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Influence of Nutrient Solutions in an NGS® Soilless System on the Yield, Quality and Shelf Life of Fresh-Cut Commercial Mint at Different Harvest Times

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Abstract: The optimal fertilizer concentration for *Mentha* plants is contingent on the growing systems and harvest time, serving as operational solutions to control and enhance quality and yield. This study aimed to determine the effects of three macronutrients concentration in hydroponic nutrient solution (HNS) during three harvest times on the growth, quality, yield, and shelf life of three mint species (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR)) grown in a New Growing System (NGS®). Total dry matter (DM), nitrate (NO_3^-), phosphate (PO_4^{3-}), and calcium carbonate ($CaCO_3$) concentrations were increased with the addition of higher levels of nutrient fertilization in three species. When the ion concentration of the HNS was increased, total fresh yield decreased. The highest total bacterial count (TBC) was obtained in MR species in the three harvests in all the levels of HNS. The lowest browning potential (BP) and soluble o-quinone (So-Q) levels were observed at second harvest in the MR species with the application of one of the two HNS high in nitrogen (N). In conclusion, the combination of optimal HNS ion concentration and appropriate species is considered essential to obtain suitable yield, quality, and ensure shelf life of mint.

Keywords: biochemicals; fertilizer; *Mentha* sp.; post-harvest quality; soilless system

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1. Introduction

The demand for medicinal and aromatic plants as sources of phytochemical composition in the food, medicine and cosmetic industries has recently increased [1]. In medicinal and aromatic plants, growth, yield, phytochemical composition, and shelf life are influenced by various environmental factors, growing practices, and nutrient supply [1,2].

Mint belongs to the Lamiaceae family, which are stoloniferous or rhizomatous, annual or perennial, medicinal plants that constitute one of the most popular aromatic plants in the world [1]. The genus *Mentha* is characterized by 62 taxa belonging to 18 species and 11 hybrids worldwide [3]. Also, an increasing number of mint cultivars are constantly being introduced. Mint species and cultivars differ widely in their characteristics, such as plant appearance, flavour, and architecture [2].

As described in the literature, the genus *Mentha* has been used in ethnomedicine for the treatment of bronchitis, flatulence, anorexia, nausea, ulcerative colitis, and liver problems due to its anti-emetic, anti-inflammatory, antispasmodic, sudorific, analgesic, and stimulating activities [2,3]. Moreover, it is well reported in the literature that some *Mentha* species, including *M. spicata* and *M. piperita*, possess antimicrobial and antioxidant activities [4,5]. Mint species have a wide global dispersal and are extensively cultivated in temperate or subtropical climates in the Mediterranean region, America, China, Europe,

India, and Brazil. Mint can be grown outdoors or in controlled environments, but in colder climates the year-round production is more possible in controlled environments [3,6].

The Piedmont Region (Italy) is famous for its production of the Lamiaceae genera. The main local species is black peppermint (*M. piperita* L. var. *officinalis* forma *rubescens* Camus), but other mints are also cultivated, such as red spearmint (*M. spicata* L. var. *rubra*) and *M. spicata* L. var. *viridis* (syn. *M. viridis* Auct.) [7].

Nowadays, the demand for fresh mint has increased, and it is necessary to find sustainable and efficient systems for its cultivation. One solution can be using the soilless culture systems (SCSs) which have several advantages compared to the traditional culture system (TCS) in soil which include an increase in yield, cost-efficiency of fertilizer application and water, lower use of products for plant protection, and lower environmental pollution [8]. Because of the water shortage and land availability, it is highly recommended to conduct more investigations on the SCSs and their potential [9].

SCSs represents an interesting approach to growing plants efficiently because they allow for the precise control of plant nutrition [10]. The concentration, composition, and interval of application of the HNS used in the SCS are among the main variables/factors in the success of crop production [11]. The regulations of these factors are of utmost importance because high concentrations of nutrients induce nutrient imbalance, osmotic stress, and ion toxicity, while low concentrations usually lead to nutrient insufficiency stress [12]. As SCSs are highly productive in high plant densities and short culture cycles, they are frequently used for the production of high-value-added crops [9,13]. Moreover, SCSs allow for a reduction in the use of products for the plant protection contributing to the potential decrement of the environmental pollution [14]. There are several settings of SCS which are typically used all over the world, mainly including floating systems, aeroponic systems, pot systems, the nutrient film technique (NFT) system, and others derivatives [8]. The New Growing System (NGS®) is a SCS similar to NFT based on the recirculation of water in a closed-cycle system of the HNS through a multi-channel film [9]. The multilevel system has been developed to favour HNS aeration and avoid the bung effect due to root growth. The HNS can be pumped into the system intermittently according to the growth conditions and the plant needs [15]. One advantage of the NGS® is the reduced volume of HNS necessary for plant production. This, consequently, reduces the energy required by the system both for the recirculation and the maintenance of the optimal water temperature [9,16]. Concerning the safety aspects, as in other SCSs [17,18], microbial contamination might be better controlled in NGS® than in TCS in the open field [8]. Therefore, the optimum fertilization regime is important for improving growth, yield, and quality, as well as reducing production costs of aromatic plants [11,12].

