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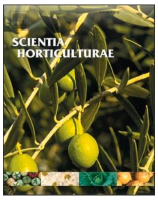

Research Paper

Reducing nitrate accumulation through the management of nutrient solution in a floating system lettuce (*Lactuca sativa*, L.)

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ABSTRACT

Lettuce is a leading greenhouse-grown vegetable highly appreciated by consumers for its nutritional properties texture and flavor. However, leafy vegetables are known to accumulate an excessive amount of nitrate and this constitutes a strong health concern in Europe. Among the factors that most affect the yield and quality of hydroponic crops, the composition and management of the nutrient solution stand out. In this work, different strategies have been studied in the management of the nutrient solution for hydroponic lettuce cultivation. Production nutritional status, and quality parameters were evaluated. The concentrations of nutrients in the nutrient solution significantly influence the harvested product, 100 to 50 % H treatment resulted in highest yields with significant increase of 14 % compared to 50 % H at 34 DAT, from the point of view of both biomass production and quality, regarding the contents of anthocyanins, chlorophylls, and calcium, constituents that add value to the product due to their importance in the human diet. A significant reduction of nitrates in lettucewas observed one (14 %) and two (22 %) weeks after modifying the nutrient solution (100 to 50 % H). After one week there was no reduction in lettuce growth. In addition, no significant reduction in the concentration of most macro- and micronutrients was observed.

1. Introduction

One of the major advantages of hydroponics is the precise control of the nutrient solution, which can be modified several times throughout the growing cycle, optimizing the nutrient supply according to the vegetative cycle of the crop, improving yield and product quality (Velazquez-Gonzalez et al., 2022). Currently, hydroponic leafy vegetable cultivation is gaining popularity all over the world because of efficient resource management and quality food production (Sharma et al., 2018). Lettuce (*Lactuca sativa*, L.) is one of the most important vegetable crops. World's production was 27,660,187 Mt and the cultivated area was 1226,370 ha (FAOSTAT, 2024). The main producers are China, India, and Spain. The increase in production in the last two decades (2000–2020) has been 50 %. The explanation is that lettuce is considered a moderate source of important antioxidant and health-related molecules, which can provide important traits related to the nutritional quality of these foods. These compounds include phenolics, anthocyanins and carotenoids, among others (Simko, 2019). Its regular consumption improves protection against cancer, cardiovascular diseases, and other chronic diseases (Nicolle et al., 2004). It is eaten in salads, as a garnish in many dishes, or in sandwiches, highlighting the constant growth in the consumption of fresh-cut salads (Damerum et al., 2020; Stuart, 2011) due to changes in consumption habits; hence, there is an increased demand for lettuce with a variety of colors, textures, and flavors.

Nitrogen (N) is one of the most important plant nutrients and the main source of nitrogen in hydroponics systems are nitrates (calcium and potassium nitrate). N is the most yield limiting nutrient and plays an important role in the quality of leafy vegetables (Thapa et al., 2022), being an integral part of protein and chloroplast structure and its function (Barker and Bryson, 2007). Moreover, hydroponic crop yield is directly related to mineral nutrient supply during critical growth stages (Mahlangu et al., 2016) and lettuce requires a high rate of nitrogen for growth and development (Yoldas et al., 2008). On the other hand, an excessive N application can result in negative quality and nutritional features such as the accumulation of nitrates in edible tissues

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(Santamaria, 2006). In this sense, lettuce grown as baby leaves, especially when grown without soil even in nutrient mixtures with only small quantities of nitrates tend to accumulate large amounts of nitrate in vacuoles (Guffanti et al., 2022). Nitrates have low toxicity to humans; however, the higher risk for human health is related to the nitrate reduction to nitrite in the mouth and stomach. On the one hand, nitrite can react with other compounds as amines or amino acids contained in food, leading to nitrosamine formation, some of which are considered carcinogenic if consumed excessively (Ciriello et al., 2021). The nitrate accumulation in leafy vegetables is highlighted in EU Regulation 1258/2011, which sets upper limits on nitrate levels in lettuce depending on the growing season (European Union 2011): 5000 mg/kg fresh mass (FM) in winter and 4000 mg/kg FM in summer for lettuce grown.

There are several factors promoting the accumulation of nitrates in a protected environment cultivation system, including low light availability, light quality variation, high water availability, high temperatures, pH, aeration management (high CO_2 level) and nutrient solution quality (Guffanti et al., 2022). In an essay with hydroponic lettuce, Ciriello et al. (2021) were able to reduce nitrate tissue concentration by reducing the electrical conductivity (EC) of the nutrient solution by adding water or completely substituting the nutrient solution with water in the last days before harvest can help in reducing the amount of nitrate in leaves. However, this operation could negatively affect the plant growth rate and negatively affect the final product quality (Umar and Iqbal, 2007). The application of three different concentrations of nitrogen (7.14, 10.71, and 14.29 mmol/L), showed increases in the fresh biomass and dry matter production, and in the foliar concentrations of nitrate, potassium, and magnesium, with the in-crease in the nitrogen supply (Petropoulos et al., 2016). The effect of a reduction in the nitrogen concentration in the nutrient solution (NS) on the productive development of the plant and the final concentration of nitrogen in the leaf has been studied (Tsouvaltzis et al., 2020). This work concluded that the production of high-quality baby-size lettuces grown on floating systems is feasible, provided that proper N concentration based on the type of lettuce to be grown. In addition, the authors concluded that the N reduction in NS did not cause a decrease in the foliar content of nitrogen, potassium, phosphorus, calcium, magnesium, or manganese, because an amino acid supplement was included in the latter treatment. In the last few years, numerous fertilizer management strategies have been development in order to reduce nitrate concentration in leafy vegetables grown in indoor hydroponic system. Decreasing nitrate concentrations in nutrient solutions has shown to notably reduce nitrate buildup in hydroponically grown lettuce (Liu and Yang, 2012; Wang and Shen, 2011; Bian et al., 2020). Nevertheless, reducing nitrate levels through restricted nitrate supply can lead to diminished yields, particularly when nitrate deficiency occurs without simultaneous adjustments to other environmental factors impacting plant development and nitrate absorption (Fu et al., 2017).

Despite numerous studies addressing nitrate accumulation in leafy vegetables and strategies for its mitigation (Bian et al., 2020), there is still a lack of comparative studies examining how variations in nutrient concentration throughout the crop cycle can effectively reduce nitrate content without compromising crop yield and quality. Our work addresses this gap by evaluating nutrient solution management. Our hypothesis state that a controlled reduction of nutrient solution concentration in the final phase of the vegetative cycle can significantly decrease the nitrate concentration in the harvested plant.. Therefore, the aim of the present study was to evaluate different nutrient solution management strategies, for reducing the nitrate content in lettuce grown in a hydroponic system maintaining crop yield and overall quality. In addition, these strategies not only will sustains crop quality and yield, but it also enhances nutrient utilization, thus fostering more sustainable agricultural practices and enhancing human health safety

2. Materials and methods

2.1. Experimental set-up

The experiment was carried out in a greenhouse module of the Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) located in Santomera (Murcia, Spain), with coordinates 38° 6′ 26″ N and 1° 2′ 7″ W. The module used has an area of 462.5 m^2 (18.5 × 25.0 m), it is made of polycarbonate and the height of the channel is 5.0 and that of the ridge is 7.5 m. The greenhouse is oriented north-south. Inside the module, sensors monitor the environmental temperature, relative humidity, and radiation. For climate control, it has butterfly-type overhead ventilation with an automatic opening and closing system; a shade screen, and an automatic opening and closing system; an evaporative panel with four extractors protected with automatic opening-closing shutters; fog-system installation, and a pressure group (JH 15 5 M) of 25 L (1 HP) with a 250 L accumulator, and a 4 HP air compressor. Throughout the experiment, the evaporative panel was used for cooling and the destratifiers were used to improve the homogeneity of the interior climate.The mean values of the daytime and nighttime temperatures were 21.2 °C and 14.6 °C respectively.

The NS was automatically prepared in a 2000 L capacity mixing tank. Fertilizers were added in appropriate amounts to achieve the final nutrient concentration. Six concentrate fertilizer tanks were utilized, containing potassium nitrate, calcium nitrate, monopotassium phosphate, magnesium sulfate, micronutrients and iron, and nitric acid. Deionized water from a desalination plant (model HRO-20-P, HIDRO-TEC, Cartagena, Spain) was used. The pH was adjusted to 5.5 with nitric acid. Once the NS was ready, it was transferred to three storage tanks, each with a capacity of 900 L (one for each treatment).

Plants were cultivated in a floating culture system, consisting of a table with a tray (Stal and Plast A/S) measuring 205.0 × 122.0 × 4.5 cm for length, width, and depth, respectively, with a capacity of 112.5 L. A recirculation system was employed to maintain a constant volume of the tray throughout the experiment. To renew the NS in the tray, a submersible pump (SICCE brand MULTI 5800 L/h model) was installed in a 500 L reservoir tank. The pump was programmed for 15 min on and 10 min off during the culture cycle. When the reservoir tank's volume dropped below 200 L, the NS was automatically transferred from the 900 L capacity storage tank.

