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Lettuce growth response to increased sodium chloride and reduced potassium concentrations in the nutrient solution in the plant characterization unit

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Bioregenerative life support systems (BLSSs) will be essential in long-term space missions to reduce the requirement for supplies from the Earth. Most of these BLSSs will include crops to generate oxygen (O_2), water, and food needed by the astronauts, while capturing carbon dioxide (CO_2) from the atmosphere. While previous research studies have shown that plants provide these services when grown under optimal mineral nutrition, we consider it also important to study the impact of suboptimal plant nutrition as any decrease in O_2, water, and food production or CO_2 capture could have long-term effects in the BLSS. To this end, we conducted four crop tests in the Plant Characterization Unit (PCU) of the European Space Agency, located at the University of Naples Federico II, examining the impacts of adding sodium chloride (NaCl) and reducing potassium (K) in nutrient solutions on lettuce (*Lactuca sativa* L.). The control treatment with the standard nutrient solution composition was run twice (Control_1 and Control_2) to investigate the repeatability of the PCU crop tests. Plant growth during the experiment was monitored by quantifying O_2 and water production, the projected leaf area, and canopy temperature throughout the crop tests. At harvest, photosynthesis-related parameters (F_v/F_m, SPAD, stomatal conductance, and transpiration rate) were collected, and the leaf, stem, and roots were weighed and analyzed for total elemental composition. Assessing the impact of the suboptimal mineral nutrition on plants was challenged by high plant-to-plant variability within each test. Furthermore, considerable differences in plant growth were observed in the two control treatments. Comparisons with previous and similar experiments suggested that Control_2 provided a more realistic representation of the control treatment. Compared to Control_2, the addition of 27 mM NaCl in the nutrient solution did not result in any significant decrease in biomass production, or in photosynthesis-related parameters at harvest. The net water and O_2 productions were also comparable to those observed in Control_2. However, the projected leaf area

showed decreased plant development compared to Control_2. The reduction in K in the nutrient solution decreased plant development, as reflected in the projected leaf area, and a 20% decrease in shoot dry biomass. This, in turn, led to led to a decrease in O_2 and water production of the PCU relative to Control_2. Our results show that a nutrition strategy that is not adapted to plant needs can lead to decreased plant performance in BLSSs. The impact of such a decrease on the sustainability of long-duration crewed Space missions will need to be studied.

KEYWORDS

hydroponics, lettuce, low K condition, MELiSSA loop, NaCl addition

1 Introduction

The European Space Agency (ESA) is developing the Micro Ecological Life Support System Alternative (MELiSSA) closed-loop system for continuously producing sufficient food, water, and oxygen (O_2), and capturing carbon dioxide (CO_2) for long-term crewed missions in space (Lasseur et al., 2010). The MELiSSA loop (European Space Agency, 2022; Vermeulen et al., 2023; Frossard et al., 2024) includes five major compartments. The crew (compartment V in MELiSSA's terminology) consumes food, water, and O_2 and produces urine, other organic waste, and exhales CO_2. Organic waste (except urine) is transferred to compartments I and II, in which microorganisms liquefy and mineralize the waste in the absence of O_2 to produce water-soluble mineral salts and CO_2. The CO_2 produced from compartment I is guided to compartment IV-A, a photobioreactor containing algae, and compartment IV-B, a growth chamber containing higher plants that are grown in hydroponics. The mineral salts from compartment II, the urine from compartment V, and O_2 produced in compartment IV-A and -B are transferred to compartment III, where ammonium (NH_4) is nitrified. Mineral elements enriched in nitrate (NO_3) produced in compartment III are then transferred to compartments IV-A and IV-B to grow algae and higher plants that provide food and O_2 to the crew while capturing CO_2. In addition, higher plants from compartment IV-B release clean water through transpiration for the crew.

The optimal performance of crops in the long term, in terms of food, O_2, and water production, and CO_2 capture, depends on adequate plant nutrition. By "adequate," we mean that the nutrients are in a chemical form available to plants, that the nutrient concentration and the ratios between them allow plants to achieve their growth potential and ensure healthy development, and that the solution should always be free from toxic compounds. Such a goal can be achieved when using an adequate nutrient solution or slow-release fertilizer pillows, as shown by Wheeler et al. (1994), Wheeler et al. (1996), and Massa et al. (2017). Frossard et al. (2024) however, showed that this goal will be difficult to achieve in the MELiSSA loop, where all nutrients will be derived from organic waste and urine as it is currently extremely inefficient and requiring substantial costs to separate nutrients from each other and reinject each of them at the proper concentration and form in the nutrient solution. Problems that might arise include the following: (i) a lack of phosphorus (P) as P can precipitate in many areas of the loop; (ii) a lack of potassium (K) if the crop residues are not properly recycled; (iii) an excess of NH_4 for ineffective nitrification; (iv) an excess of sodium (Na) and chloride (Cl) as both the elements are present in high concentrations in human excreta; (v) the release of toxic elements from the machinery used in the loop or produced by organisms (Frossard et al., 2024). Thus, we need to study how suboptimal plant nutrition can affect the performance of BLSSs. Such results will thereafter have to be included in BLSS models, including a higher plant model, to be able to predict how changes in food, O_2, and water production and CO_2 capture occurring in the short term will impact the functioning of the BLSS over long-term space missions. This paper describes a short-term experiment with lettuce but does not touch upon modeling.

To study the effect of suboptimal nutrition in BLSSs, experiments need to be conducted in completely sealed growth chambers to measure O_2 and water release and CO_2 captured at the crop level, which allow precise control of air temperature (T), relative humidity (rH), light intensity, air flow, and nutrient solution composition to set the plant under the desired conditions. The National Aeronautics and Space Administration (NASA) developed the Biomass Production Chamber (PBC) in the 1980s and summarized the results in Wheeler et al. (1996). ESA has developed two growth chambers of this type: a higher plant growth chamber at the MELiSSA Pilot Plant (MPP) at the Universitat Autònoma de Barcelona (Spain) named the "Higher Plant Chamber" (HPC) (Peiro et al., 2020) and another at the University of Naples Federico II (Italy) named the "Plant Characterization Unit" (PCU) (Pannico et al., 2022). Since we are studying MELiSSA loop, we thereafter focus on the work done with ESA. While Peiro et al. (2020) showed the importance of a homogenous air flow in the chamber for lettuce growth, Pannico et al. (2022) focused on the design and technical description of the PCU. More recently, Schiefloe et al. (2023) used the PCU to study the impact of the NH_4:NO_3 ratio in the nutrient solution on lettuce growth. They showed that the biomass and O_2 production per plant were significantly decreased in a nutrient solution with a high NH_4:NO_3 ratio, thus demonstrating the importance of the nitrification rate and illustrating the implications and possibilities for nutrient management strategies in a closed loop. A limitation of these growth chambers, however, is that they are single-channel systems, which do not allow simultaneous treatments to be run. Furthermore, they have restricted availability and high operation costs, and the experiments are extremely labor-intensive, which altogether strongly limits the number of experiments conducted.

In the present study, we used a slightly modified version of the PCU described by Pannico et al. (2022) to assess the impact of an increased concentration of sodium chloride (NaCl) and a

decreased concentration of K in the nutrient solution on lettuce growth; production of plant biomass, O_2, and water; and the capture of CO_2 compared to that in lettuce grown in a standard nutrient solution. Here, we hypothesize that lettuce grown with increased NaCl concentration will produce less biomass and, therefore, less O_2 and water and capture less CO_2 than lettuce grown in the control nutrient solution. Furthermore, we expect that the presence of NaCl in the nutrient solution will reduce the K and magnesium (Mg) concentrations in the biomass, as reported by Breś et al. (2022). Similarly, we hypothesize that because of the importance of K for photosynthesis and carbohydrate assimilation and transfer (Rengel et al., 2022), lettuce grown in the presence of a low K concentration in the solution will produce less biomass and lower leaf area, as shown by Zhang et al. (2017), and, therefore, less O_2 and water and capture less CO_2 than when grown in the control nutrient solution. In order to test these hypotheses, we conducted four tests in the modified PCU, including two control tests with the optimal nutrient solution, one test with increased NaCl concentration in the nutrient solution, and the last with decreased K concentration in the nutrient solution. This is the first time that a full crop test has been conducted in the PCU with low macronutrient concentration and a high NaCl concentration and that a single treatment has been run twice.