Aromatic plants grown in SCSs require an accurate setting of water and nutrient management because the buffer effect of the substrate is minimum while the plant density is high. Hence, the optimization of the nutrient supplementations is necessary for the farmers to quantitatively and qualitatively maximize their production. Also, the nutrient concentration in the HNS used in SCSs is one of the most important factors for improving crop production [19].

Similar to other aromatic plants, in the cultivation of mint, the HNS can affect the plants appearance, growth, nutritional value, and shelf life. Several authors have presented experiments on mint with the aim of determining the best relation between the ratio of essential nutrients with plant growth and yield [20,21].

It is well established that in SCSs, the commercial production of aromatic plants requires adequate and regular levels of macro-, meso- and micro-nutrients to provide high-quality post-harvest characteristics required for long shelf life [11]. For instance, supplementation of high levels of N usually leads to storage disorders quality loss in post -harvest [22,23] and low contents of vitamin C, sugars, and dry weight [24]. Conversely, a low level of N leads to a decrease in the growth of the aerial part. Also, some studies indicated that utilizing N at higher values led to increased growth, yield, and essential oil content. Consequently, this resulted in higher essential oil content, specifically an increased

menthol content in the essential oil of *M. piperita* L. and *M. spicata* L., conversely, a deficit in N application reduces mint growth [25,26]. On the other hand, potassium (K) is involved in stomata closure and K deficit increases susceptibility to pathogens and may also interfere with the absorption of calcium (Ca^{2+}) [27].

In Italy, mint is usually cultivated in soil and the demand for the fresh-cut product is increasing in processing industry, cooking, and home consumption. The cultivation of mint in open fields gives yield only in a specific and limited period of the year (mainly in summer). The setting of NGS® in the greenhouse may be considered as a solution to alleviate the seasonal shortages of this crop. To the best of our knowledge, SCSs have not been adequately considered in the cultivation of mint. An innovative growing system such as NGS® can become an alternative to TCS in soil for mint cultivation to increase health, quality (without soil pollutants), and yield of this plant. This may also help to standardize the cultural practices and decrease the growing period and the production costs. Moreover, NGS® may be suitable for continuous harvests, regrowth, and exploiting the same root system [9]. The timing of harvesting significantly impacts the quantity and quality of mint. The yield, quality characteristics, and shelf life in mint plants exhibit a species-specific pattern, influenced by the expression of genes at various harvest stages. Consequently, research has delved into the impact of growth stages on mint [25,26,28,29], shedding light on their growth, yield, quality characteristics, shelf life, metabolites, and related biological activities, including antimicrobial and antioxidant properties. Research indicates that the timing of harvest significantly impacts both the yield and quality of *M. piperita*. The peak yields are achieved at the commencement of flowering, particularly concerning drug leaf yield, while optimal conditions for all other characteristics occur during the 100% flowering period [29,30].

Another critical issue in the cultivation of leafy vegetables is the frequency distribution of NO_3^- concentrations [31]. Due to the adverse effects of NO_3^- on human health, much attention has always been paid to the accumulation of this ion in vegetables [32]. Therefore, finding the best nutrition treatment to achieve a safe concentration of NO_3^- (not harmful to human health) is also considered in a part of our study.