2.2. Plant material and germination conditions

Seeds of the lettuce (*Lactuca sativa*, L., var. secalina cv. E01.30190), supplied by Enza Zaden (Enkhuizen, The Netherlands), were used. This type of lettuce that is highly appreciated by consumers for its red color and slightly bitter taste, and is widely consumed in salads combined with sprouts of different cultivars and other leafy vegetables.

Seed germination took place at an average temperature of 18 °C and an average relative humidity of 80 %, in dark conditions. Each seed was placed in a cylinder of rock wool (diameter × height = 2 × 3 cm), inserted in polystyrene trays (length × width = 60 × 40 cm) with a capacity for 240 cylinders per tray. The cylinders were thoroughly soaked in a 50 % Hoagland NS. The pH and EC were 5.5 and 1.4 dS/m, respectively. The development of the seedlings took place on the hydroponic cultivation tables in the greenhouse module, irrigating them with 100 % Hoagland NS and with a renewal of the NS every 30 min. The definitive transplanting was carried out using expanded polystyrene plugs with a central hole into which the rock wool plugs were inserted. The plants were placed in 120 cm × 50 cm × 3 cm extruded polystyrene trays (Chovafoam T-III), placing four of these trays on each of the tables.

2.3. Experimental design

The treatments used consisted of i) Modified Hoagland NS (Epstein, 1972) throughout the crop cycle ("100 % H"); ii) Hoagland NS at 100 %

at the beginning of the cycle, changed to a Hoagland NS diluted to 50 % (“100 to 50 % H”) 20 days after transplanting (14 days before commercial size), and iii) Hoagland NS diluted to 50 % (“50 % H”) throughout the cycle (Tables 1, and 2). When “50 % H” is used, the NS of Table 1 will be adjusted to 50 %. In both NS (“100 % H” and “50 % H”), the concentration of micronutrients is always the same, as in Table 2.

At 27 days after transplanting (DAT),7 days before commercial size, a harvest of 30 lettuces was made, ten per treatment. The plants were regrouped to maintain a density of 16 plants/m^2. The fresh weights of the aerial parts and the roots were determined, together with the length of the stem, and a fraction of the leaves and the entire root system were dried for subsequent mineral analysis. At 34 DAT (commercial size) ten plants per treatment were harvested. The fresh weights of the aerial parts and the roots, the length of the stem, and the leaf color were determined. Samples were also taken for mineral, anthocyanin, and photosynthetic pigment analysis.

Throughout the experiment, the values of temperature (°C), pH, EC (dS/m), and dis-solved oxygen (mg/L) in the NS deposits were measured three times a week, using a digital multimeter (HACH HQ40D digital two-channel, Colorado, USA). If the pH was not optimal (between 5.5 and 6), it was adjusted by adding nitric acid (1 M) or sodium hydroxide (1 M). The NS of the storage tanks (900 L) was disinfected weekly by adding 100 mL of hypochlorous acid, to maintain 1 ppm of free chlorine. In addition, for the nutritional re-balancing of the NS, a 100 mL sample of NS was taken weekly from, each of the deposits. With the results of the anion and cation analyses, deficiencies with respect to the original composition of the NS were identified and corrected.

2.4. Determination of the production parameters

The fresh weight (FW, g/plant) was measured for the aerial parts and the roots at the samplings carried out 27 and 34 DAT, in 10 lettuces per treatment, using a precision balance with an uncertainty of ±0.01 g (RADWAG WLC 2/A2, Warsaw, Poland). It was also determined at 8 and 16 DAT in five lettuces per treatment, to evaluate the evolution of FW during the crop cycle. The productive yield per unit surface area (g/m^2) was calculated at 27 and 34 DAT, from the FW values of the aerial parts.

The dry matter production per plant was measured at 27 and 34 DAT, in 10 lettuces per treatment, for both the aerial parts and the roots. The samples were left in a drying oven (MEMMERT model UF1060, Schwabach, Germany) at 70 °C for 72 h. The dry matter content of the aerial parts and the roots and the water content (%) were determined. The stem length was determined by cutting the lettuce longitudinally and subsequently measuring it with a ruler. It was measured at 27 and 34 DAT, in six lettuces per treatment.

2.5. Mineral analysis

The analyses were carried out in the aerial parts and the roots of six lettuces per treatment, for the samplings carried out at 27 and 34 DAT. The dried plant material was ground in a blade mill (IKA a10 basic, 25,000 rpm. IKA-Werke GmbH and Co. KG, Stau-fen, Germany). For the determination of cations, digestion was carried out for a sample of 0.1 g, weighed on an analytical balance (GRAM FS-120, Barcelona, Spain), with a solution containing 5 mL of nitric acid (HNO_3) and 3 mL of hydrogen peroxide (H_2O_2). The digestion of the organic matter was carried out in a microwave oven (CEM Mars X, Bucking-hamshire, UK) programmed in stages, in which a temperature of 200 °C was reached in 20 min and maintained for 2 h. Once the residue resulting from the digestion had cooled to room temperature, each digester tube was filled to 25 mL with milliQ water and the samples were stored in scintillation vials until analysis. For the determination of anions, 0.05 g of sample was homogenized with 10 mL of deionized water and then incubated under continuous stirring for 30 min at 25 °C. The samples were then centrifuged at 10,000 g for 20 min (Hettich, model UNIVERSAL 320. Andreas Hettich GmbH and Co., Tuttlingen, Germany). The supernatants were collected and filtered (0.45 μm pore size, CHROMAFI Xtra PA-45/25), and an aliquot was used for anion analysis. The cation and anion extracts were analyzed using an ICP-OES (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA).

Table 1
Concentrations of salts and macronutrients used in the preparation of the NS (“100 % H”).

Compound	Concentration (mM)	Element	Final Concentration (mM)
KNO_3	6	N	14
$Ca(NO_3)_2\ 4H_2O$	4	K	7
KH_2PO_4	1	Ca	4
$MgSO_4\ 7H_2O$	1	P	1
		S	1
		Mg	1

Table 2
Concentrations of salts and micronutrients used in the preparation of the NS.

Compound	Concentration (μM)	Element	Final Concentration (μM)
H_3BO_3	25.00	B	25.00
$MnSO_4\ H_2O$	2.00	Mn	2.00
$ZnSO_4\ 7H_2O$	2.00	Zn	2.00
$CuSO_4\ 5H_2O$	0.50	Cu	0.50
$(NH_4)6Mo7O_{24}\ 4H_2O$	0.071	Mo	0.50
Fe-EDTA	20.00	Fe	20.00

2.6. Determination of the quality parameters

The anthocyanins content was measured in six lettuces per treatment at 34 DAT. A 95 % ethanol:1.5 N HCl (85:15 ratio) extractant was prepared and 1.5 mL was used to ex-tract the anthocyanins from 300 mg of fresh plant material. The absorbance at 530 nm and 657 nm was measured in a UV/Vis spectrophotometer (PowerWave XS2, BioTek, Winoo-ski, Vermont, USA).

The color parameters were measured at 34 DAT. For each treatment, 18 measurements were made on three lettuces (six per lettuce). A tristimulus colorimeter (Minolta CR400, Osaka, Japan) was used, which records the parameters L, luminosity, a*, green-red, b*, blue-yellow, C, chroma or saturation, and h, hue (McGuire, 1992). For each lettuce, three measurements were made in external leaves and three in internal leaves, selecting healthy leaves, without symptoms of chlorosis or damage, and taking the measurements in the upper part of the leaf without veins.

The chlorophyll content (SPAD units) was measured, for fully expanded leaves with-out chlorosis symptoms, at 34 DAT in five lettuces per treatment. A portable instrument (Hansatech CL-01, King’s Lynn, UK) was used.

The content of carotenoids and chlorophylls (a, b, and total) was measured at 34 DAT in five lettuces per treatment. Leaf samples were homogenized and centrifuged. The supernatants were diluted and their absorbance at 470, 663, and 647 nm for carotenoids (xanthophylls and carotenes) and chlorophylls a and b, respectively, was determined using a UV/Vis spectrophotometer (PowerWave XS2, BioTek, Winooski, Vermont, USA). The corresponding concentrations were obtained according to Lichtenthaler and Buschmann (2001).

2.7. Statistical treatment of the data

The statistical analysis included a one-way ANOVA with the statistical package SPSS version 26 (Chicago, IL, USA). The values shown for each treatment are the means of at least 5 repetitions ($n = 5–10$). When the variables were significant ($p < 0.05$), the treatment means were separated using Duncan’s posthoc test multiple comparison test.

3. Results

3.1. Production

3.1.1. Growth parameters

The biomass parameters are shown in Fig. 1. The treatments produced significant differences in the fresh weight of the aerial parts (Shoot FW), and in the production per unit area (yield), at 27 and 34 days after transplanting (DAT). At 27 DAT, no significant differences were observed between treatments “100 % H” and “100 to 50 % H”. However, at 34 DAT, the parameters determined showed significant differences among all treatments, with the “100 % H” treatment producing the highest yields. At 34 DAT plants receiving “100 % H” NS, the FW and yield values were 94.89 g/plant and 1518 g/m^2, with “100 to 50 % H” they were 84.60 g/plant and 1353 g/m^2, and with “50 % H” they were 75 g/plant and 1201 g/m^2. Therefore, the “100 % H” treatment produced increases in these variables of 12 % compared to “100 to 50 % H” and 26 % compared to “50 % H” (Fig. 1).