2 Materials and methods

2.1 Experimental settings

The experiments were carried out in the PCU at the University of Naples Federico II between November 2022 and May 2023 (the dates of the different tests are reported in Supplementary Material A). The experimental conditions used in this work were identical to those presented by Pannico et al. (2021) and Schiefloe et al. (2023), except for an upgrade of the hardware, the composition of the nutrient solutions, and the number and position of the plants. Only ten plants were installed in the growth chamber in the present study instead of 18 in the two above-mentioned papers.

We used lettuce as a fast-growing model crop (*Lactuca sativa* L.), cultivar Grand Rapids as in Peiro et al. (2020), Pannico et al. (2021), and Schiefloe et al. (2023). Seeds (0.4 g) (obtained from West Coast Seeds, Vancouver, Canada) were disinfected with 0.7 M sodium hypochlorite solution for 15 min and rinsed with water purified by reverse osmosis (roH_2O). The seeds were then dispersed onto previously autoclaved absorbent paper, moistened with roH_2O, and incubated at room temperature under natural light. At 48 h after the beginning of seed germination, 84 pre-germinated seeds were selected and transferred to a polystyrene germination tray filled with vermiculite. The tray was placed in a plastic tank external to the PCU, floating in half-strength ACSA nutrient solution at an electrical conductivity (EC) of 1 ms cm^{-1} and a pH of 5.9 (Peiro et al., 2020). pH and EC were monitored using a portable multi-meter (model HI-991301, Hanna instruments, Padova, Italy) and corrected manually every day. The seedlings were cultivated for 10 days at non-controlled room temperature (ranging between 23 °C and 25 °C) and air humidity (ranging between 50% and 60%). The illumination regime was 16/8 h provided by an LED panel unit (K5 Series XL750, Kind LED, Santa Rosa, CA, USA) of 300 µmol m^{-2} s^{-1} (PPFD, represented by 30% blue, 60% red, and 10% white). Ten days after the seeds were transferred to vermiculite, 30 to 40 homogenous and healthy seedlings were selected. The selected seedlings were gently removed from the vermiculite, washed with roH_2O to remove the vermiculite from the roots, and transferred to rockwool cylinders surrounding the plant roots. The cylinders were then placed in bottomless 50-mL polyethylene tubes. After adding to the tubes, the surface of the rockwool was sealed with grafting mastic (Cortimax, Cisa Adriatica S.r.l., Italy) to minimize gas exchange between the hydroponic and atmospheric parts of the PCU during the experiments. The selected seedlings in the polyethylene tubes were then repositioned on a perforated lid designed to accommodate these tubes and allowed to grow for 6 more days under the same conditions as in the vermiculite. After these 6 days in rockwool, homogeneous healthy seedlings were selected for transplanting into the PCU. Top-view pictures of the seedlings were taken to estimate their projected leaf area (PLA) segmenting plant pixels with a free image analysis software application (ImageJ, version 1.53e, US National institutes of Health, MD, USA). In the first test, the 10 seedlings with the PLA closest to the median were selected for transplantation into the PCU. For the following tests the procedure was the same, except that the 10 selected seedlings were the ones with the PLA closest to the median of the first test so as to increase the comparability of the four crop tests. The seedlings of the four tests exhibited an average PLA of 25.6 cm^2 $plant^{-1}$ ranging between 21.9 and 30.4 cm^2 $plant^{-1}$.

We grew 10 plants per test, which was equivalent to a plant density of 5.6 m^{-2}. Each plant had an identification number which is shown in Supplementary Material B. The duration of each crop test was 28 days. The day/night cycle was 16/8 h, with an average light intensity during the day of 420 ± 50 µmol m^{-2} s^{-1} (PPFD represented by 23% blue, 20% green, and 57% red). The light intensity for each plant position is reported in Supplementary Material J. The air temperature was set at 26 °C during the day and 20 °C during the night, while the relative air humidity was set at 50% during the day and 70% during the night. Compared with the optimal conditions for lettuce growth in hydroponics (Amitrano et al., 2021), higher air temperature and lower air humidity were applied to enhance plant transpiration. The CO_2 concentration in the atmosphere was set at 1,000 ppm, with a single-sided control system allowing the CO_2 concentration to increase above 1,000 ppm due to plant respiration during night. O_2 concentration in the atmosphere accumulated at ambient condition and was left to accumulate with time as a function of photosynthesis, respiration, and leakage (which was relatively low, see Supplementary Material C). Equations used to calculate net O_2 and water production are given in Section 2.3.1.

2.2 Preparation of nutrient solutions for different treatments

The treatments differed only in the nutrient solution composition, while all the other environmental conditions and the plant density were kept constant between the experiments. We conducted four tests, including three treatments. Control_1 and Control_2 were the two tests during which lettuce was grown with the standard ACSA nutrient solution. The ACSA nutrient solution was chosen because in former studies, it was used in both

the HPC and PCU for growing the lettuce cultivar Grand Rapids (El Nakhel et al., 2021; Peiro et al., 2020; Pannico et al., 2021) to ensure comparability between studies. In the second treatment (+NaCl), lettuce was grown in a modified ACSA solution containing 27 mM NaCl. This NaCl concentration was chosen because in a pre-test it caused an increase in the leaf Na concentration, reaching the N: Na molar ratio of human urine. Considering a hypothetical scenario where human urine is continuously added to replenish the N taken up by the plants, if the lettuce N: Na ratio is equal to that of human urine, then the Na concentration in the nutrient solution would not increase further. This is because the Na input in the nutrient solution provided by human urine would be equal to the output via leaf harvest (more details on this pre-test are provided in Supplementary Material D). In the third PCU treatment (-K), lettuce was grown in a modified ACSA nutrient solution with a K concentration of 0.5 mM instead of the 4.8 mM present in the control treatments. The design of a K-deficient treatment was selected because human urine contains a large amount of N, but low concentrations of K (Rose et al., 2015). Feeding the plants to meet their N needs with human urine can, therefore, potentially lead to K deficiency. The ACSA nutrient solution has an N: K molar ratio of 3.1, whereas fresh and stored human urine have a higher N: K molar ratio of 11.7 (Udert et al., 2006). Determining the appropriate reduction in K concentration that would impose a mild stress on the plants without limiting their growth too severely was a challenge since the nutrient solution contains a surplus of nutrients and the response to K deficiency is cultivar-dependent (Zhang et al., 2017; Zhang et al., 2020; Yang et al., 2007). The concentration of 0.5 mM was determined based on the results of a pre-test, where we observed an effect on the leaf biomass production of Grand Rapids only when the K concentration was below 0.5 mM.