The study aims to investigate the impact of varying N concentrations, specifically at doses of 6 and 12 mmol·L^{-1}, while maintaining constant levels of phosphor (P) and K (2 and 6 mmol·L^{-1}, respectively), or by doubling their concentrations in the HNS, on the yield and quality traits of three mint species grown in NGS® in a greenhouse. The evaluation also included assessing the shelf life of the fresh-cut product obtained from these mint species. The hypothesis posits that manipulating macronutrient concentrations in the nutrient solution will significantly influence the growth, yield, and quality characteristics of the mint plants, ultimately affecting the shelf life of the fresh-cut product obtained. Additionally, the study aims to address the issue of NO_3^- concentration in leafy vegetables, emphasizing the importance of finding optimal nutrition treatments that achieve safe NO_3^- levels, ensuring the produced mint is not harmful to human health. The results of the research are expected to contribute valuable insights into the potential benefits of using SCSs, particularly NGS®, for mint cultivation, offering a sustainable and efficient alternative to traditional soil-based methods or other soilless systems.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The study was conducted at the Experimental Center of the Department of Agricultural, Forest and Food Sciences (DISAFA) (44°53′11.67″ N; 7°41′7.00″ E—231 m a.s.l.), in Tetti Frati, Carmagnola (TO), Italy, from January to July in an automatically controlled greenhouse. A factorial in time experiment was carried out in a randomized complete block design (RCBD) with three replications. The factorial design included two levels of N (6 mmol·L^{-1} (N6) and 12 mmol·L^{-1} (N12)), and two ratios of P and K (constant K-P and doubled, with levels of 2 and 6 mmol·L^{-1} (P2K6) and 4 and 12 mmol·L^{-1} (P4K12)). The plant materials used were commercial mints (*M. spicata* L. var. *viridis* (MV);

M. piperita L. (MP); *M. spicata* L. var. *rubra* (MR) (S.A.I.S. S.p.A., Cesena (FC), Italy) propagated from mother plant cuttings in 60-cell Styrofoam trays; then, the plants were subsequently transferred into pots utilizing the Neuhaus Huminsubstrat N17, which is a peat-based horticultural medium. Plant lets were kept at 20 °C day and night and were overhead irrigated twice daily for 1 min (until transplanting). The nursery phase was conducted following standard cultural practices. When the plants were at an appropriate development stage, they were moved into a Lab-scale Pilot Plant (LSPP) based on the NGS® technology [9]. Max, min, and mean temperatures during the growing period in the greenhouse were 43 °C, 2 °C, and 17.3 °C, respectively.

During the cultivation in NGS®, four levels of HNS were tested (Table 1). Salts (purity > 98%) were dissolved in tap water with a known salt content to create the HNS. The tap water has an electrical conductivity (EC) of 440 $\mu S \cdot cm^{-1}$, with a pH of 7.5 and 24 °F of hardness. During the cultivation in NGS®, the nutrient solution was used in this protocol, which contained the following macroelements: (NH_4) H_2PO_4, $(NH_4)_2SO_4$, K_2SO_4, KH_2PO_4, $Ca(OH)_2$, $MgSO_4$, $7H_2O$ NH_4NO_3, and microelements (Oligogreen 30 mg L^{-1} with the following composition: Fe EDTA: 2%, Mn EDTA: 4%, Zn EDTA: 3%, Cu EDTA: 1%, B: 0.05%, and Mo 0.05% and Kelagreen 10 mg L^{-1} with the following composition: Fe 11%, Mn 13%, Zn 14% and Cu 14%; Green Has Italia S.p.a., Canale d'Alba (CN), Italy) were added to the HNS. All the HNS prepared had a ratio of 40/60 N-NO_3^-/N-NH_4^+. The EC, pH, and temperature were measured in the HNS by means of a Waterproof CyberScan PC 650 (Eutech Instruments Pte Ltd., Singapore), equipped with an EC/temperature probe (CONSEN9203J) and a submersible pH electrode (ECFC7252203B). The pH in the HNS was monitored continuously and kept close to 5.5, while the EC was between 2.0 and 2.5 dS m^{-1}. The temperature of the HNS was 20–25 °C, and the dissolved oxygen was measured by using an oximeter (YSI 550A; YSI, Inc., Yellow Springs, OH, USA), and was between ca. 7 and 9 ppm throughout the growing cycle. The total number of plants used was ca. 360 (ca. 40 plants/m^2).

Table 1. Hydroponic nutrient solutions (HNS) composition used in the experiment (mmol·L^{-1}). N6: 6 mmol·L^{-1}; N12: 12 mmol·L^{-1}; P2: 2 mmol·L^{-1}; P4: 4 mmol·L^{-1}; K6: 6 mmol·L^{-1}, and K12: 12 mmol·L^{-1}.