The treatments mainly affected the growth parameters of the root system (Table 3). Although there were no significant differences between treatments, the length of the stem and the dry matter content of the aerial parts tended to increase as the concentration of nutrients in the NS increased throughout the crop cycle. There were also no differences in water content (WC). Regarding to vegetative growth parameters in roots, the FW tended to be greater as the NS concentration increased as shown the data in the first harvest (27 DAT). This trend became more visible in the second harvest (37 DAT) where significant differences between treatments were observed. The results showed the values recorded in the “100 % H” and “100 to 50 % H” treatments were higher than for the “50 % H” treatment. Regarding dry matter, the differences among treatments appeared earlier and with greater intensity when analyzing the dry matter content at second harvest (34 DAT). At 27 DAT the dry matter content of the roots in the “100 % H” treatment was 42 % higher than for “50 % H”. At 34 DAT, the dry matter value in the “100 % H” treatment was 109 % higher than that of “50 % H”. Also, the WCR in “50 % H” treatment at 27 DAT was significantly higher than that recorded in “100 % H”. At 34 DAT, the differences in the WCR between the “50 % H” and “100 to 50 % H” treatments and “100 % H” were significant.

3.1.2. Evolution of growth

The fresh weight of the aerial parts and the roots increased progressively throughout the crop cycle in all treatments (Fig. 2). At 27 DAT, some differences began to appear in the fresh weight of the aerial parts among the treatments, it is greater for the plants of the “100 % H” treatment than for those of the other two treatments. At 34 DAT the differences among the three treatments were significant, the fresh weight of the aerial parts decreasing in the order “100 % H”, “100 to 50 % H”, and “50 % H”. In the 100 % H treatment, the root fresh weight increased practically linearly throughout the experiment. At 34 DAT, the root fresh weight of the plants of the “50 % H” treatment was lower than that of the other two treatments, between which there was no significant difference.

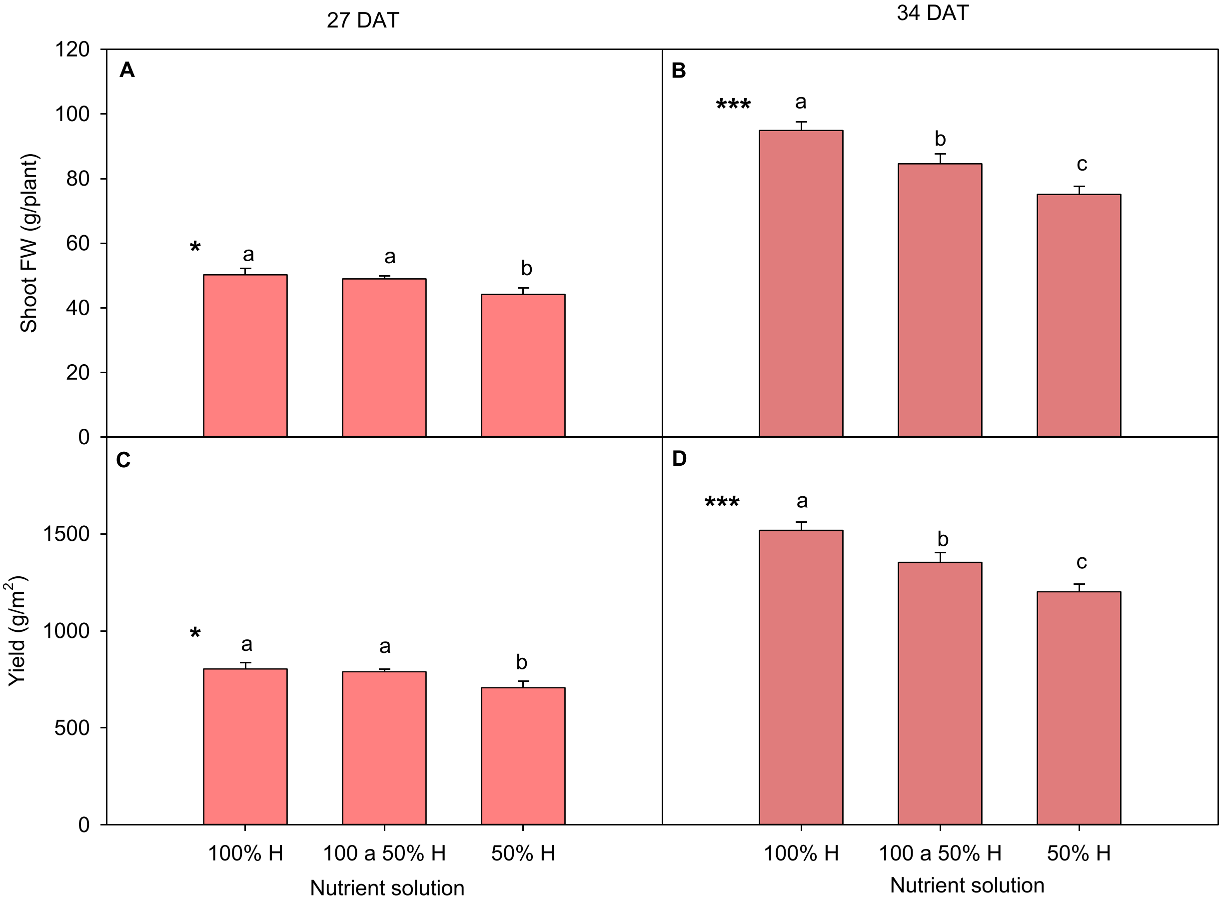

Fig. 1. Fresh weight of the aerial parts and yield for “100 % H”, “100 to 50 % H”, and “50 % H” plants at 27, and 34 DAT. (A) Fresh weight of the aerial parts (g/plant) at 27 DAT. (B) Fresh weight of the aerial parts (g/plant) at 34 DAT. (C) Yield (g/m^2) at 27 DAT. (D) Yield (g/m^2) at 34 DAT. The data represent the mean value ± SE; $n = 10$. * and *** indicate degrees of significance at $p < 0.05$, 0.001, respectively. Different letters indicate significant differences between treatments for $p < 0.05$, according to Duncan’s multiple comparison test.

Table 3

Stem length (cm/plant), dry matter content of aerial parts (g/plant), water content of aerial parts (%), fresh weight of roots (g/plant), dry matter content of roots (g/plant), and water content of roots (%) for treatments “100 % H”, “100 to 50 % H”, and “50 % H” at 27, and 34 DAT.

		Aerial parts			Roots		
Harvest	Treatment	Stem length (cm/ plant)	Dry mass (g/ plant)	WC_{AP} (%)	Fresh mass (g/ plant)	Dry mass (g/ plant)	WC_R (%)
27 DAT	100 % H	0.78	3.00	94.0	5.93	0.37 a	93.7 b
	100 to 50 % H	0.76	2.87	94.0	5.85	0.33 ab	94.4 ab
	50 % H	0.74	2.59	94.1	5.57	0.26 b	95.2 a
	ANOVA	ns	ns	ns	ns	*	**
34 DAT	100 % H	1.23	5.13	94.5	8.13 a	0.46 a	94.0 b
	100 to 50 % H	1.12	4.11	95.1	7.67 a	0.34 b	95.7 a
	50 % H	1.07	3.90	94.8	6.32 b	0.22 c	96.5 a
	ANOVA	Ns	ns	ns	***	***	**

WC_{AP} Water content of the aerial part and WC_R: water content of root part. Mean values of stem length, shoot dry matter, shoot water content, root fresh weight, root dry matter, and root water content. In all cases $n = 6$, except for the fresh weight of the root ($n = 10$). ns indicates not significant ($p \geq 0.05$), while *, **, and *** indicate degrees of significance at $p < 0.05$, 0.01 and 0.001, respectively. Different letters indicate significant differences between treatments for $p < 0.05$, according to Duncan’s multiple comparison test.

3.2. *Crop nutritional status*

3.2.1. *Nutritional status of the aerial parts*

At 27 DAT, the treatments used gave rise to significant differences in the concentration of nitrate. The mean value was 3994 mg/kg FW in the plants treated with “100 % H”, 14 % and 25 % higher than in the “100 to 50 % H” and “50 % H” treatments, respectively. On the other hand, at 27 DAT no differences were found in the concentration of chloride among the treatments, the average value was 630 mg/kg FW (Fig. 3). At 34 DAT, the treatments caused significant differences in the contents of nitrate and chloride. In the case of nitrate, the “100 % H” treatment gave an average value of 5762 mg/kg FW, much higher than the values of 4506 and 4194 mg/kg FW for the treatments “100 to 50 % H” and “50 % H”, respectively. The “100 % H” and “100 to 50 % H” treatments did not differ significantly in the concentration of chloride, although their values were on average 32 % higher than the “50 % H” treatment.

At 27 DAT, there were significant differences in the foliar concentrations of potassium, calcium, sulfur, magnesium, and sodium among the treatments (Fig. 4). The potassium concentrations in the “100 % H” and “100 to 50 % H” plants were higher than that for the “50 % H” treatment. The “100 % H” treatment gave higher calcium concentrations than “50 % H”. The concentrations of sulfur, sodium, and magnesium were similar in the treatments “100 to 50 % H” and “50 % H”, being higher in the plants supplied with “100 % H”. At 34 DAT, the potassium concentration in the “100 % H” treatment was higher than in the other two treatments, which presented similar values. The concentrations of calcium, magnesium, and sulfur in the “100 % H” plants were higher than with the “50 % H” treatment. Throughout the crop cycle, there were no significant differences among the treatments in the phosphorus concentration (Fig. 4).