Nutrient solutions were prepared in all four experiments by adding two stock solutions to approximately 270 L of roH_2O to reach an EC of 1.9 m cm^{-1}. During the entire duration of the Control_1, Control_2, and -K treatments, this EC was kept constant by adding the two stock solutions to replenish the nutrient uptake of the plants. In the +NaCl treatment, when an EC of 1.9 m cm^{-1} was reached, NaCl was added gradually over the first 3 days of the experiment to avoid osmotic shocks for the plants and reach the final concentration of 27 mM and an EC of 4.6 m cm^{-1}. This final EC was then maintained until the end of the experiment by adding the two stock solutions. In the ACSA solution, K is provided in the form of potassium nitrate (KNO_3). In the nutrient solution of the -K treatment, we compensated for the reduction in KNO_3 with the addition of sodium nitrate ($NaNO_3$) to maintain the same concentration of nitrate (NO_3) as in the control. Sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) at a concentration of 0.5 M were added during nutrient solution preparation and throughout the tests to maintain the pH at 5.9. The ion concentration in the nutrient solution at the beginning of the experiments is shown in Table 1. The recirculating nutrient solution was filtered in three steps using cartridge filters installed downstream of the plant gullies and upstream of the continuously stirred nutrient solution tank. The filters had different pore sizes (1.2, 0.65, and 0.45 μm; PP3 Sartopure–Sartobran, Sartorius, Goettingen, Germany). More details on the nutrient solution preparation and refeeding strategy can be found in Supplementary Material E.

TABLE 1 Elemental composition of the nutrient solutions at the beginning of the crop tests (Control_1, Control_2, +NaCl, and -K). In the +NaCl treatment, NaCl was added over the first 3 days of experiment, and the Na and Cl concentrations at the fourth day are reported in brackets. Differences between the theoretical concentrations shown in Supplementary Material H and the measured concentrations shown here arise from imprecisions in estimating the total nutrient solution volume, from the addition of H_2SO_4 and NaOH to adjust pH, and from inaccuracies in both procedural and analytical steps.

Ion	Control_1	Control_2	+NaCl	-K
	mM			
NO_3	10.1	11.4	11.3	12.3
NH_4	2.5	2.2	2.9	2.3
Total N	12.6	13.5	14.2	14.6
K	4.0	4.5	4.4	0.7
P	1.1	1.2	1.3	1.3
Ca	3.3	3.4	3.6	3.2
Mg	0.9	1.0	1.0	1.1
S	1.6	1.6	1.7	1.4
Na	0.6	0.5	0.6 (24)	4.8
Cl	0.5	0.3	0.3 (24.5)	0.3

2.3 Monitoring of plant growth during the experiment

2.3.1 Net O_2 and water production

The atmospheric O_2 levels were monitored continuously in the PCU using a California Analytical Instruments NDIR analyzer (700 Series, SN 1906036), with data recorded at 5-s intervals. These measurements were then used to calculate the O_2 balance, allowing us to estimate the net daily O_2 production by the plant through Equation 1:

$$System_n[O_2]_{S,n} + Compress\,Tank_n[O_2]_{CT,n} + O_2\,Injected - Leakage[O_2]_{S,av} + O_2\,Plant\,Production = System_{n+1}[O_2]_{S,n+1} + Compress\,Tank_{n+1}[O_2]_{CT,n+1}. \quad (1)$$

Here, $System_n[O2]_{S,n}$ and $System_{n+1}[O_2]_{S,n+1}$ represent the moles of O_2 in the system at instants n and n+1, respectively, which were calculated by multiplying the measured O_2 concentration by the mol of gas in the PCU (estimated using the ideal gas law). *Compress* $Tank_n[O_2]_{CT,n}$ and *Compress* $Tank_{n+1}[O_2]_{CT,n+1}$ denote the moles of O_2 in the pressure compensation system at instants n and n+1, respectively, which were calculated by multiplying the moles of gas in the pressure compensation system (estimated using the ideal gas law) by an estimation of the O_2 concentration in the pressure compensation s,ystem which was measured only at the chamber closure and opening. O_2 *Injected* is the total moles of O_2 injected between instants n and n+1. $Leakage[O_2]_{S,av}$ is the total moles of O_2 leaked over the same interval. Finally, O_2 *Plant Production* is the total moles of O_2 produced by the plants between instants n and n+1.

Water production during the experiment was estimated as follows: considering the two instants n and n+1 at stable and similar pressure, temperature, and relative humidity conditions, the amount of water contained in the recirculating air can be considered equal. Then, it was possible to estimate the amount of water transpired by the plants based on Equation 2:

$$Dehumidifier_n + water\,Injected + water\,Plant\,Production = Dehumidifier_{n+1}. \quad (2)$$

Here, $Dehumidifier_n$ and $Dehumidifier_{n+1}$ represent the water collected by the dehumidifier at instants n and n+1, respectively, *water Injected* is the water injected by the humidifier between instants n and n+1, and *water Plant Production* is the water transpired by the plants between instants n and n+1.

2.3.2 Projected leaf area and canopy temperature

The projected leaf area of all the tests was estimated once per day with pixel counts of the top-view color photos taken inside the PCU with an HD red–green–blue (RGB) camera (BFS-U3-200S6C-C Blackfly S, Flir, Wilsonville, OR, USA). For PCU plant experiments, we performed the plant pixel segmentation automatically with a random forest-trained MATLAB script that is described in Supplementary Material F. Leaf temperature was monitored with a thermal camera (A65, Flir, Wilsonville, OR, USA) from the top of the PCU. The canopy temperature was estimated daily at 15 h after the beginning of the light phase on all the plants. We identified the pixels based on the top-view color pictures taken by the PCU a few seconds after thermal image acquisition, and a link to the pixel of the thermal photo considering the different positions and angular field of view of the two different cameras was implemented with a MATLAB script (also described in Supplementary Material F). The temperature of each plant was estimated with this method based on the total leaf area visible from above, corresponding to approximately 300–12,000 pixels depending on the plant size.

2.4 Post-harvest measurements and nutrient solution ion content

2.4.1 Plant biomass and leaf production

Leaves and stems were weighed fresh and immediately after harvest. The leaves were then separated and counted. The total leaf area was estimated with imaging segmentation of top-view photos of all the leaves arranged on a white surface, as done in Pannico et al. (2021) and in Schiefloe et al. (2023). Right after the harvest, roots were washed first in 10 mM $CaSO_4$ solution to remove the sorbed cations and then rinsed briefly in roH_2O. Leaves, stems, and roots were then weighed after drying at 40 °C for assessing the dry biomass production.

2.4.2 Nutrient concentration in biomass

The dry biomass samples of leaves, stems and roots were first milled with a Qiagen TissueLyzer ball mill, and then 0.2 g of the milled sample was digested with nitric acid (7 M) in a single reaction chamber microwave (Turbowave, 31.5 min, 220 °C, $1.2^{*}10^{7}$ Pa). The digested samples were finally analyzed for the following total elements after dilution: P, K, calcium (Ca), Mg, Na, cadmium (Cd), cobalt (Co), cupper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) with an ICP-OES (Shimadzu ICPE-9820). Plant samples (1–4 mg) were analyzed for total C, N, and S with an elemental analyzer (vario PYRO cube, Elemental).

2.4.3 *In vivo* measurements of photosynthesis-related parameters

To access the plants for the *in vivo* measurements described in this section, the plant chamber was opened, which altered the environmental conditions. To minimize this artifact, the nutrient solution was allowed to continue circulating, the lights were kept on, and measurements were conducted as quickly as possible. Nevertheless, minor deviations from the true growth conditions cannot be completely excluded.

2.4.3.1 Maximum quantum efficiency of the photosystem II (F_v/F_m)

Immediately after opening the PCU, young, fully developed leaves were clipped with dark adaptive leaf clips. After 30 min and complete dark adaptation, the F_v/F_m parameter was measured with a portable fluorometer (Opti-Sciences Inc., Hudson, NH, USA) in the exact same position where the clips were applied. The measurement was repeated three times per plant in three different leaf positions.