	N6P2K6	N12P2K6	N6P4K12	N12P4K12
N	6.0	12.0	6.0	12.0
P	2.0	2.0	4.0	4.0
K	6.0	6.0	12.0	12.0
Ca	2.5	2.5	2.5	2.5
Mg	2.0	2.0	2.0	2.0

The experiment consisted of harvesting mint plants three times when the plants reached a suitable maturity stage. After the first canopy harvest, plants were allowed to regrow twice with the aim of evaluating the system efficiency and having an alternative to the common agronomic practice of the mint in the surrounding area [33]. Normal/conventional cultivation practices consist of two annual harvests, one in early and one in late summer.

The first harvesting took place 37, 42, and 49 days after transplanting for MV, MR, and MP, respectively, while the second harvesting took place 46, 37, and 43 days after the first harvest, and the third harvesting took place 30, 37, and 30 days after the second harvest. All the procedures followed the standard practices to obtain replicable and comparable data, and the timing efficiency of the sampling procedures [9,34]. Upon the completion of the harvest, the raw material was promptly conveyed to the post-harvest laboratory for further analysis and the preparation of the fresh-cut products.

2.2. Raw Material Analysis

The biometrical measurements recorded were the leaf fresh weight (LFW) per m^2 and LFW per plant. The dry matter (DM) was determined through a process of drying at a temperature of 40 °C.

2.3. Weight Loss

Weight loss (WL) of fresh-cut products was determined by weighing the bags daily during storage and it was calculated progressively based on the comparison with the at-harvest (d0) value as an index of freshness decay.

2.4. Fresh-Cut Processing

Samples (50 g) were packaged in thermo-sealed bags (0.25 m × 0.35 m) that had previously been prepared with polypropylene film (Alvapack S.r.l., Bologna, Italy). The packaged specimens were maintained at a temperature of 4 °C for a duration of nine days in refrigerated chambers, which were completely shielded from any form of light within the display cabinet.

2.5. Headspace Analysis

Individual packages' headspace gas composition was monitored using an O_2 analyzer with an electrochemical ceramic oxide–zirconia detector (CG-1000, Ametek, Thermox Instruments Co., Pittsburgh, PA, USA) and infrared CO_2 detectors (Via 510, Horiba Instruments Co., Irvine, CA, USA). Overall, 1 mL samples (per package) gas were measured, and the results were reported as the mean of the three packages.

2.6. Leaf Colour

The measurement of leaf colour was conducted utilizing a CR10 colorimeter (Konica-Minolta Sensing Inc., Osaka, Japan). The L^* component signifies lightness, the a^* component represents values from green (−) to red (+), and the b^* component represents values from blue (−) to yellow (+). Prior to sampling mint leaves, the instrument underwent calibration using a Minolta standard white reflector plate.

2.7. Tissue Ion and Salt Content

Nitrate (NO_3^-), phosphate (PO_4^{3-}) and calcium carbonate ($CaCO_3$) contents were determined using a refractometric kit (Merck Reflectoquant RQflex2©; Darmstadt, Germany) following the manufacturer's instructions. A frozen tissue sample (10.0 g) was stomached with distilled water for 2 min and then filtered for refractometric measurement and expressed as mg kg^{-1} LFW for NO_3^- and mg g^{-1} LFW for PO_4^{3-}, and $CaCO_3$.

2.8. Browning Potential and Soluble o-Quinone Content

The browning potential (BP) and soluble o-quinone (So-Q) content were determined from 5 g of frozen tissue according to the methodologies of Couture et al. [35] and Loaiza-Velarde and Saltveit [36]. The results were stated as raw absorbance units (Abs_{340} and Abs_{437} for BP and So-Q, respectively).

2.9. Microbial Analysis

The total bacterial count (TBC) was determined using the Plate Count Agar substrate and the mould and yeast count (MC and YC, respectively) were determined using the Yeast Extract Glucose Chloramphenicol Agar substrate. A total of 25.0 g of fresh tissue from each sample was subjected to stomaching for a duration of 2 min at normal speed, using 225.0 mL of Ringer's buffer. The resulting mixture was then diluted and subsequently transferred into Petri dishes containing the selective substrate. The TBC was performed after incubation for 48 h at 30 °C, while both MC and YC were performed after incubation for 5 days at 30 °C. The results were expressed as colony-forming units (CFU) g^{-1} LFW.