At 27 DAT, the concentrations of the rest of the micronutrients did not show significant differences due to the treatments applied, the exception being the foliar copper concentration, which was lower in the plants supplied with “50 % H” than in the plants of the “100 % H” and “100 to 50 % H” treatments. However, at 34 DAT, the concentrations of all the micronutrients analyzed, except manganese, showed significant differences among the treatments. The boron concentration in “100 % H” plants was significantly (18 %) higher than in “50 % H” plants. The behavior of the copper concentration at 27 DAT was more evident at 34 DAT, the differences for the treatments “100 % H” and “100 to 50 % H” increasing with respect to “50 % H”. The concentrations of molybdenum and iron in the “100 % H” plants were higher than with the “100 to 50 % H” and “50 % H” treatments, in which they were similar to each other. The zinc concentration showed a behavior similar to that of the copper concentration, with a significant decrease in the “50 % H” treatment compared to the “100 to 50 % H” and “100 % H” treatments, between which there were no differences (Fig. 5).

3.2.2. *Nutritional status of the roots*

The concentrations of nitrate in the root were significant at both date harvests. At 27 DAT “100 to 50 % H” and “50 % H” were significantly lower than 100 % H. These differences were more significant at 34 DAT, when nitrate concentration of 50 %H treatment was the lowest (1524 mg/kg FW). No significant differences were observed in chloride concentration at 27 DAT. However, at 34 days, treatment “100 % H” had the highest chloride concentration compared to the other two treatments which were equal to each other. (Fig. 6).

At 27 DAT, among the rest of the macronutrients analyzed, the treatments only produced significant differences in the root concentration of sodium, its value decreasing in the order “100 to 50 % H”, “100 % H”, “50 % H”. The mean root concentrations were 56, 4.7, 15, 11, and 1.3 g/kg DW for potassium, calcium, phosphorus, sulfur, and magnesium, respectively (Fig. 7).

At 34 DAT, there were significant differences among the treatments in the root concentrations of phosphorus and magnesium. The “100 % H” treatment gave the highest phosphorus concentration and the “50 % H” treatment the lowest magnesium concentration. The mean values of the potassium, calcium, sulfur, and sodium concentrations in the roots were 61, 4.9, 11, and 4.5 mg/kg DW, respectively.

At 27 DAT, the root concentration of copper was lowest and those of manganese and zinc were highest in the plants treated with “50 % H”. The root concentrations of the rest of the micronutrients analyzed did not differ significantly among the treatments and the average values were 32.7, 21.6, and 445.8 mg/kg DW for boron, molybdenum, and iron, respectively. At 34 DAT, the plants supplied with “100 % H” showed higher concentrations of copper and zinc and a lower concentration of manganese, although in the latter case without a significant difference from the “50 % H” treatment (Fig. 8).

3.3. *Quality parameters*

3.3.1. *Color, anthocyanins, and chlorophylls (SPAD)*

The chromaticity values (a*) were higher in the plants treated with “100 to 50 % H”. The saturation values (C) were higher in the “100 to 50 % H” plants than in those supplied with “100 % H”. The treatments did not produce significant differences in the rest of the parameters that determine the color of the leaves. No significant differences were observed in the concentration of anthocyanins between the control and “100 to 50 % H” treatment plants. However, the concentration of anthocyanins in the control treatment was 74 % higher in the “50 % H” plants. The number of SPAD units in the plants treated with “100 % H” was 50 % higher than in those of the “50 % H” treatment (Table 4).

The data represent means with $n = 19$ (color parameters), $n = 6$ (anthocyanins), and $n = 5$ (chlorophylls). ns indicates not significant ($p \geq 0.05$), while *, **, and *** indicate degrees of significance at 0.05, 0.01, and 0.001, respectively. Different letters indicate significant differences between treatments for $p < 0.05$, according to Duncan’s multiple comparison test.3.3.2. Photosynthetic pigments: carotenoids and chlorophylls (a, b, and total)

At 34 DAT, the concentration of chlorophyll *b* was 91 % higher in plants treated with “100 % H” than in those treated with “50 % H”. The “100 to 50 % H” plants did not show significant differences from those of

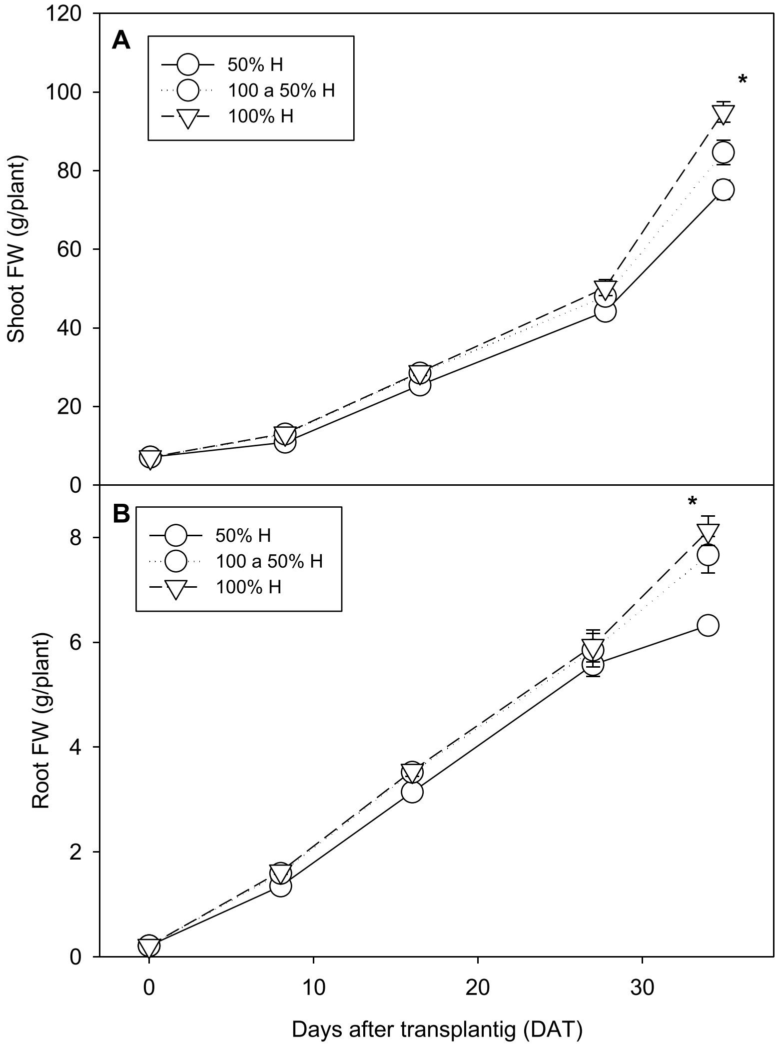

Fig. 2. Temporal evolution of the fresh weight (g/plant) of (A) the aerial parts, AP, and (B) the roots during the cultivation of lettuce, for the treatments "50 % H" (solid lines and circles), "100 to 50 % H" (dotted lines and circles), and "100 % H" (dashed line and triangles). The weights were recorded at 0, 8, 16, 27, and 34 DAT. The data represent the mean value ± SE; $n = 10$. * indicates a degree of significance at $p < 0.05$.

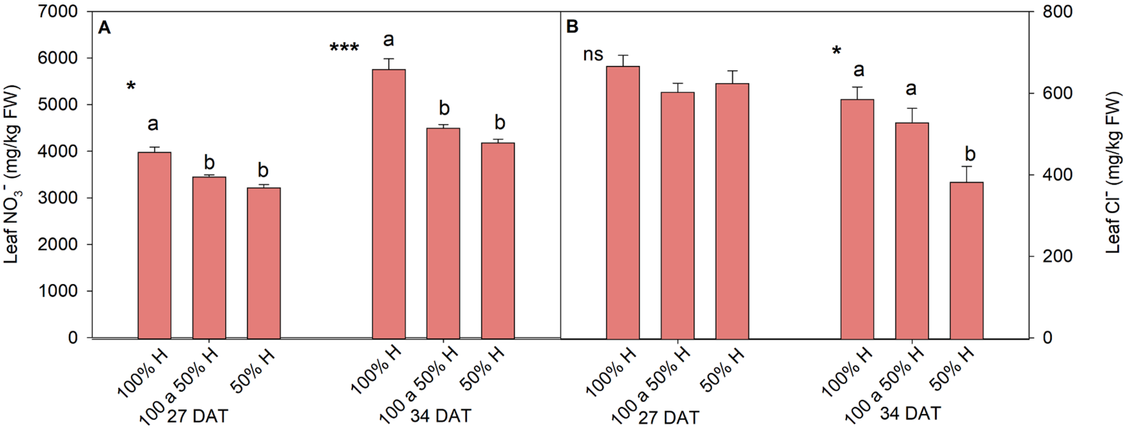

Fig. 3. Leaf concentrations of anions (mg/kg FW) for "50 % H", "100 to 50 % H", and "100 % H" plants at 27, and 34 DAT: (A) nitrate, and (B) chloride. Data represent means ± SE with $n = 6$. ns indicates not significant ($p \geq 0.05$), while * and *** indicate degrees of significance at 0.05, 0.001, respectively. Different letters indicate significant differences between treatments for $p < 0.05$, according to Duncan's multiple comparison test.

the other two treatments. No significant differences were found in the rest of the parameters analyzed (carotenoids, chlorophyll a, and total chlorophyll) (Fig. 9).