2.4.3.2 Stomatal conductance, transpiration, and net CO_2 assimilation rate

Net CO_2 assimilation rate (A), transpiration rate (E), and stomatal conductance (gs) were determined for every plant with a portable gas exchange analyzer (LCA 4; ADC BioScientific Ltd., Hoddesdon, United Kingdom) on the portion of young, fully expanded leaves that were completely exposed to light.

2.4.3.3 SPAD index

The SPAD index, an indicator of chlorophyll content, was measured 10 times for every plant on young and fully expanded leaves with a portable chlorophyll meter SPAD-502 (Minolta Co. Ltd, Osaka, Japan).

2.4.4 Ion concentration in the nutrient solution

Every other day, 40 mL of the nutrient solution was sampled from the downstream nutrient solution filters. A sub-sample of 200 µL–250 µL was taken for analyzing the concentrations of Ca, Cl, K, Mg, Na, NH_4, NO_3, SO_4, and HPO_4 by liquid ion-exchange chromatography (ICS-3000 Dionex, Thermo Fisher Scientific, Sunnyvale, CA, USA), as described by Pannico et al. (2019). The concentrations of Fe, Cu, and Zn in the solution were measured with an ICP-OES five times during every experiment from the nutrient solution samples taken in the two plant gullies and downstream from the filters.

2.5 Major accidents

CO_2 injected into the PCU was automatically quantified by a control system; however, this system failed in accurately quantifying CO_2 (more information on this can be found in Supplementary Material G). The amount of C fixed by the plants

is, therefore, quantified based on the post-harvest analyses, as described in Wheeler et al. (1996).

The PCU control system server crashed 16 days after transplantation (DAT) of the +NaCl test. This resulted in approximately 26 h of no control of the atmospheric and hydroponic systems, no light in the PCU, and complete loss of the logged data. During the night between 17 and 18 DAT, the lights were kept on, catching up 8 hours of light of the 18 lost hours during the crash.

During the test Control_2, plant 4 started to wilt after the first 3 days of normal development. The wilted plant was substituted at 5 DAT with a seedling that was still growing in a batch with the ACSA nutrient solution outside the PCU. For this operation, the plant chamber was shortly opened.

2.6 Statistical analysis

Statistical analysis and visualization of the data were conducted with the R software (version 4.2.0). For the post-harvest parameters including yields, biomass composition, and biometric data, and for the PLA and canopy temperature at different timepoints, the significance between tests was analyzed with single ANOVA and Tukey honestly significant post-tests considering the plants as an experimental measurement and the tests as a factor (package *agricolae*, version 4.2.1). Prior to performing ANOVA, assumptions of normality and homogeneity of variances were controlled with Shapiro–Wilk and Levene's tests, respectively.

3 Results

3.1 Plant performance in the PCU

3.1.1 Biomass and leaf production

Plants presented poor quality at harvest, including leaf necrosis, leaf tissue thickening, beginning of flowering, and lateral heads building, in all treatments (photos are provided as examples in Supplementary Material G). Leaf necrosis was more widespread in the Control_1 test than in the plants produced in other tests.

Due to the high standard deviation, the leaf fresh weight presented no significant differences between treatments (Table 2). Nevertheless, -K treatment developed significantly less leaf dry mass compared to Control_2. The root dry matter robustly varied between treatments ranging between 3.3 g plant^{-1} (control_1) and 6.3 g plant^{-1} (control_2), with -K and +NaCl showing intermediate values. The final leaf number and area were the maximum in +NaCl and the minimum in -K and control_1, respectively.

3.1.2 Net oxygen and water production

The net O_2 production increased from the ambient value at the start of each experiment to a range of 23.5%–24.2% by the end. Since the safety level of 25% was not exceeded, it was not necessary to vent the PCU during the experiment. The daily water production increased steadily during each experiment to finally reach values ranging between 2.0 and 2.5 L day^{-1} at day 27 for the entire PCU. The overall net O_2 and water production during the four tests are shown in Table 3. No statistical analyses could be performed as these data were obtained at the PCU level. The O_2 and water production calculated per DW g of shoot (stem dry weight + leaf dry weight) was similar for all the treatments.

3.1.3 Net CO_2 uptake

We observed a higher C content in the roots of the Control_2 and -K and lower C content in the roots of +NaCl, whereas no difference could be seen in the C content in the leaves and stems between tests (Table 4). Using the biomass reported in Table 2 we calculated that each plant had an average C content of 10.7 g ± 2.0 in the total biomass, and we could not detect any statistical significance between the tests.

3.2 Plant development

3.2.1 Projected leaf area

The PLA increased until reaching a plateau in the last days of growth (Figure 1). The PLA of +NaCl followed a different pattern with a decrease of the slope at approximately 17 DAT, followed by a subsequent increase interrupted by the harvest. Control_2 reached the plateau earlier than the other treatments, indicating that the plants reached full development more quickly. The PLA of Control_1 was constrained by the leaf necrosis negatively impacting the plant development and flattening the PLA growth curve in the second half of the experiment. At harvest, the two treatments with modified nutrient solutions, +NaCl and -K, showed a PLA between the ones of the two controls, Control_1 and Control_2. This indicates that the impact of changes in the nutrient solution on plant growth may be overshadowed by the differences between Control_1 and Control_2. The PLA at harvest covered a maximum of 40% for Control_1 of the plant chamber total surface (1.8 m^2). The average PLA of -K was lower than that of Control_2 during the entire duration of the experiment; however, this difference was significant only at 21 DAT (Table 5). At 28 DAT, the only significant difference observed between tests was between the two control treatments.

3.2.2 Canopy temperature

Over time, the canopy temperature tended to decrease from an average temperature of 22.7 °C at 0 DAT to 20.9 °C at 28 DAT (Figure 2). For +NaCl, only the temperature until 15 DAT was considered since after day 15, a server crash had occurred, and no thermal pictures were collected thereafter. The differences between tests were considerably lower than between plants. Leaf temperature was affected by the distance between the plants and the lights, with the plants in the middle of the gullies, which were closer to the lights, showing a higher leaf temperature. The canopy temperature was significantly affected by the tests only during the first 2 weeks of the experiment (Table 6). At 7 DAT, +NaCl showed a significantly higher canopy temperature compared to the three other tests. At 14 DAT, Control_1 and -K had higher canopy temperatures than Control_2.

3.2.3 Changes in element concentrations in the nutrient solution

The ion concentrations in the nutrient solution were measured every other day, and the Zn, Cu, and Fe concentrations in the nutrient solution for each test are given in Supplementary Material H. In the description and

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TABLE 2 Leaf, stem, and shoot (leaf + stem) fresh (FW) and dry weights (DW), root DW, leaf number, and leaf area ± standard deviations, at lettuce harvest of the four tests Control_1, Control_2, +NaCl, and -K. The letters indicate significance grouping in accordance with Tukey HSD (p value <0.05) of values in the same column.

Crop test	Leaf FW	Leaf DW	Stem FW	Stem DW	Shoot FW	Shoot DW	Root DW	Leaf number	Leaf area
	g plant^{-1}							leaves plant^{-1}	cm^2 plant^{-1}
Control_1	231 ± 72^{a}	17.8 ± 3.3^{ab}	42.6 ± 15.3^{ab}	4.2 ± 1.0^{a}	275 ± 87^{a}	22.0 ± 4.3^{ab}	3.6 ± 0.5^{c}	77.6 ± 21.6^{b}	2,691 ± 925^{c}
Control_2	302 ± 77^{a}	21.1 ± 3.9^{a}	49.1 ± 14.8^{a}	4.4 ± 0.7^{a}	351 ± 92^{a}	25.5 ± 4.5^{a}	6.3 ± 0.6^{a}	84.1 ± 16.0^{ab}	3,289 ± 827^{abc}
+NaCl	289 ± 37^{a}	18.7 ± 2.3^{ab}	52.6 ± 14.7^{a}	4.3 ± 1.0^{a}	342 ± 50^{a}	23.0 ± 3.0^{ab}	4.8 ± 0.5^{b}	102.6 ± 14.8^{a}	4,053 ± 573^{a}
-K	252 ± 80^{a}	17.0 ± 2.3^{b}	31.8 ± 11.6^{b}	3.5 ± 0.7^{a}	283 ± 92^{a}	20.6 ± 3.0^{b}	5.0 ± 0.9^{b}	71.5 ± 16.0^{b}	2,936 ± 828^{bc}

TABLE 3 Net total O_2 production expressed in mol plant^{-1} and mmol (g DW shoot)$^{-1}$ and water total production expressed in L plant^{-1} and L (g shoot)$^{-1}$ during the four tests Control_1, Control_2, +NaCl, and -K.

