (1991) suggested that a NS with an EC of 1.5 dS m^{-1} is sufficient to meet the nutrient requirements of fruiting vegetables when the mutual ratios between the macronutrients are optimal. For lettuce, the maximum recommended EC in the root zone of the plants is 2.6 dS m^{-1} (Sonneveld and Voogt, 2009), beyond which salinity stress may occur. Conversa et al. (2021) reported a 20 % yield reduction due to an increase of the EC from 2.5 to 3.5 dS m^{-1}, indicating the need to maintain root-zone EC below critical levels. In tomato and sweet pepper, Giannothanasis et al. (2024) and Voogt et al. (2021, 2023), respectively, have shown that maintaining the EC threshold by reducing the nutrient concentrations to compensate for Na^+ and Cl^- accumulation in closed soilless cropping can be a viable strategy for extending NS recirculation, while preventing salinity stress. The results of the current study show that this strategy might be successfully applied also for lettuce production in hydroponic systems, if the Na^+ concentration in the raw water used to prepare NS is suboptimal (1 to 3 mM).

The K and Ca concentrations in lettuce leaves were influenced only by the EC level of the NS, while the leaf total-N content was influenced by both the EC and the N:K ratio without interaction between the two experimental factors. In all treatments, the leaf macronutrient concentrations measured, which ranged from 25–50 mg g^{-1} for total-N, 29–78 mg g^{-1} for K, 14–30 mg g^{-1} for Ca, and 2.5–4.5 mg g^{-1} for Mg, were within the sufficiency range for optimal growth suggested by Hartz et al. (2007). Sonneveld and Voogt (2009), reported similar optimal ranges for total-N, K and Mg, though lower for Ca (8–12 mg g^{-1}), suggesting that lower Ca concentrations may induce tip-burn. The maintenance of nutrient concentrations within the optimal range for plant growth across all treatments agrees with the lack of any yield differences between them. The absence of tip-burn in the lettuce irrespective of the treatment indicates that the leaf Ca concentrations remained adequate even in the treatment with the lowest Ca supply. This finding agrees with previous suggestions that other factors beyond the Ca concentration in the root zone are the main causal factors for tip burn (De Freitas and Mitcham, 2012). This notion is further supported by Ferrarezi et al. (2024), who found no tip-burn in lettuce with leaf Ca concentrations of 12.7–14.5 mg g^{-1}, which are lower than those found in the current study.

It is well known that increasing the N availability in the root zone typically enhances N uptake (White, 2012). Indeed, in the present study the treatments with lower N concentration in the NS exhibited lower N UC. Specifically, the combined application of low EC and reduced N:K molar ratio decreased the UC of N by 13 % compared to the standard treatments. The reduced N UC resulted in lower N input requirements in both low N treatments, thereby reducing the fertiliser consumption without negatively affecting yield, and concomitantly increasing the AE_N by 15–21 %. These results pave the way for application of cultural practices in soilless cropping systems that can substantially contribute to achieving the EU goals for reducing nutrient losses and fertiliser consumption in agriculture by 50 % and 20 %, respectively (European Green Deal, 2019). Additionally, the elevated Cl^- UC observed when Cl^- partially replaced NO_3^- in the NS aligns with its passive absorption via channels in plant cells membrane through a mass-flow uptake mechanism (Raven, 2017; White, 2012), which increases proportionally with the root-zone Cl^- concentrations. In support of this, Neocleous et al. (2024) demonstrated an exponential increase in Cl^- uptake with elevated Cl^- concentration in the root zone when NO_3^- was partially replaced by Cl in hydroponically grown lettuce.

Differences in K^+ and Ca^{2+} UC were related to the respective concentration differences in the root environment. Luxury consumption of K when supplied in excess, is a well-documented response (Hawkesford et al., 2012). The higher Ca UC observed under standard EC conditions is likely due to its primary uptake mechanism, where uptake increases with transpiration through mass-flow (White, 2012). Sonneveld and Voogt (2009) suggested a K^+ UC of 11 mM for hydroponic lettuce crops, which is consistent with those found in the current study, and 4 mM Ca^{2+}, which is lower than those found here. However, recent findings from Neocleous et al. (2024) under Mediterranean climatic conditions report even lower Ca^{2+} UC (2.85 mM using the plant tissue method and 3.13 mM using the mass-balance method). Notably, under low nutrient concentrations, corresponding to an EC of 1.2–1.6 dS m^{-1}, Vought et al. (2024) found that NO_3^- and K^+ tend to deplete, while Ca^{2+} to accumulate, suggesting that actual Ca^{2+} UC may often be lower than the standard recommendations of Sonneveld and Voogt (2009). Liu et al. (2025) and Pace and Williams (2024) report similar results for NO_3^-, K^+ and Ca^{2+} concentrations in the root zone, highlighting the importance of precise nutrient management in closed-loop hydroponic systems for avoiding nutrient imbalances.