2.10. Experimental Design and Statistical Analysis

During the data analysis, the effects of various factors were determined through the utilization of analysis of variance (ANOVA) employing the general linear model (GLM) procedure within the Statistical Analysis System (SAS 9.4) software. The PROC UNIVARIATE function within SAS was employed to assess the assumptions of ANOVA, and it was determined that the residuals exhibited a normal distribution. To check significant differences between the means, the least significant difference (LSD) test with p values < 0.05 was used. In the case of significant interaction, the LS means procedure was used to compare significant interactions. When an F-test indicated statistical significance at $p < 0.05$, the protected least significant difference was used to separate the means of main effect and the significant interactions were separated by the slicing method. When the interactions were not significant, we only discussed the main effects. When the main effects, two-way, and the three-way interaction effects of the traits were significant, we only discussed the three-way interaction effects or when the main effects, or two-way interaction traits were significant, we only discussed the two-way interaction effects.

3. Results

3.1. Quantitative Characteristics

The results of analysis of variance of DM, LFW, yield, and LDG are shown in Table S1. The highest amount of LFW and yield in all HNS treatments was related to the third harvest time. The results of HNS and harvest time interaction showed that the highest amount of LFW was related to the application of N6P2K6 and N12P2K6 treatments at the second harvest (29.99 and 30.04 g/plant, respectively), and the lowest amount of LFW was observed in the application of N12P4K12 treatment at the third harvest time (11.76 g/plant).

The results of HNS and harvest time interaction showed that the highest yield was observed in the application of N6P2K6 and N12P2K6 at the second harvest with 1199 and 1201 $g \cdot m^{-2}$, respectively (Table 2). In contrast, the lowest yield was observed in the application of N12P4K12 at the third harvest with 470 $g \cdot m^{-2}$.

Table 2. Influence hydroponic nutrient solutions (HNS) and harvest time (first harvest: I harvest, second harvest: II harvest, regrowth and third harvest: III harvest, regrowth) on the leaf fresh weight per plant (LFW), leaf daily growth (LDG) and yield of mint species in a new growing system (NGS®). N6: 6 mmol·L^{-1}; N12: 12 mmol·L^{-1}; P2: 2 mmol L^{-1}; P4: 4 mmol L^{-1}; K6: 6 mmol L^{-1} and K12: 12 mmol L^{-1}.

HNS	Harvest Time	LFW (g/plant)	Yield (g m^{-2})	LDG (g/plant/d)
N6P2K6	I harvest	17.9 ± 1.3 cd	719.4 ± 52.9 cd	0.43 ± 0.03 c–e
	II harvest, regrowth	29.9 ± 2.6 a	1199.5 ± 105.7 a	0.72 ± 0.07 a
	III harvest, regrowth	21.9 ± 3.5 b	877.6 ± 138.3 b	0.67 ± 0.09 a
N12P2K6	I harvest	19.2 ± 0.9 bc	766.7 ± 36.4 bc	0.45 ± 0.02 cd
	II harvest, regrowth	30.1 ± 2.0 a	1201.8 ± 80.2 a	0.72 ± 0.05 a
	III harvest, regrowth	18.2 ± 2.1 cd	729.4 ± 85.1 cd	0.56 ± 0.06 b
N6P4K12	I harvest	13.6 ± 1.2 ef	544.7 ± 46.4 ef	0.32 ± 0.03 f
	II harvest, regrowth	20.3 ± 1.9 bc	812.9 ± 79.2 bc	0.49 ± 0.05 bc
	III harvest, regrowth	15.1 ± 1.7 de	605.9 ± 66.2 de	0.46 ± 0.04 c
N12P4K12	I harvest	14.3 ± 0.5 ef	571.5 ± 20.0 ef	0.34 ± 0.01 ef
	II harvest, regrowth	20.1 ± 1.7 bc	802.8 ± 66.9 bc	0.49 ± 0.03 bc
	III harvest, regrowth	11.8 ± 1.6 f	470.2 ± 65.8 f	0.36 ± 0.05 d–f

Means followed by different letters in the same column for the same factor are significantly different ($p \leq 0.05$) according to the LSD test.

The results of LDG also showed that LDG was affected by the interaction of HNS and harvest time and its highest value was obtained in the treatment of N12P2K6 at the second harvest (0.72 g/plant/d) and N6P2K6 at the second and third harvests (0.72 and 0.67 g/plant/d, respectively). In contrast, the lowest amount of LDG was observed in

N6P4K12 treatment at the first harvest (0.32 g/plant/d). The results showed that the amount of LDG at the first and third harvests was the lowest, and the amount at the second harvest was the highest in all HNS treatments.