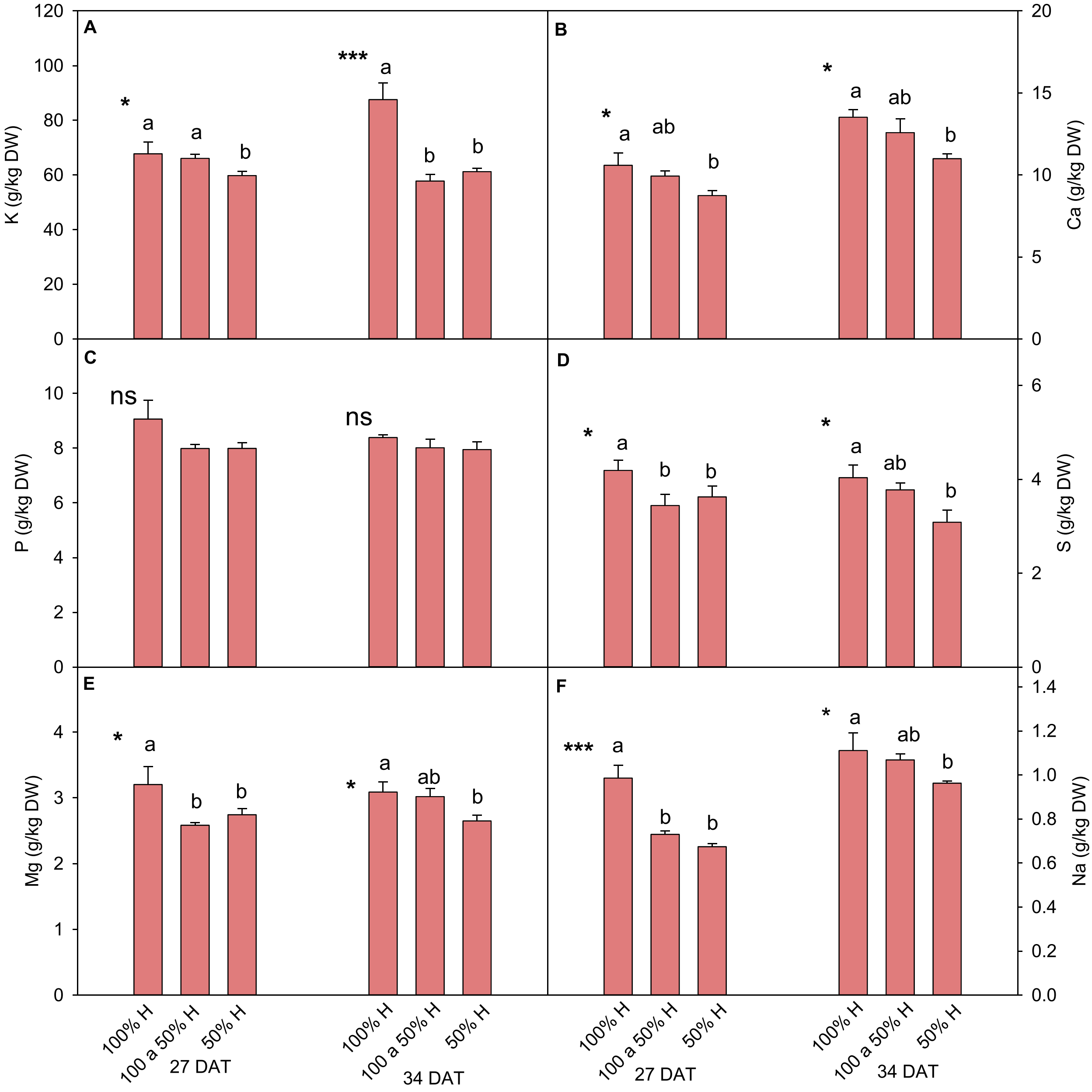

Fig. 4. Leaf concentrations of macronutrient (g/kg DW) for “50 % H”, “100 to 50 % H”, and “100 % H” plants at 27, and 34 DAT: (A) potassium, (B) calcium, (C) phosphorus, (D) sulfur, (E) magnesium, and (F) sodium. The data represent means ± SE with $n = 6$. ns indicates not significant ($p \geq 0.05$), while * and *** indicate degrees of significance at 0.05, and 0.001, respectively. Different letters indicate significant differences between treatments for $p < 0.05$, according to Duncan’s multiple comparison test.

4. Discussion

4.1. Growth

This work aims to obtain hydroponic lettuces with a lower concentration of nitrates in the leaves. For this, experimental plants were grown in a complete Hoagland nutrient solution, reducing the nutrient concentration by 50 % on day 20 after transplant. From the productive point of view, results showed similar productions between “100 to 50 % H” and control (“100 % H”) treatments after 7 days of reducing the concentration of the nutrient solution. However, after 14 days (34 DAT) of modifying the nutrient solution, the yield of the “100 to 50 % H” treatment was significantly reduced. Probably because when the aerial parts reached a mass of about 40 g (approximately at 20 DAT), the demand for nutrients was too high to be satisfied by the “100 to 50 % H” treatment. The fact that the best agronomic yield of the lettuces was achieved with “100 % H” may have been because in the final phase of the crop this treatment was the only one that provided the amount of nutrients necessary for maximum plant growth. In future work, “50 % H” could be supplied until 20 DAT, at which point, coinciding with the onset of the phase of plant development in which the demand for nutrients is higher, the NS could be changed to “100 % H”. On the other hand, “50 % H” treatment, decreased yield from 27 DAT (Fig. 1). It is well known that the management of mineral nutrition is a key preharvest factor determining the yield and quality of leafy vegetable crops (Fallovo et al., 2009). Other studies also demonstrated the lettuce response to the nutrient composition and concentration. For example, Petropoulos et al. (2016) reported that in-creasing N rate from 100 to