Crop test	O_2 produced	Water produced	O_2 produced	Water produced
	mol plant^{-1}	L plant^{-1}	mmol (g DW shoot)$^{-1}$	L (g DW shoot)$^{-1}$
Control_1	0.86	3.21	0.039	0.15
Control_2	0.94	3.34	0.037	0.13
+NaCl	0.92	3.11	0.040	0.14
-K	0.77	2.71	0.038	0.13

TABLE 4 Carbon content ± standard deviation expressed in g C (Kg dry weight)$^{-1}$ of the plants of the four experiments at harvest divided into leaves, stems, and roots. The letters indicate significance grouping in accordance with Tukey HSD (p value <0.05) of values in the same column.

Crop test	Leaves	Stem	Roots
	g C (Kg DW)$^{-1}$		
Control_1	391 ± 7^{a}	408 ± 8^{a}	370 ± 13^{b}
Control_2	403 ± 6^{a}	414 ± 12^{a}	385 ± 5^{c}
+NaCl	376 ± 7^{a}	378 ± 7^{a}	322 ± 6^{a}
-K	413 ± 7^{a}	414 ± 3^{a}	385 ± 4^{c}

discussion of the results, we focused on changes in the Na concentration in the +NaCl test, in the K concentration in the -K treatment, and on changes in Cu and Zn concentrations in the four tests.

The Na concentration in the nutrient solution ranged between 24 mM at 4 DAT (when the NaCl addition was finalized) and 29 mM at harvest. The gap between the target Na concentration (27 mM) and the Na concentration measured at 4 DAT was probably attributed to inaccuracies in the estimation of the volume of the nutrient solution circulating in the PCU. The increase in the Na concentration observed in the -K test was due to the addition of $NaNO_3$ instead of KNO_3, and the increase in the Na concentration observed in all the tests was due to the addition of NaOH for pH control. The measured K concentration in the nutrient solution ranged from 0.7 mM at the beginning of the -K crop test to 0.1 mM at the harvest. The reason for this decrease is attributed to the plant K uptake. The difference between the target K concentration and the measured K concentration at the beginning of the experiment is probably caused by the presence of K contained in roH_2O used for preparing the nutrient solution. Finally, the results show much higher Cu and Zn concentrations in the nutrient solution at the beginning of the first test (control_1) than the targeted values. These values increased linearly with time during the first test. The Cu and Zn concentrations strongly diminished at the beginning of the second test, but they were still above the target values and increased again linearly with time. The lowest values of Cu and Zn concentrations in the solution were observed in the last test (Control_2). These results indicate that during the first and the second tests significant amounts of Cu and Zn were released from the hardware.

3.3 Post-harvest measurements

3.3.1 Element concentrations in plant organs

Ca, K, Mg, N, Na, Cu, and Zn concentrations in the leaves, stems, and roots of plants grown in the four tests are reported in Table 7. This table also shows the optimal nutrient range proposed for lettuce by Hartz et al. (2007). Sulfur, Fe, Mn, and P concentrations

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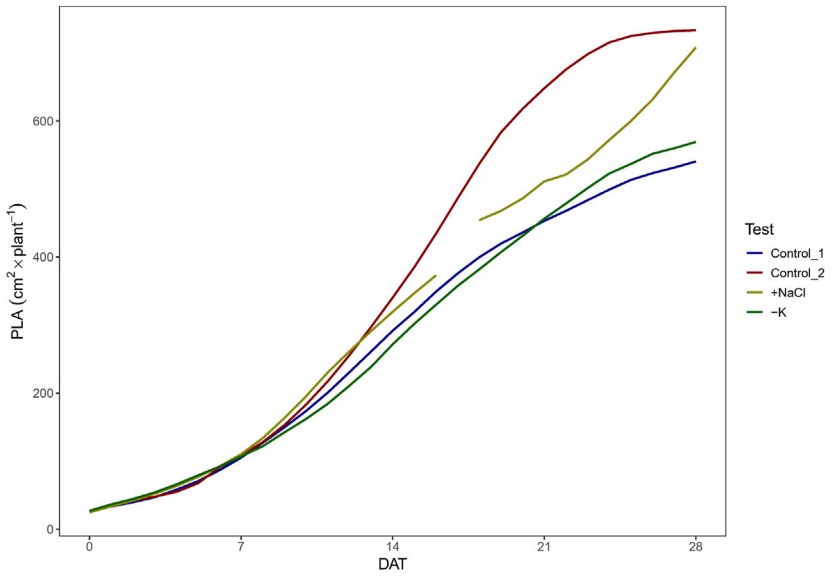

FIGURE 1
Average projected leaf area (PLA, cm^2 $plant^{-1}$) measured as a function of the days after transplantation (DAT) for the entire duration of each test Control_1, Control_2, +NaCl, and -K.

TABLE 5 Average projected leaf area (cm^2 $plant^{-1}$) of the four tests Control_1, Control_2, +NaCl, and -K at 7 days after transplantation (DAT), 14 DAT, 21 DAT, and 28 DAT ± standard deviation. The letters indicate significance grouping in accordance with Tukey HSD (p value < 0.05) of values of the same column.

Crop test	7 DAT	14 DAT	21 DAT	28 DAT
	cm^2 $plant^{-1}$			
Control_1	105 ± 17^a	292 ± 95^a	453 ± 150^b	540 ± 143^b
Control_2	108 ± 22^a	340 ± 94^a	641 ± 210^a	733 ± 235^a
+NaCl	110 ± 25^a	320 ± 73^a	511 ± 90^{ab}	708 ± 83^{ab}
-K	107 ± 20^a	272 ± 42^a	456 ± 85^b	569 ± 83^{ab}

are reported in Supplementary Material I. The data show a strong decrease in K concentrations in all organs in the -K test compared to the other tests, while in the +NaCl test, a strong increase in the Na concentration can be observed in the leaves. The final Na concentration in the leaves of the +NaCl test is close to what was expected from the pre-test (Supplementary Material D). In addition, these results showed a suboptimal Ca level in the leaves of Control_1 and suboptimal levels of Mg in the leaves of each test compared to values given by Hartz et al. (2007). Finally, we observed Cu and Zn levels in leaves that were above the optimal range in Control_1 and +NaCl and in -K (only Zn).

3.3.2 *In vivo* measurements of photosynthesis-related parameters

The ratio F_v/F_m was minimum in Control_1 and maximum in -K and +NaCl, while the transpiration rate and stomatal conductance were minimum in Control_1 and maximum in Control_2 (Table 8). The treatments had no effect on the photosynthesis rate and SPAD.

4 Discussion

The discussion is sectioned into two parts. First, we discuss the variability of the results, both within individual tests and between different tests, and then we discuss the impacts of the addition of NaCl and low K concentration in the nutrient solution on the plants.