The interaction of harvest time and species showed that the highest amount of DM was related to MP species at the third harvest (22.75%), whereas the lowest amount of DM was observed in MR species in the second harvest (16.06%) (Table 3). The results showed that in all species, the highest amount of DM was related to the third harvest and the lowest amount was observed at the second harvest.

Table 3. Influence of harvest time (first harvest: I harvest, second harvest: II harvest, regrowth and thrid harvest: III harvest, regrowth) on the dry matter (DM), leaf fresh weight per plant (LFW), yield and leaf daily growth (LDG) of three commercial mint (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR) cultivated in a new growing system (NGS®).

Species	Harvest Time	DM (%)	LFW (g/plant)	Yield (g m^{-2})	LDG (g/plant/d)
MW	I harvest	18.2 ± 0.3 c	14.1 ± 0.9 e	562.1 ± 37.6 e	0.38 ± 0.0 de
	II harvest, regrowth	16.0 ± 0.4 de	30.8 ± 1.6 a	1233.9 ± 63.9 a	0.67 ± 0.04 ab
	III harvest, regrowth	19.7 ± 0.3 b	17.4 ± 1.6 d	695.5 ± 65.3 d	0.58 ± 0.05 c
MP	I harvest	17.2 ± 0.3 cd	16.6 ± 0.9 de	662.4 ± 37.1 de	0.34 ± 0.02 e
	II harvest, regrowth	17.2 ± 0.3 cd	18.4 ± 1.4 d	734.8 ± 57.4 d	0.44 ± 0.03 d
	III harvest, regrowth	22.7 ± 0.6 a	10.4 ± 1.5 f	417.1 ± 58.4 f	0.35 ± 0.05 e
MR	I harvest	17.8 ± 0.3 cd	18.2 ± 1.1 d	727.2 ± 45.1 d	0.43 ± 0.03 d
	II harvest, regrowth	16.1 ± 0.3 e	26.1 ± 2.1 b	1044.1 ± 84.2 b	0.71 ± 0.06 a
	III harvest, regrowth	19.3 ± 0.4 b	22.5 ± 2.1 c	899.8 ± 83.5 c	0.61 ± 0.06 bc

Means followed by different letters in the same column for the same factor are significantly different ($p \leq 0.05$) according to the LSD test.

Among the three studied species, MP had the highest amount of DM and MW and MR species did not differ significantly in terms of DM (Table 3). In addition, the highest amount of LFW was related to MW species at the second harvest (30.85 g/plant), while the lowest amount was observed in MP species in the third harvest (10.43 g/plant). Among the three studied species, MW and MR species had the highest amount of LFW and, while MP species had the lowest amount of LFW among all studied species, no significant differences were found between the species. The highest and the lowest yield in the interaction effect of harvest time and species were related to MW at the second harvest (30.85 g/plant) and MP at the third harvest (10.43 g/plant) (Table 3). Among the three studied species, MP had the lowest yield and MW and MR had the highest yield. Also, in all the studied species, the second harvest had the highest yield compared to the time of the first and third harvests.

The amount of LDG was affected by the interaction effect of harvest time and species. The lowest amount of LDG was related to MP species. On the other hand, the highest amount of LDG were observed in the MW and MR species. The highest level of LDG in MR species was related to the second harvest (0.71 g/plant/d). In MP species, the first and third harvests had the lowest LDG, with 0.34 and 0.35 g/plant/d, respectively (Table 3). Furthermore, LDG was affected by the osmotic stress of high-concentration treatments; however, the amount of N did not induce a significant difference in LDG.

LFW and yield were affected by the interaction of species and HNS. The highest LFW and yield were related to MR species and N6P2K6 (Figure 1a,b). In contrast, the lowest amount of LFW was observed in MP species in N6P4K12 and N12P4K12 treatments. Among different HNS treatments, N6P2K6 and N12P2K6 had the highest amount of LFW and yield and no significant difference was observed between them. In contrast, the lowest amount of LFW and yield were related to N6P4K12 and N12P4K12 treatments.