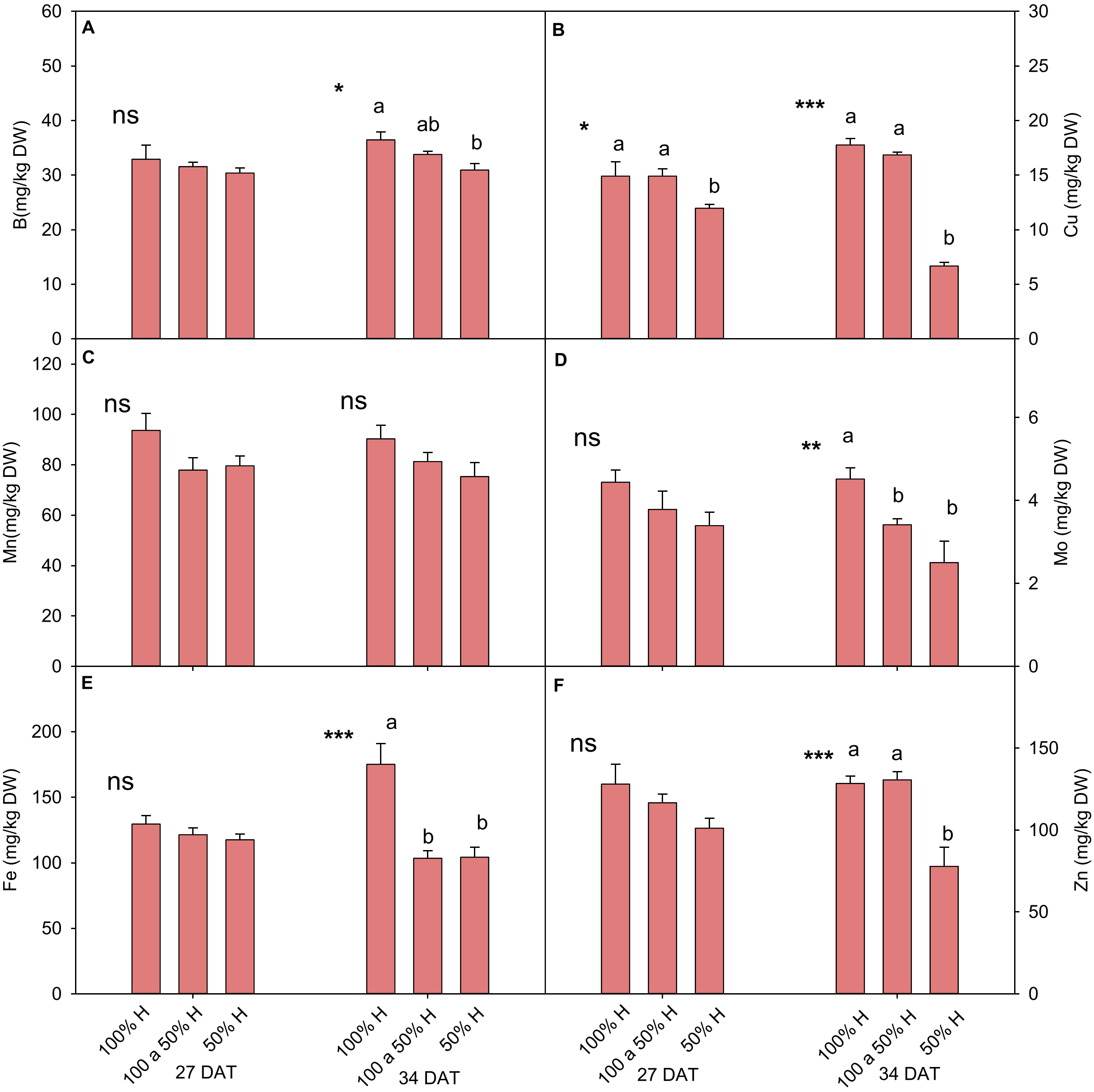

Fig. 5. Leaf concentrations of micronutrients (mg/kg DW) of “50 % H”, “100 to 50 % H”, and “100 % H” plants at 27, and 34 DAT: (A) boron, (B) copper, (C) manganese, (D) molybdenum, (E) iron, and (F) zinc. The data represent means ± SE with $n = 6$. ns indicates not significant ($p \geq 0.05$), while *, **, and *** indicate degrees of significance at 0.05, 0.01, and 0.001, respectively. Different letters indicate significant differences between treatments for $p < 0.05$, according to Duncan’s multiple comparison test.

200 mg/L increased fresh lettuce plant weight by 23.5 %–113 % (from 12 to 41.9 kg/m^2) in hydroponic culture. Similarly, Sapkota et al. (2019) reported an increase in lettuce yield proportional to the increase in the concentration of the nutrient solution. However, this authors also observed that an excessive level of nutrient solution concentration could have negative effects on plant growth.

In this experiment the nutrient solution concentration did not significantly affect the relative water content, which determine the dry matter, in the aerial part of lettuce (Table 3). These results are consistent with the findings by Fallovo et al. (2009), who observed that dry aerial weight was unaffected by nutrient solution composition. If we take into account that the dry matter content in lettuce is considered a quality parameter because a valuable indica-tor of the nutritional value, texture, shelf life, and overall quality of lettuce (Nicolle et al., 2004), the “100 to 50 % H” treatment maintains quality values similar to control treatment at 27 DAT. The evolution of the fresh weight of lettuce plants is shown in Fig. 2. As the cultivation progresses, the growth differences between the treatments become more pronounced. The results obtained suggest not extending the exposure of lettuce to nutrient solutions with low nutrient concentration beyond 7 days, as it negatively impacts lettuce yield. Probably, fresh weight reduction of lettuce after 14 days of reducing the nutrient solution was caused by macronutrient deficiency (Ciriello et al., 2021).

4.2. *Nutrients and nitrate concentrations*

The nutritional status of the hydroponic leafy vegetables is critical to establish the growth rate of the plants (Burns, 1992). When growing

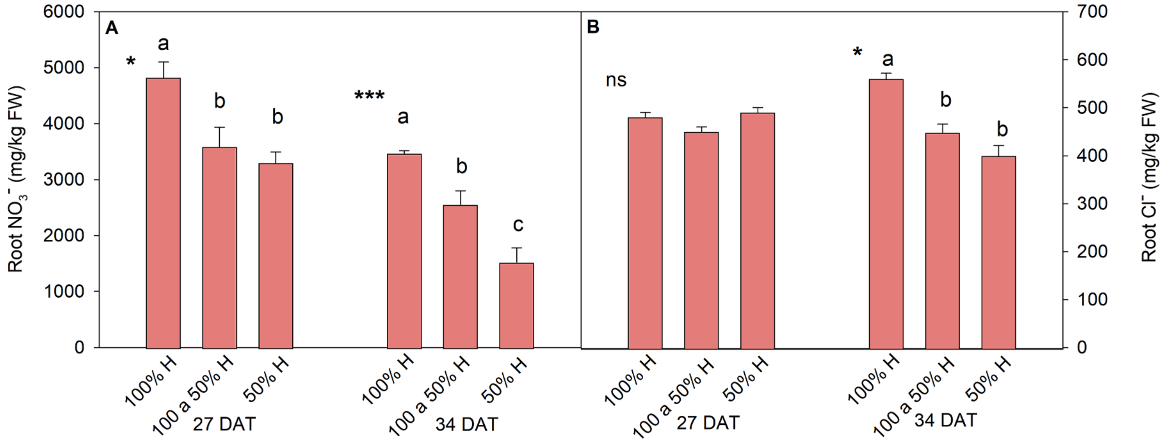

Fig. 6. Concentrations of anions in the roots (mg/kg DW) of "50 % H", "100 to 50 % H", and "100 % H" plants, at 27, and 34 DAT: (A) nitrate and (B) chloride. The data represent means ± SE with $n = 6$. ns indicates not significant ($p \geq 0.05$), while * and *** indicate degrees of significance at 0.05, and 0.001, respectively. Different letters indicate significant differences between treatments for $p < 0.05$, according to Duncan's multiple comparison test.

lettuce in a plant factory, it is important to optimize the composition of the nutrients in the irrigation water. To achieve equal leaf minerals and vitamin content and, second, to not decrease the growth potential (Celine et al., 2019). In our work, the plants of the three treatments had foliar concentrations of nutrients within the normal ranges defined by Kim et al. (2016), not showing deficiencies or toxicities in any case. In the "50 % H" and "100 to 50 % H" treatments, no deficiency problems were observed, probably due to decreased growth, which produced an effect of nutrient concentration, masking an insufficient nutrient supply. Macronutrients, except phosphorus, potassium and calcium, were significantly reduced in the "100 to 50 % H" treatment at 27 DAT. These differences were increased at 34 DAT, now observing a significant reduction of concentration in potassium (Fig. 4). Phosphorus is an essential plant nutrient that is required as a main constituent of plasma membranes, also involved in energy and nucleic acid synthesis and phosphorylation processes. Until now it seemed that, lettuce exhibits higher P fertilizer requirements than most other vegetables and shows a pronounced yield and quality response to P fertilization under most conditions (Hoque et al., 2010). However, in a phosphorus supply limitation work, Neocleous and Savvas (2019) reduced the dose of phosphorus needed lower than those currently recommended (1.8 mM) to 1.3 mM in nutrient solution of lettuce crops grown in closed hydroponic systems. In addition, our results indicate that the dose of phosphorus can still be reduced further, without producing nutritional deficiencies in the leaves at 34 DAT (Fig. 4). The calcium concentration in lettuce from the "100 to 50 % H" treatment showed similar values to the control treatment until 34 DAT. Calcium plays an essential role in plant development and overall plant health because it is a structural component of cell walls, and it is necessary for cell growth and division. In addition, calcium content in the leafy vegetables could further improve their nutritional benefits to the consumers (Grusak, 2002). The potassium concentration was significantly reduced only at 34 DAT, so with an efficient reduction of the nutrient solution before harvest there should be no nutritional deficiencies in the lettuces. Therefore, the data presented here may contribute to the precise control and saving of macronutrient supply, especially phosphorous and calcium improving the sustainability and efficiency in hydroponics lettuce system production. Regarding micronutrients, no significant effects were observed between the control and "100 to 50 % H" treatments indicating that the micro nutritional status of the lettuces at 27 DAT was optimal. However, seven days later at 34 DAT there was a significant reduction in iron (Fe) and molybdenum (Mo). Although there was a significant reduction in iron in the "100 to 50 % H" treatment, no symptoms of iron deficiency were observed in the younger leaves. Furthermore, some authors had observed that mild Fe-deficiency could increase biomass and improve the quality of leafy vegetables, probably due to the enhancement of N, P and K acquisitions via the roots (Jin et al., 2013). In this case we have not observed this increase in production, probably due to the dilution of the rest of the macronutrients in the nutrient solution.

The build-up of an excessive amount of nitrate ions is frequently observed in vegetables grown through soilless cultivation, mainly due to the frequent application of nitrate fertilizers. For this, the leaf nitrate content is one of the main quality components in lettuce cultivation (Da Luz et al., 2008; Maynard et al., 1976). Although nitrate itself is not directly toxic, it has the potential to be transformed into metabolites like nitrite and N-nitroso compounds, which can have ad-verse effects on human health (Santamaria, 2006). Consequently, a heightened intake of dietary nitrate is considered undesirable, as it poses a health risk to humans. A practical approach for individuals to mitigate this risk is to reduce the accumulation of nitrate in vegetables. In the European Union, this limit is established by Regulation (EU) No. 1258/2011 of the Commission of December 2, 2011, which modifies Regulation (EC) no. 1881/2006 regarding the maximum content of nitrate in food products. It establishes, for fresh lettuce grown in greenhouses and harvested between October 1 and March 31, a maximum limit of 5000 mg NO$_3^-$/kg fresh weight. In our experimental conditions, a direct relationship between the nitrate concentration in the NS and the accumulation of nitrate in the leaf was observed, with the "100 % H" treatment giving the highest value of NO$_3^-$ concentration (3994 and 5862 mg/kg FW at 27 DAT and 34 DAT respectively). The nitrate concentration at 34 DAT exceeded the maximum limit established by legal regulations by 17 %. However, the "100 to 50 % H" treatment significantly reduced the concentration of nitrates in leaves by 14 % at 27 DAT and by 22 % at 34 days compared to the control treatment. This approach enables producers to meet stringent health regulations regarding nitrate levels, enhancing consumer confidence and potentially accessing premium markets. Our results are in concordance with previous works confirming that a reduction in nitrate concentrations in nutrient solutions significantly decreased the accumulation of nitrates in hydroponic lettuce (Liu and Yang, 2011, 2012; Wang and Shen, 2011; Bian et al., 2020). However, nitrate concentration reduction in vegetables by a limited nitrate supply often results in yield and quality losses (Fu et al., 2017). Therefore, the optimization of the NS for floating-root lettuce crops must be taken into account, to reduce the foliar concentration of nitrate without affecting yield and quality.