Nutrient UCs calculated using the mass-balance method correspond to nutrient losses from the soilless culture system (apparent UCs), whereas those calculated via the plant tissues method correspond to the net nutrient absorption by the plant (Cedeño et al., 2024; Xaxiri et al., 2023). The significantly higher UC of N, when estimated through the mass-balance method, are presumably due to N losses through gaseous pathways including denitrification as N_2 or N_2O as well as volatilization

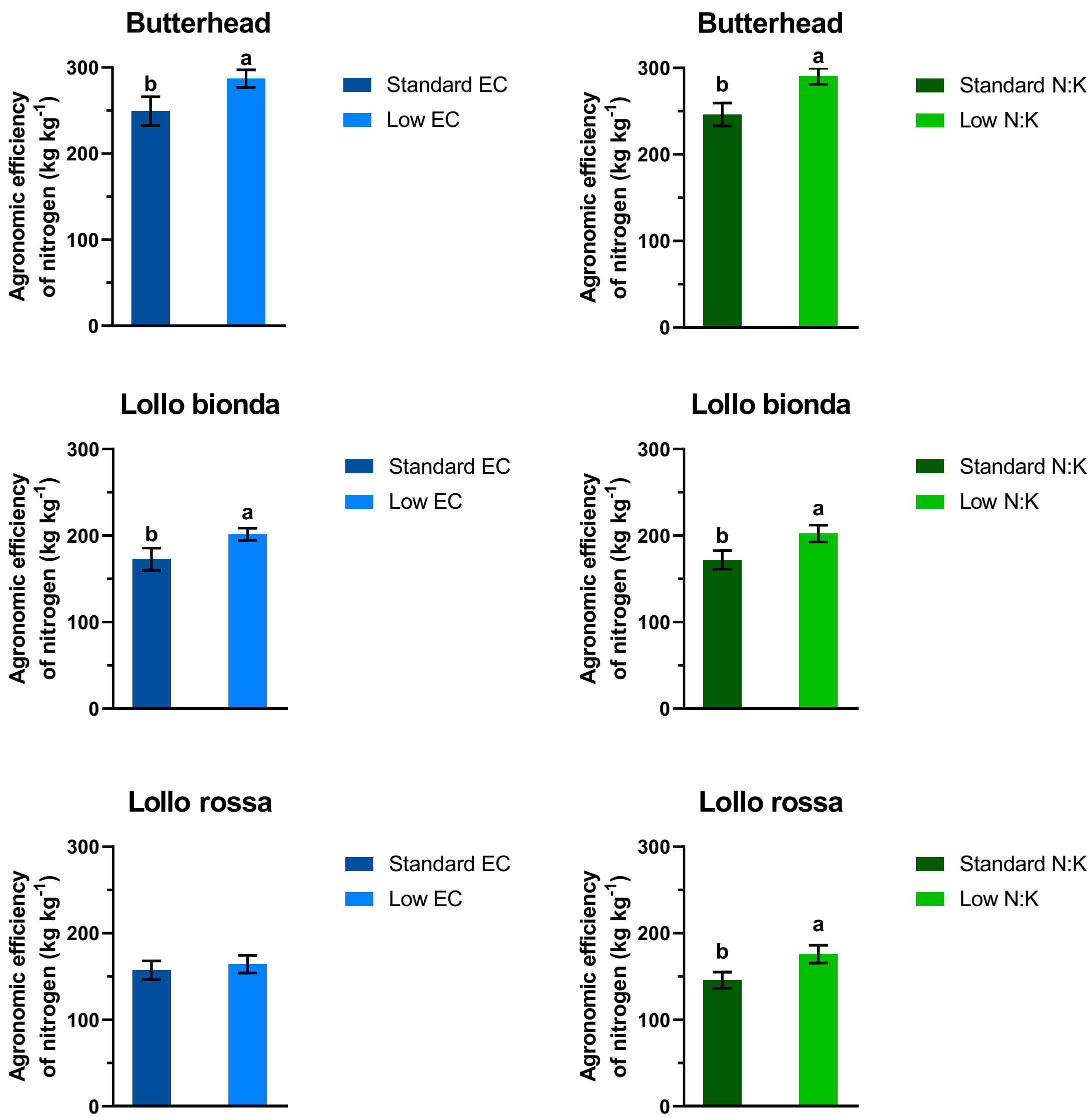

Fig. 4. Impact of EC and N:K ratio on agronomic efficiency of nitrogen (kg^{-1}). Different letters indicate significant differences between means according to the ANOVA test and the Tukey Honest Significant Difference test ($p \leq 0.05$). Vertical bars indicate ± standard errors of means (data pooled over the two experiments).