4.1 Variability of plant growth in the PCU

4.1.1 Variability of plant growth within the test

We observed a coefficient of variation (cv, calculated dividing the standard deviation by the average) ranging between 15% and 34% for fresh weight shoots (leaves + stems) in the four tests. Wheeler et al. (1994) observed coefficient of variations of fresh shoot mass ranging between 21% and 25% for lettuce cv Waldmann's Green grown in the BPC at NASA. Higher inter-plant variability (cv of 42%) was reported in fresh shoot production in PCU experiments with Grand Rapids (Schiefloe et al., 2023), while Pannico et al. (2021) reported a cv of 11% for the same cultivar in the PCU. This variability may stem from both genetic and environmental factors. Heterogeneous microclimatic conditions (light, temperature, and air velocity) are a well-known cause of plant biomass variability in growth chambers (Potvin et al., 1990; Wheeler et al., 1994; Wheeler et al., 1996). Usually, this problem is solved by moving the plants in the chamber during growth so that each plant can experience variable growth conditions, but this is not possible in a tight system such as the PCU unless an automatic system can be developed. In Pannico et al. (2021), biomass was minimum

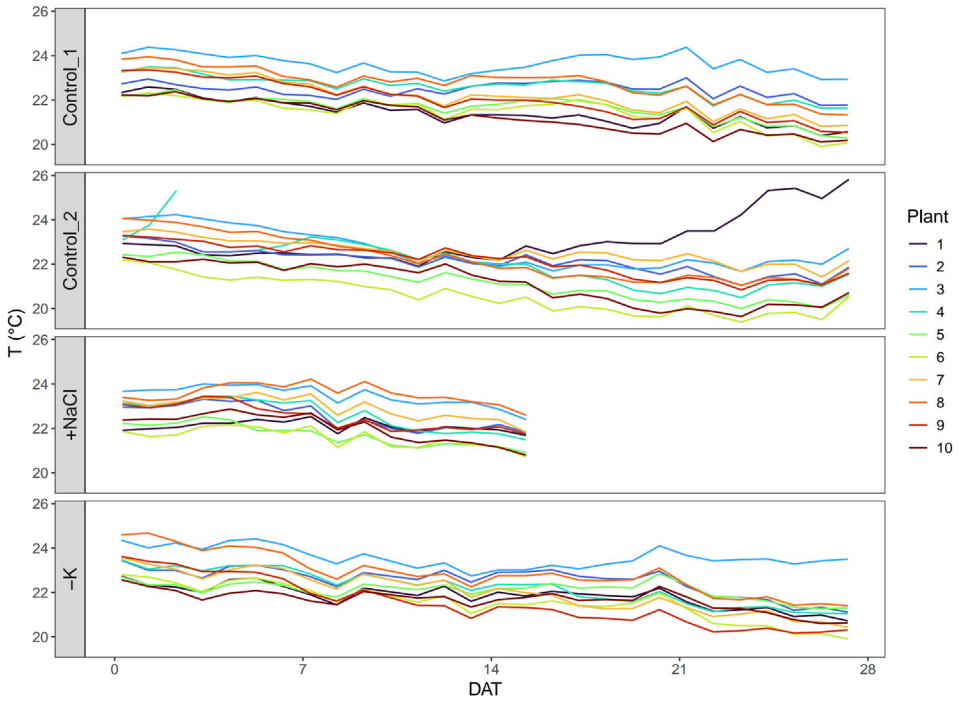

FIGURE 2
Canopy temperature (°C) measured daily 15 h after switching from the night phase to the day phase for every plant during the four crop tests Control_1, Control_2, +NaCl, and -K.

TABLE 6 Average canopy temperature (°C) of the four tests Control_1, Control_2, +NaCl, and -K at 7 days after transplantation (DAT), 14 DAT, 21 DAT, and 27 DAT ± standard deviation. The letters indicate significance grouping in accordance with Tukey HSD (p value <0.05) of values of the same column.

Crop test	7 DAT	14 DAT	21 DAT	27 DAT
	°C			
Control_1	22.4 ± 0.7^{b}	22.2 ± 0.6^{a}	22.2 ± 1.0^{a}	21.0 ± 1.3^{a}
Control_2	22.6 ± 0.7^{b}	21.7 ± 0.7^{b}	21.4 ± 0.9^{a}	21.9 ± 0.9^{a}
+NaCl	23.0 ± 0.6^{a}	22.0 ± 0.7^{ab}	-	-
-K	22.4 ± 0.7^{b}	22.2 ± 0.7^{a}	21.9 ± 1.1^{a}	21.0 ± 1.3^{a}

at the highest leaf temperature and light intensity and at the lowest air velocity. We measured varying light intensity and air flows at different plant positions in the empty plant chamber (Supplementary Material J). This might partly explain the variability between plants. Peiro et al. (2020), who grew the cultivar Grand Rapids in the HPC of Barcelona, improved the homogeneity of the plants by reducing the air flow variability. Ventilation is currently produced from the side walls of the PCU, which probably leads to an increased transpiration from older leaves. As Ca moves with water flow in the plant, this higher transpiration from older leaves probably leads to a preferential transfer of Ca to older leaves and to a limited transfer of Ca to younger leaves (de Bang et al., 2021). This would explain the appearance of necrosis on young leaves, a known Ca deficiency symptom, which has been observed throughout the tests but more strongly in Control_1. Furthermore, the higher leaf temperature observed in the plants close to the lamps indicates heterogeneous temperature inside the chamber. The calculation of vapor pressure deficit (VPD) using the equation provided by Abtew and Melesse (2013) including the set air relative humidity of 50% and air temperature of 26 °C, delivers 1.68 kPa, whereas the optimum VPD for lettuce ranges from 0.5 to 0.8 kPa (Amitrano et al., 2021; Inoue et al., 2021). The additional heating of the lamps at the foliage level likely caused a local increase in the VPD, which led to increased stress on the plants. Such high VPD values can lead to a reduction in the biomass production (Amitrano et al., 2021). The plant-to-plant variability inside the PCU observed in this work and in a previous study conducted with the same cultivar (Schiefloe et al., 2023) make it impossible to analyze factors that have a small impact on plant growth. Only factors that can trigger a large plant reaction can deliver significant results. For instance, Schiefloe et al. (2023) could detect a decrease of 58% in the leaf fresh yield in the cultivar Grand Rapids after having changed the NH_4:NO_3 ratio from 0.14 to 0.95 mol mol^{-1} in the nutrient solution despite the high interplant variability observed in their experiments.

4.1.2 Variability of plant growth between tests

The two tests conducted under identical standard conditions (Control_1 and Control_2), first at the very beginning of our test series and then at the end of our test series, yielded very different results (5.1 vs. 6.3 g dry matter m^{-2} day^{-1}). In comparison, three experiments conducted with lettuce (cv Waldmann's Green) in the

TABLE 7 Total nutrient contents in leaves, stems, and roots ± standard deviation of the plants growing in the four crop tests (Control_1, +NaCl, -K, and Control_2). The letters indicate significance grouping in accordance with Tukey HSD (p-value <0.05) of values of the same elements in the same plant parts (leaf, stem, and root) of different tests. Ca content in the roots is not shown as the roots were washed in 10 mM $CaSO_4$ solution after harvest.