Finally, the optimized nutrient management strategy "100 to 50 % H" can lead to cost savings by reducing the amount of nitrate fertilizers required, contributing to more sustainable and cost-effective production practices. For stakeholders, including retailers and consumers, the benefits include a safer product with reduced health risks associated

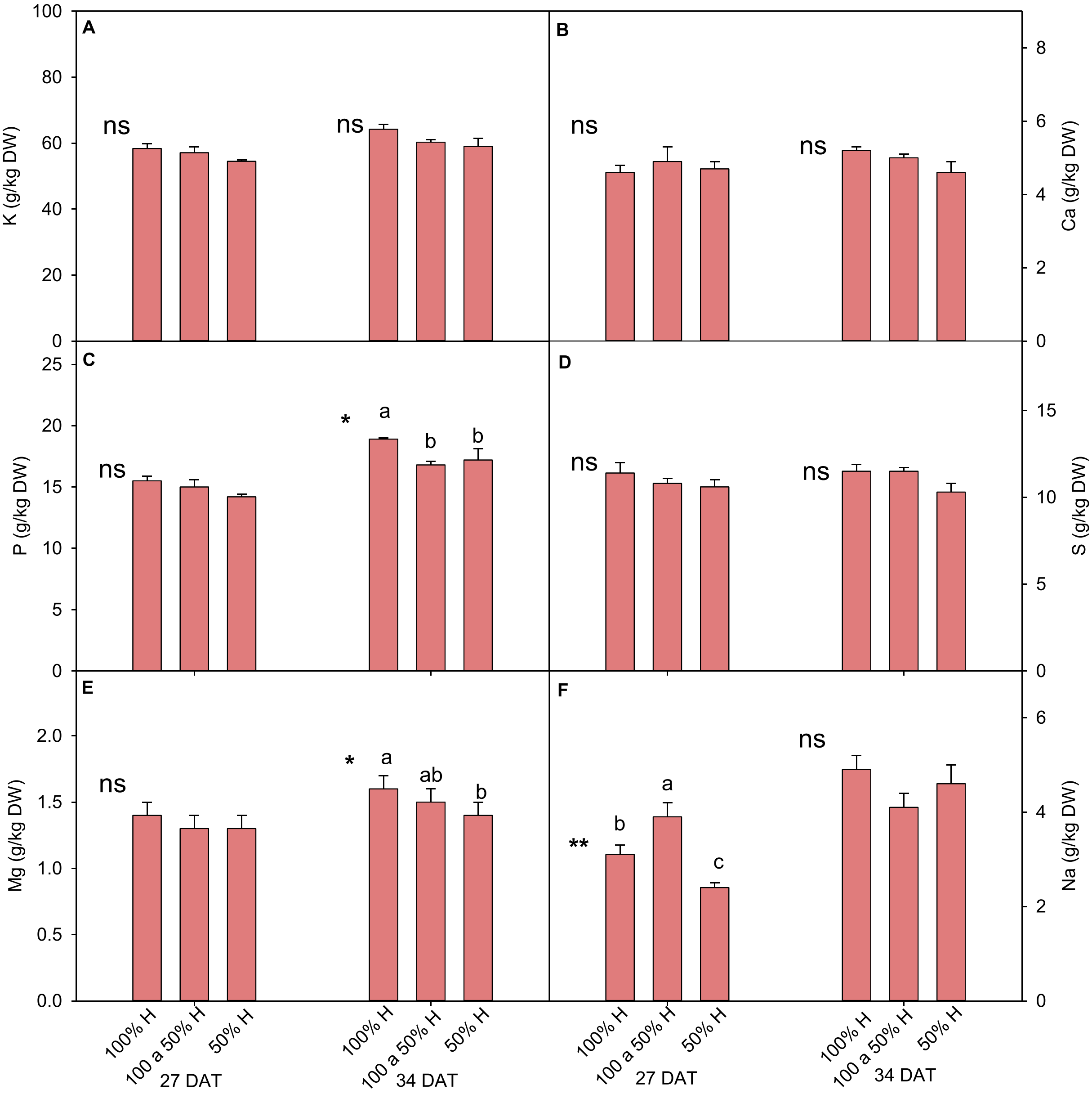

Fig. 7. Macronutrient concentrations in the roots (g/kg DW) of "50 % H", "100 to 50 % H", and "100 % H" plants, at 27, and 34 DAT: (A) potassium, (B) calcium, (C) phosphorus, (D) sulfur, (E) magnesium, and (F) sodium. The data represent means ± SE with $n = 6$. ns indicates not significant ($p \geq 0.05$), while * and ** indicate degrees of significance at 0.05, and 0.01, respectively. Different letters indicate significant differences between treatments for $p < 0.05$, according to Duncan's multiple comparison test.

with high nitrate consumption (Rajaseger et al., 2023).

4.3. Quality

As a commercial product, lettuce plays a vital role in the human diet because they are rich in a wide range of beneficial compounds, including vitamins, minerals, and secondary metabolites (Wang and Shen, 2011). In addition, in recent years, there has been a change in consumer attitudes towards vegetables shopping. Recent concerns over pesticide use and food safety have made consumers more selective and demanding in terms of the health and quality of food in general and vegetables in particular. The nutrients decrease in nutrient solution significantly affects some quality parameters (Table 4). The "50 % H" treatment significantly reduced the concentration of anthocyanins, which are directly related to the intensity of red pigment and are also important food-bioactive compounds, improving the senso-rial properties of vegetable products (Assefa et al., 2021). However, the concentration of anthocyanins was similar in the "100 to 50 % H" and control treatments. Previous experiments have also evaluated the organoleptic quality of lettuce about the composition of the NS. Thus, similar to our results Tsouvaltzis et al. (2020) did not observe changes in the concentration of anthocyanins in red lettuce (cv. Carmesi) grown with different concentrations of nitrogen. Regarding color parameters, Fallovo et al. (2009) in lettuce (var. Acephala, cv. 'Green Salad Bowl') grown in a floating hydroponic system with five different concentrations of nutrients in the NS (2, 18, 34, 50, and 66 meq/L), found that the colour values increased in response to the increase in nutrient concentrations. Although no significant reduction was observed in photosynthetic pigments (total chlorophyll and carotenoids), a tendency for decreased pigment levels was noted in treatments with lower concentrations of