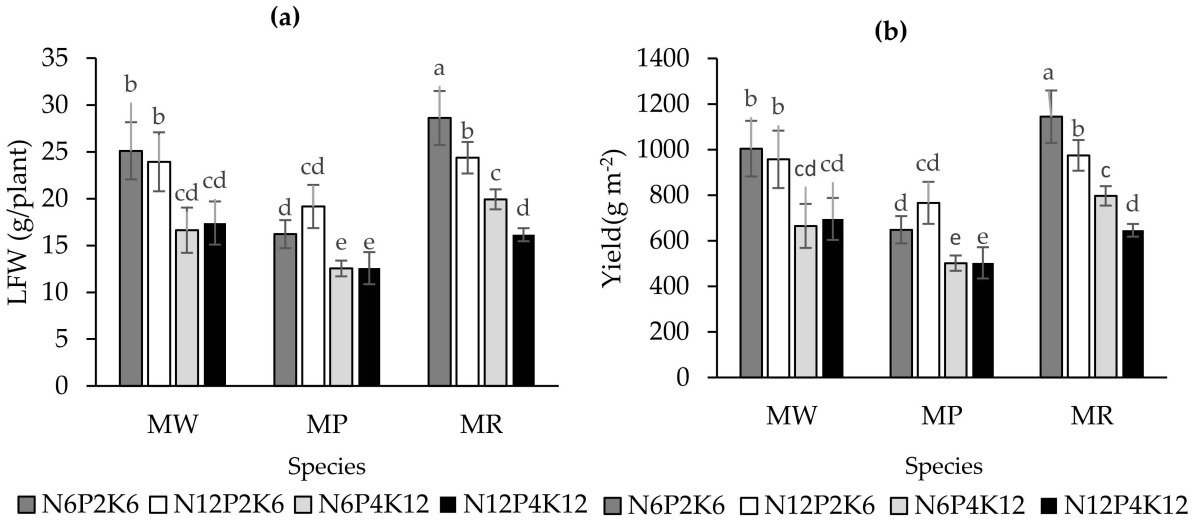


Figure 1. Influence hydroponic nutrient solutions (HNS) on the leaf fresh weight per plant (LFW) (**a**) and yield (**b**) of commercial mint (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR) in a new growing system (NGS®). N6: 6 mmol·L^{-1}; N12: 12 mmol L^{-1}; P2: 2 mmol·L^{-1}; P4: 4 mmol L^{-1}; K6: 6 mmol L^{-1} and K12: 12 mmol L^{-1}. Means followed by different letters in the same column for the same factor are significantly different ($p \leq 0.05$), according to LSD test.

3.2. Qualitative Characteristics

3.2.1. Colour Parameters

Red-green (*a**) colouring was also affected by species and harvest time interaction (Table S1). The highest amount of *a** was related to MR species at the second harvest time (11.93). In contrast, the lowest amount of *a** was observed in MP species at the second harvest time (7.83) (Figure 2).

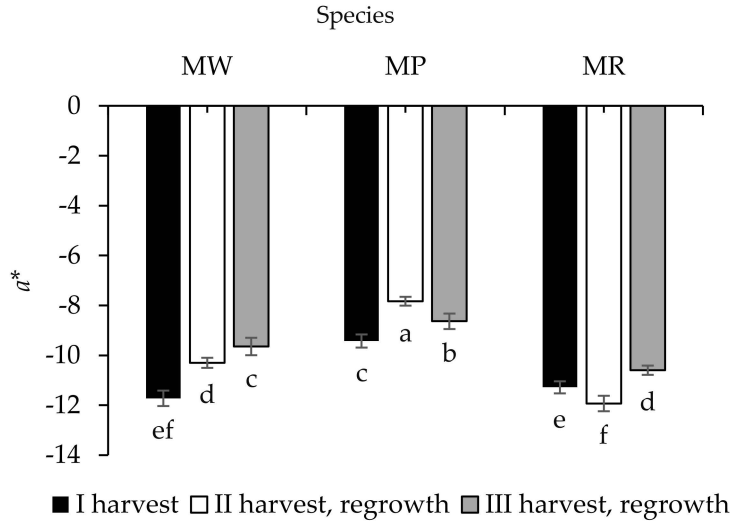


Figure 2. Influence of harvest time (first harvest: I harvest, second harvest: II harvest, regrowth and third harvest: III harvest, regrowth) on the colour parameter (*a**) of commercial mint (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR) in a new growing system (NGS®). Means followed by different letters in the same column for the same factor are significantly different ($p \leq 0.05$) according to LSD test.