Crop test	Plant part	Ca	K	Mg	N	Na	Cu	Zn
		mg (g DM)$^{-1}$					μg (g DM)$^{-1}$	
Control_1	Leaf	4.0 ± 0.9^{b}	33.3 ± 6.1^{b}	1.4 ± 0.2^{b}	38.9 ± 6.6^{a}	1.5 ± 0.2^{c}	14 ± 5^{a}	95 ± 27^{b}
	Stem	2.7 ± 0.7^{ab}	22.8 ± 4.0^{a}	1.3 ± 0.2^{b}	43.1 ± 5.4^{b}	2.0 ± 0.3^{b}	13 ± 4^{a}	91 ± 11^{a}
	Root	-	51.6 ± 7.04^{b}	1.5 ± 0.2^{a}	57.4 ± 5.0^{b}	2.6 ± 0.4^{b}	136 ± 36^{ab}	504 ± 107^{c}
Control_2	Leaf	7.1 ± 1.3^{a}	44.2 ± 5.9^{a}	1.8 ± 0.2^{ab}	43.3 ± 5.4^{a}	1.4 ± 0.2^{c}	4 ± 1^{b}	73 ± 15^{b}
	Stem	2.9 ± 0.6^{ab}	27.0 ± 4.5^{a}	1.6 ± 0.3^{a}	54.2 ± 7.1^{a}	2.2 ± 0.3^{b}	9 ± 2^{a}	76 ± 9^{a}
	Root	-	65.4 ± 3.6^{a}	1.7 ± 0.1^{a}	53.6 ± 4.0^{b}	2.5 ± 0.5^{b}	112 ± 24^{b}	327 ± 47^{b}
+NaCl	Leaf	5.9 ± 0.5^{a}	48.9 ± 3.0^{a}	0.5 ± 0.1^{c}	47.1 ± 2.5^{a}	12.6 ± 1.7^{a}	15 ± 1^{a}	206 ± 29^{a}
	Stem	2.1 ± 0.4^{b}	24.4 ± 7.4^{a}	0.5 ± 0.1^{c}	29.2 ± 2.3^{c}	2.9 ± 3.1^{ab}	12 ± 7^{a}	103 ± 6^{a}
	Root	-	33.5 ± 3.2^{c}	1.2 ± 0.2^{b}	57.0 ± 1.5^{b}	7.5 ± 5.9^{a}	185 ± 84^{a}	740 ± 169^{a}
-K	Leaf	6.5 ± 1.4^{a}	16.0 ± 3.0^{c}	1.8 ± 0.3^{a}	41.9 ± 9.3^{a}	6.2 ± 1.4^{b}	6 ± 2^{b}	83 ± 21^{b}
	Stem	3.0 ± 0.8^{a}	8.7 ± 1.9^{b}	1.2 ± 0.2^{b}	40.8 ± 8.3^{b}	4.6 ± 0.7^{a}	6 ± 2^{a}	75 ± 14^{a}
	Root	-	18.3 ± 1.0^{d}	1.7 ± 0.2^{a}	62.6 ± 2.4^{a}	7.8 ± 1.1^{a}	122 ± 15^{b}	341 ± 68^{c}
Optimum range*	Leaf	6–11	29–78	2.5–4.5	33–48	-	5–8.4	25–73

*From Hartz et al. (2007).

TABLE 8 Average of photosynthesis-related parameters including maximum quantum efficiency of PSII (Fv/Fm, unitless), transpiration rate (E, mmol water m^{-2} s^{-1}), stomatal conductance (gs, mmol water m^{-2} s^{-1}), photosynthesis rate (A, μmol CO_2 m^{-2} s^{-1}), and SPAD (unitless) measured on lettuce leaves in the four tests (Control_1, +NaCl, -K, and Control_2). Mean and standard deviation are obtained with plant individual measurements. The letters indicate significance grouping in accordance with Tukey HSD (p value <0.05) of values in the same columns.

Crop test	F_v/F_m	E	gs	A	SPAD
	Unitless	mmol water m^{-2} s^{-1}	mmol water m^{-2} s^{-1}	μmol CO_2 m^{-2} s^{-1}	Unitless
Control_1	0.83 ± 0.01^{b}	2.6 ± 0.5^{b}	0.2 ± 0.1^{a}	12.5 ± 1.7^{a}	23.7 ± 0.9^{a}
Control_2	0.84 ± 0.01^{ab}	3.5 ± 0.5^{a}	0.4 ± 0.1^{b}	15.0 ± 2.6^{a}	24.2 ± 2.1^{a}
+NaCl	0.85 ± 0.01^{a}	2.9 ± 0.7^{ab}	0.3 ± 0.1^{ab}	12.5 ± 2.9^{a}	24.2 ± 2.1^{a}
-K	0.85 ± 0.01^{a}	3.2 ± 0.7^{ab}	0.3 ± 0.2^{ab}	12.7 ± 4.8^{a}	23.8 ± 2.9^{a}

BPC at NASA under the same conditions (nutrient solution, light, etc.) delivered between 7.5 and 7.7 g dry matter m^{-2} day^{-1}; i.e., they delivered repeatable results (Wheeler et al., 1996). By comparing the results obtained in our work in the PCU to results from literature, we identified the test that provided results close to what could have been expected, and then we discuss the possible reasons that could have led to these differences.

Lettuce production data from different studies on lettuce conducted in growth chambers in ESA and NASA facilities are summarized in Table 9. Comparison between different experiments is challenged by various factors. One of them is the difference in seedling size and developmental stage at the start of the experiment. For example, Peiro et al. (2020) started with 7-day-old seedlings, whereas studies conducted in the PCU used 15- or 16-day-old seedlings. However, even seedlings of the same age may exhibit different developmental stages depending on the growth conditions. However, when the production over 28 days is expressed as g dry matter m^{-2} d^{-1}, as proposed by Wheeler et al. (1996), the highest and comparable growth rates were reported by Peiro et al. (2020) and Pannico et al. (2021), followed by those reported by Wheeler et al. (1996), by Schiefloe et al. (2023) under low NH_4:NO_3 ratio conditions, and by the Control_2 treatment of

TABLE 9 Comparison of results on the lettuce growth rate, water and O_2 production, and CO_2 fixation in different growth systems in ESA (MELiSSA) and NASA facilities to results obtained in this study. The photoperiod was 16 h day and 8 h night in all studies.

References	Treatment	Growth system	Cultivar	Light intensity	Air temperature	Plant density	Total dry matter per plant	Relative dry matter growth	Water produced		O_2 produced		CO_2 fixed[a]	
				μmol m^{-2} s^{-1}	day/ night °C	plant m^{-2}	g $plant^{-1}$	g m^{-2} day^{-1}	L m^{-2}	L m^{-2} day^{-1}	mol m^{-2}	mol m^{-2} day^{-1}	mol m^{-2}	mol m^{-2} day^{-1}
Wheeler et al. (1996) [b]	Optimal nutrient solution	BPC/NASA	Waldmann's green	293	23/23	19.2	11.0	7.6	53.3	1.9	6.5	0.2	6.4	0.2
Peiro et al. (2020) [c]	Optimal nutrient solution	MPP/MELiSSA	Grand rapids	460	26/20	20.0	12.7	9.1	nr	nr	nr	nr	nr	nr
Pannico et al. (2021)	Optimal nutrient solution	PCU/MELiSSA	Grand rapids	430	26/20	10.0	26.3	9.4	40.9	1.5	9.2	0.3	nr	nr
Schiefloe et al. (2023)	High NH_4:NO_3	PCU/MELiSSA	Grand rapids	430	26/20	10.0	13.0	4.6	nr	nr	4.1	0.1	4.5	0.2
	low NH_4:NO_3	PCU/MELiSSA	Grand rapids	430	26/20	10.0	21.0	7.5	nr	nr	6.9	0.2	6.6	0.2
This work	Control_1	PCU/MELiSSA	Grand rapids	430	26/20	5.6	25.6	5.1	17.8	0.6	4.8	0.2	4.7	0.2
	+NaCl	PCU/MELiSSA	Grand rapids	430	26/20	5.6	27.8	5.5	17.3	0.6	5.1	0.2	4.8	0.2
	-K	PCU/MELiSSA	Grand rapids	430	26/20	5.6	25.5	5.1	15.1	0.5	4.3	0.2	4.9	0.2
	Control_2	PCU/MELiSSA	Grand rapids	430	26/20	5.6	31.8	6.3	18.6	0.7	5.2	0.2	6.0	0.2

[a]CO_2 fixed was calculated using the C concentration and biomass of the different plant parts.
[b]The yield data shown here are taken from the tests 911, 921, and 931; the test 902 was not taken into account because it was performed under different light conditions (Wheeler et al., 1994).
[c]The yield data shown here are takenfrom the test done with an homogeneous air flow.
nr, not reported.