losses in the form of ammonia (Karlowsky et al., 2023; Lin et al., 2023; Llorach-Massana et al., 2017; Pitton et al., 2021). In the case of the Ca UC, the significant differences recorded between the two calculation methods can be attributed to Ca^{2+} precipitation due to formation of sparingly soluble salts with P and SO_4^{2-} ions (Cerozi and Fitzsimmons, 2016; De Rijck and Schrevens, 1998). Similar differences in the Ca^{2+} UC calculated with these two methods were also reported by Xaxiri et al. (2023) in a floating hydroponic system, by Neocleous et al. (2024) in a nutrient film technique lettuce production, and by Cedeño et al. (2024) in a substrate soilless system. This study also revealed also for Fe significant differences in the UC calculated with the two different methods. Similar differences have been reported previously by other researchers (Neocleous et al., 2024; Xaxiri et al., 2023), which are ascribed to analytical limitations, i.e. Fe losses during the extraction procedure in plant tissues analysis, or to Fe precipitation in form of iron phosphate salts. The quantification of micronutrients like Fe in dried plant material can vary significantly depending on the digestion technique used, particularly with respect to ashing temperature and duration, which can lead to fluctuations in the detected concentrations (Hoenig, 2005).

Based on the results of this study, practical recommendations can be provided to growers aiming to optimise lettuce yield and quality while minimising fertiliser use. Growers can adopt the nutrient strategy of the T4 treatment, characterized by reduced EC (2.00 dS m^{-1}) and a lower N:K ratio (2.28). This strategy sustains growth while in parallel reduces nutrient use and input costs. To apply this strategy commercially, the growers need to use a starter NS with the composition of the RS in T4, with an EC of 2.0 dS m^{-1} and a N:K ratio of 2.28. This composition should be also the target composition for the root solution (RS) during the cultivation of the lettuce plants. To replenish the water and nutrients absorbed by the plants, the AS supplied during the cultivation to compensate for plant uptake must have a nutrient concentrations equal to the nutrient UCs found for T4 in the current study. Nevertheless, the RS should be regularly monitored, and the AS composition must be adapted accordingly to maintain optimal conditions in the root zone using suitable decision support systems for the calculations (Savvas et al., 2023). The frequent adaptation of the composition of the AS allows for balancing cultivar differences in UC. This fertigation scheme ensures balanced lettuce nutrition, sufficient nutrient concentrations in plant tissues and reduced NO_3^- concentrations in edible plant parts, while the recycling of the NS by growing the plants in closed-loop hydroponic systems prevents nutrient losses to the environment.

5. Conclusions

This study showed that reducing nitrogen, along with potassium (K), calcium (Ca), and magnesium (Mg) concentrations thereby lowering the electrical conductivity (EC) of the root solution in hydroponically grown lettuce, significantly decreases nitrate accumulation in the leaves, while maintaining yield performance. The partial substitution of NO_3^- with Cl^- proved effective without any negative impact on the crop, as Cl^- at the applied concentrations functioned as an osmotic agent without inducing toxicity or any other stress to the plants. The current study revealed that the N, Cl, K, Ca and Fe uptake concentrations were influenced by EC, N:K ratio and the calculation method, with the mass-balance method of calculation consistently estimating higher values, presumably due to nutrient losses from the NS. These results confirm that optimised fertigation strategies based on lower N:K ratios and EC levels, adjusted to actual plant requirements, can improve nutrient efficiency and food safety by reducing nitrate accumulation. For practical application, fertigation programs for hydroponic lettuce should prioritize moderate EC levels combined with reduced N:K ratios, thereby lowering fertiliser inputs without compromising the commercial yield. This approach not only supports sustainable lettuce production but also contributes to the development of environmentally responsible fertilization practices in modern agriculture.

CRediT authorship contribution statement

Evangelos Giannothanasis: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Theodora Ntanasi:** Writing – original draft, Methodology, Investigation, Data curation. **Ioannis Karavidas:** Writing – review & editing, Validation. **George P. Spyrou:** Writing – original draft, Visualization. **Damianos Neocleous:** Writing – review & editing, Conceptualization. **Georgia Ntatsi:** Writing – review & editing, Supervision, Conceptualization. **Dimitrios Savvas:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The co-author Georgia Ntatsi is an Associate Editor of Scientia Horticulturae and the corresponding author Dimitrios Savvas is member of the Editorial Advisory Board. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2025.114458.

Data availability

Data will be made available on request.

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