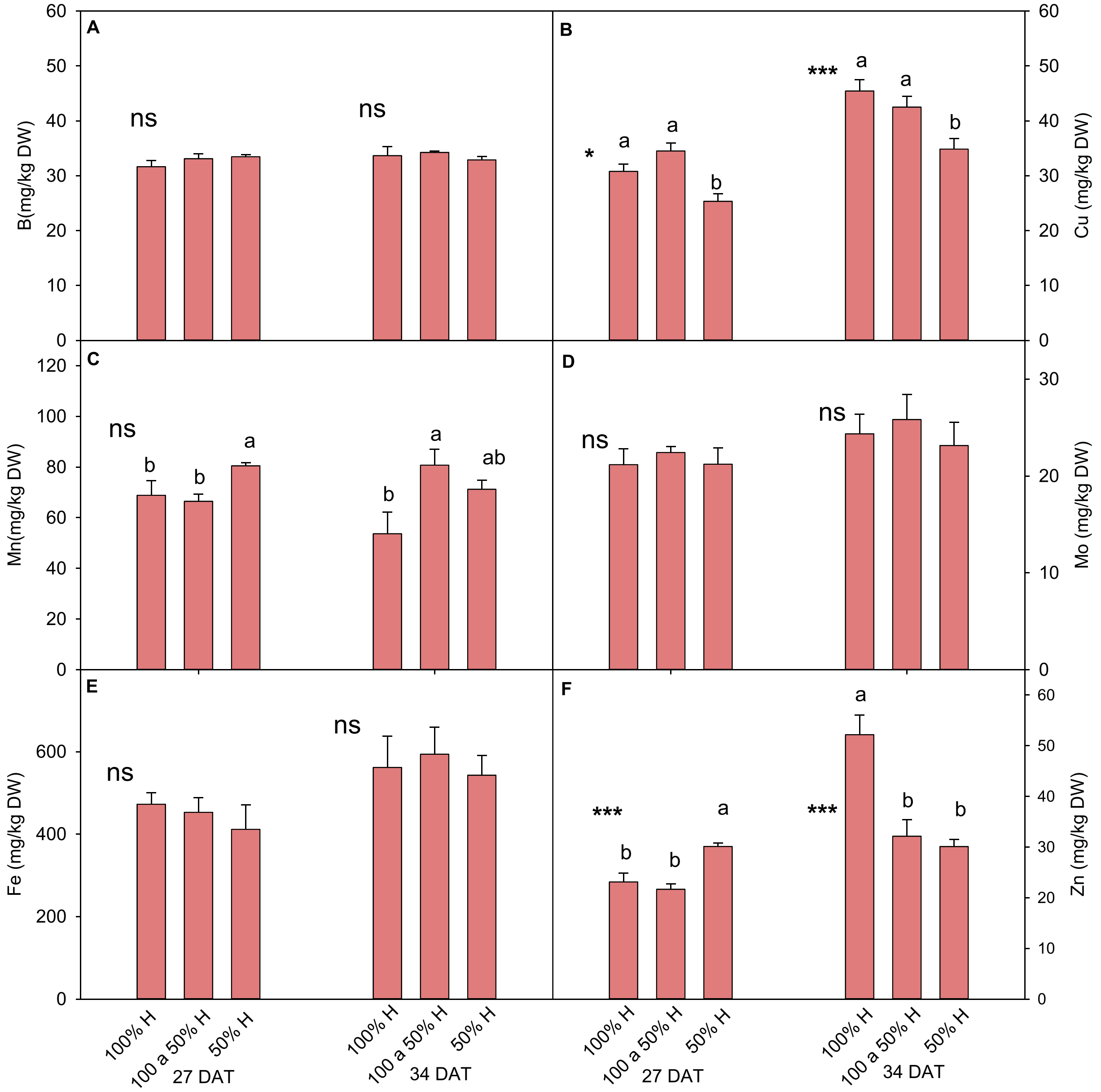

Fig. 8. Concentrations of micronutrients in the roots (mg/kg DW) of “50 % H”, “100 to 50 % H”, and “100 % H” plants at 27, and 34 DAT: (A) boron, (B) copper, (C) manganese, (D) molybdenum, (E) iron, and (F) zinc. The data represent means ± SE with $n = 6$. ns indicates not significant ($p \geq 0.05$), while *, **, and *** indicate degrees of significance at 0.05, 0.01, and 0.001, respectively. Different letters indicate significant differences between treatments for $p < 0.05$, according to Dun-can’s multiple comparison test.

Table 4

Parameters that determine the leaf color: lightness L (scale 0–100), chromaticity A and B (scale 0–100), saturation C (scale 0–100), and hue angle (°). Anthocyanins content (mg cyanidin/kg FW) and chlorophyll (SPAD units) in leaves for “50 % H”, “100 to 50 % H”, and “100 % H” plants at 34 DAT.

Treatment	Lightness L	Chromacity A	Chromacity B	Saturation C	hue angle (°)	Anthocyanins (mg cyanidin/kg FW)	Chlorophyll (SPAD units)
100 % H	36.0	4.8 a	14.7	16.1 b	69.7	17.2 a	4.3 a
100 to 50 % H	38.2	4.4 ab	15.9	18.4 a	67.8	15.1 ab	3.9 ab
50 % H	36.6	3.9 b	16.5	17.1 ab	75.5	9.9 b	2.8 b
ANOVA	Ns	**	ns	*	ns	***	*

nutrients in the solution (Fig. 9). This decline in photosynthetic pigments could potentially explain the observed reduction in productive yield. Mahlangu et al. (2016) in previous works on lettuce, and, for *Oxalis regnellii* and *Oxalis triangularis*, observed that an increase in nitrogen in the NS produced a proportional increase in chlorophylls. Meanwhile, Urlić et al. (2017) did not observe any significant effect of the nitrogen concentration in the NS on the production of chlorophylls in rocket plants. In our experiment, the highest concentration of nitrogen in the NS, in the “100 % H” treatment, produced lettuce with a higher concentration of chlorophylls. Other quality parameters such as

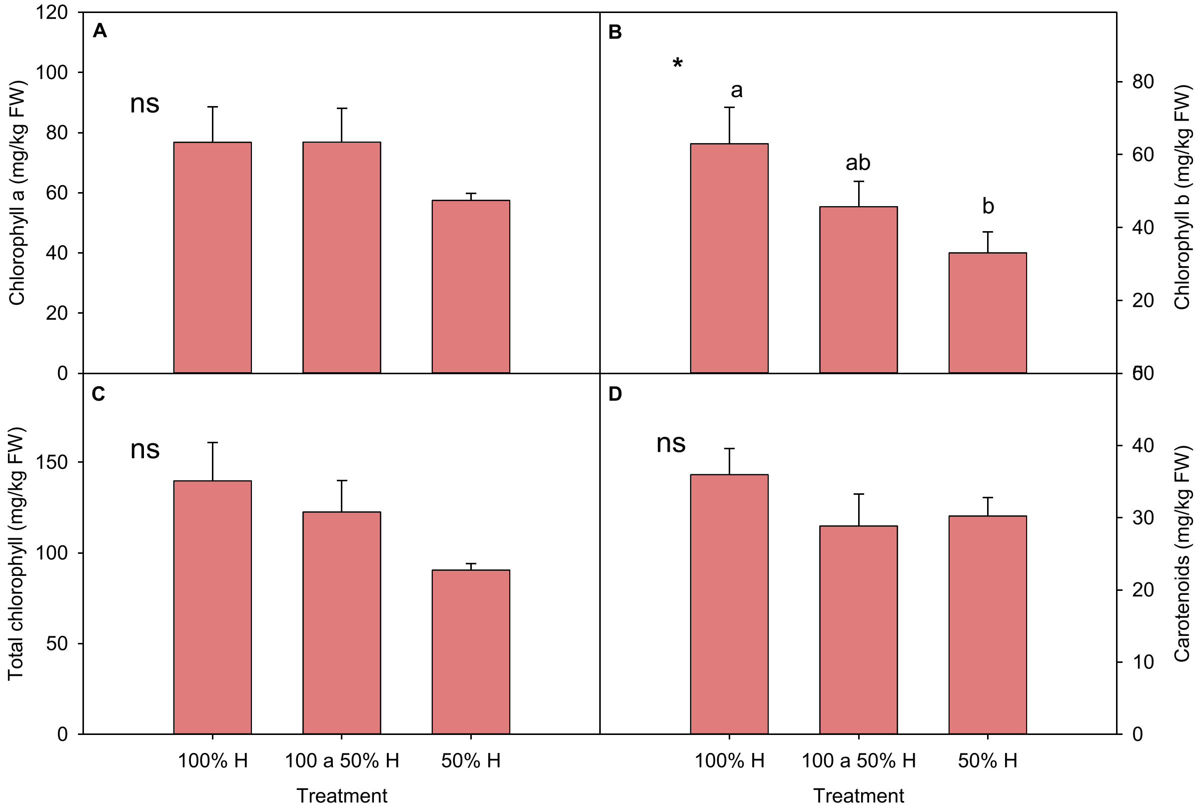

Fig. 9. Concentrations in leaves (mg/kg FW) of "100 % H", "100 to 50 % H", and "50 % H" plants, at 34 DAT: (A) chlorophyll a, (B) chlorophyll *b*, (C) total chlorophyll, and (D) carotenoids. The data represent means ± SE with $n = 5$; ns indicates not significant ($p \geq 0.05$), while * indicates significance at 0.01. Different letters indicate significant differences between treatments for $p < 0.05$, according to Duncan's multiple comparison test.

carotenoids did not vary significantly among the treatments in our experimental conditions. Similar to our results, Alberici et al. (2008) in a hydroponic lettuce system destined for the IV range industry, applied three solutions that differed in the concentrations of nutrients (100, 50, and 25 %) and observed that the total carotenoids in the leaf did not vary with the concentrations of nutrients.

5. Conclusions

Excessive accumulation of nitrates in leaf lettuce is a common issue that poses a potential threat to human health. In this work, different management of the NS in a floating system for growing lettuce, have been studied to reduce nitrate leaf concentration. The data reported here confirm that a modification of NS concentration (20 DAT) reduce significantly the nitrate concentration in leaf lettuce. The reduction of NS concentration does not affect the yield of lettuce after 7 days of application, in addition, the phosphorus potassium and calcium concentrations were also not affected. However, after 14 days, the lettuce yield was negatively affected. On the other hand, NS reduction (100 % H to 50 % H) modified the colour parameters reducing the red colour in lettuce and significantly reducing the anthocyanin concentration after 7 days of application. Our results suggest that is possible to reduce nitrate concentration without affecting lettuce yield. For this reason, future trials should focus on optimizing the management of nutrient elements of the NS and the timing of the nutrient solution change, to increase the crop yield and nutrition quality.

CRediT authorship contribution statement

Alejandro Martínez-Moreno: Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Investigation, Formal analysis. **Juan Carmona:** Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Formal analysis. **Vicente Martínez:** Writing – review & editing, Software, Methodology, Investigation, Data curation. **Francisco Garcia-Sánchez:** Supervision, Resources, Funding acquisition, Conceptualization. **Teresa C. Mestre:** Validation, Methodology, Investigation, Formal analysis. **Valeria Navarro-Pérez:** Validation, Software, Investigation, Formal analysis. **José M. Cámara-Zapata:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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