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the present study. Similarly, the amount of C fixed by the lettuce was highest and similar to that observed by Wheeler et al. (1996) and Schiefloe et al. (2023) in their treatment with low NH_4: NO_3 ratio, and our Control_2. Notably, in all these cases, plants were grown with an optimal nutrient solution. The treatment Control_1 of this study, on the contrary, had a very low productivity, close to that obtained in the treatment high NH_4: NO_3 ratio by Schiefloe et al. (2023) conducted with the same seedling size and the same experimental settings as the tests in this study. These results suggest that the plant growth during Control_1 was not representative of that of a lettuce growing under optimal conditions. Another argument in support of this claim is the widespread leaf necrosis observed on the plants growing in Control_1 and the suboptimal leaf Ca content. Although the productivity of Control_2 was lower than that observed for other plants growing under optimal conditions, which is probably due to the low plant density, we consider it as being representative for lettuce growing under optimal conditions at this density. An additional argument to consider Control_2 as the test providing the most relevant results for plants growing under optimal conditions is the fact that it reached a plateau in terms of the projected leaf area at approximately 25 DAT, as could have been expected from lettuce growing under optimal conditions (Wheeler et al., 1994). Following that, we will compare the performance of the plants grown in +NaCl and -K to that of the Control_2 treatment. The results presented in this table suggest that O_2 production expressed in mol m^{-2} and day was similar across the studies and that water production was lower in this study compared to that in Wheeler et al. (1996) and Pannico et al. (2021).

How can the poor plant productivity observed in the treatment Control_1 be explained? No objective reason could be found as the conditions were the same as those used for Control_2. Our results indicate a release of Zn and Cu from the hardware during the first tests. Although these values did not reach phytotoxic levels, they caused leaf concentrations that were above the optimal concentrations in Control_1 and +NaCl and in -K (only Zn) (Table 7). The accumulation of toxic compounds is a real risk in closed systems (Frossard et al., 2024).

4.2 Impact of suboptimal nutrient solutions on plant growth

4.2.1 Effect of NaCl addition to the nutrient solution on plant growth

Plants growing in solution enriched with NaCl showed significantly higher Na content in leaves (9× higher) and roots (3× higher) compared to Control_2. Furthermore, the Mg content of all plant organs of the +NaCl test was significantly lower than that in Control_2, which was probably due to the competition between Na and Mg in absorbance. The +NaCl plants showed a retardation in leaf growth compared to the Control_2 treatment. The server crash at 16 DAT may have contributed to the leaf growth retardation in +NaCl plants, despite a decrease in the PLA development of the +NaCl already being observed before the server crash. However, NaCl addition did not cause major changes in the O_2, water, and fresh and dry matter shoot production, or on photosynthesis-related parameters. The absence of a negative impact of NaCl and the increased EC on productivity is in contrast with previous publications (see Supplementary Material K). For instance, Breś et al. (2022) showed a significant decrease in fresh matter production in the lettuce Zeralda F1 following the addition of 20 mM NaCl in the nutrient solution compared to a solution without added NaCl; however, they observed no effect of NaCl addition on F_v/F_m as in our results. Similarly, Xu and Mou (2015) demonstrated that an input of 30 mM NaCl and 15 mM $CaCl_2$ led to a decrease in fresh weight and often in dry weight in most of the tested 178 lettuce cultivars. In contrast, this saline stress did not systematically affect SPAD readings and never affected the F_v/F_m ratio. The absence of response of Grand Rapids to an addition of 27 mM NaCl indicates that this cultivar tolerates salinity and high EC.

4.2.2 Effect of low K concentration on the nutrient solution on plant growth

The reduction in K concentration in the nutrient solution led to a decrease in O_2 and water production by 18% compared to that in Control_2. Since the O_2 and water productivity per mass of shoot is similar for all tests, this decrease can be explained by the reduction of biomass production of the -K treatment (−19% of leaf DW compared to Control_2). As expected, the K content in all the plant parts (leaf, stem, and roots) was significantly lower than that in the control. The plants growing in -K had a significantly lower PLA at 21 DAT compared to Control_2. However, this effect was not visible at harvest, which was likely because the Control_2 plants had reached full development before 28 DAT and because of the discussed high growth variability of Grand Rapids in the PCU. These results agree with what has been reported from the literature. Yang et al. (2007) grew the lettuce cultivar "Honglong" and "Lvling" in hydroponics in the presence of 1 and 4 mM K. They reported a decrease of approximately 400% of shoot dry matter production for both cultivars after 49 days of growth when the plants were grown with 1 mM K compared to 4 mM K. The decrease from 4 to 1 mM did not affect the stomatal conductance (gs), evaporation rate (E) and the ratio F_v/F_m. Similarly, Zhang et al. (2017) showed that a decrease in K concentration from 4 to 1 mM in the nutrient solution decreased lettuce dry matter shoot production by approximately 180% in three lettuce types (Green Leaf, Boston, and Romaine). The gs and E decreased significantly from 4 to 1 mM in the Green Leaf, and Boston lettuce, while the SPAD readings decreased in the Green Leaf and Romaine lettuce. The decreases in plant production observed in the two above-mentioned studies were much stronger than those observed in this study, indicating that Grand Rapids shows also a good tolerance to low concentration of K.

5 Conclusion

Under the tested conditions, lowering the K concentration in the nutrient solution reduced dry shoot biomass by 20%, resulting in lower water and O_2 production compared with the Control_2. These results, as those obtained by Schiefloe et al. (2023), show as expected that a plant nutrition strategy that is not adapted to plant needs can lead to decreased plant performance, such as decreased biomass and O_2 production, after a single crop cycle. The impact of such a decrease in plant performance on the sustainability of long (up to several years)-duration crewed Space missions will need to be modeled, e.g., by integrating a higher

plant model in an overall BLSS model (Fountain et al., 2025; Poughon et al., 2009; Poulet et al., 2016). Identifying plant cultivars resistant to different stresses will be essential to these missions. For instance, our study showed a good tolerance of Grand Rapids to salt stress, which limited its impact on biomass development and gas exchange. However, to obtain data that can be used to calibrate and validate models, it is essential to be able to conduct experiments under conditions providing precise and repeatable results. It is, therefore, urgent to continue developing plant production systems such as the MPP or the PCU that can work in totally closed systems, thus minimizing environmental variability at the plant level (light, temperature, and air velocity) as much as possible.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Author contributions

GP: Methodology, Visualization, Data curation, Formal Analysis, Conceptualization, Writing – review and editing, Investigation, Validation, Writing – original draft. AP: Conceptualization, Writing – review and editing, Methodology, Data curation, Investigation, Resources. MG: Writing – review and editing, Software, Methodology, Investigation. ØJ: Methodology, Conceptualization, Investigation, Writing – review and editing, Resources. NK: Software, Investigation, Writing – review and editing, Visualization, Methodology. SD: Writing – review and editing, Supervision, Resources. EF: Data curation, Resources, Visualization, Validation, Methodology, Writing – review and editing, Investigation, Conceptualization, Writing – original draft, Supervision, Funding acquisition.

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Conflict of interest

Author MG was employed by EnginSoft S.p.A.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors AP, SD declared that they were an editorial board member of Frontiers at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fspas.2026.1687731/full#supplementary-material

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