Among the species, MR had the highest lightness (*L**) and yellow-blue colouring (*b**). In addition, the comparison between different HNS indicated that N6P2K6 treatment had the highest levels of *L** and *b**. Also, the comparison between different harvest times

showed that the first and third harvest times had the highest amount of L^* and b^*, while the second harvest time had the lowest amount of L^* and b^* (Table 4). The effect of harvest time, species and HNS interaction showed that the amount of L^* in N6P2K6 treatment among MR species and N6P4K12 treatment in MR species had the highest values out of all harvest times. On the other hand, the highest amount of b^* was observed in N6P2K6 and MW species at the first and third harvest times. However, at the second harvest, N6P2K6 treatment in MR species had the highest amount of b^* (Table 4).

Table 4. Influence of harvest time (first harvest: I harvest, second harvest: II harvest, regrowth and third harvest: III harvest, regrowth) and hydroponic nutrient solutions (HNS) on the colour parameters value of three commercial mint (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR) cultivated in a new growing system (NGS®). N6: 6 mmol·L^{-1}; N12: 12 mmol L^{-1}; P2: 2 mmol L^{-1}; P4: 4 mmol L^{-1}; K6: 6 mmol L^{-1} and K12: 12 mmol L^{-1}.

HNS	Species	Harvest Time					
		I Harvest		II Harvest, Regrowth		III Harvest, Regrowth	
		L^*	b^*	L^*	b^*	L^*	b^*
N6P2K6	MW	43.7 ± 0.3 a–c	27.0 ± 0.1 a	39.5 ± 0.9 cd	19.8 ± 0.8 cd	44.8 ± 0.4 a	23.4 ± 1.1 a
	MP	42.8 ± 0.8 b–d	21.5 ± 0.9 cd	38.3 ± 0.7 de	14.2 ± 0.7 fg	42.9 ± 0.9 bc	20.1 ± 1.1 b
	MR	44.1 ± 0.4 ab	24.6 ± 0.5 b	43.9 ± 0.8 a	23.6 ± 1.6 a	44.6 ± 0.8 ab	22.6 ± 0.5 a
N12P2K6	MW	39.7 ± 0.1 e	19.2 ± 1.0 e	38.7 ± 0.4 de	15.8 ± 0.3 ef	37.8 ± 0.3 g	13.5 ± 0.3 e
	MP	38.9 ± 0.3 ef	15.1 ± 0.4 f	37.5 ± 0.2 e	12.3 ± 0.3 g	39.2 ± 1.1 fg	13.9 ± 1.1 de
	MR	39.9 ± 0.1 e	17.7 ± 0.1 e	40.9 ± 0.4 bc	18.8 ± 1.3 cd	40.9 ± 0.2 de	16.5 ± 0.1 c
N6P4K12	MW	42.7 ± 0.3 cd	25.3 ± 0.6 ab	41.4 ± 0.1 b	21.0 ± 0.1 bc	41.6 ± 0.5 cd	18.9 ± 0.2 b
	MP	43.6 ± 0.2 a–c	22.9 ± 0.2 c	38.1 ± 0.5 de	13.3 ± 0.6 fg	40.7 ± 0.6 def	16.6 ± 1.0 c
	MR	44.3 ± 0.3 a	24.6 ± 0.5 b	44.3 ± 0.5 a	23.6 ± 1.4 ab	43.4 ± 0.4 ab	19.8 ± 0.5 b
N12P4K12	MW	38.3 ± 0.3 f	18.9 ± 0.6 e	39.3 ± 0.7 cd	17.7 ± 0.7 de	39.2 ± 0.4 efg	16.0 ± 0.3 c
	MP	39.9 ± 0.2 e	17.6 ± 0.4 e	38.1 ± 0.2 de	12.4 ± 0.5 g	39.5 ± 0.4 ef	15.5 ± 0.6 cd
	MR	41.6 ± 0.1 d	21.1 ± 0.7 d	40.9 ± 0.5 bc	18.6 ± 0.8 cd	40.7 ± 0.2 def	16.2 ± 0.1 c

Means followed by different letters in the same column for the same factor are significantly different ($p \leq 0.05$) according to the LSD test.

3.2.2. Weight Loss

Different concentrations of HNS did not significantly affect the WL in different harvest time; however, in different species with different harvesting times, we observed a significant difference in WL (Table S2). After nine days of storage, the variation in WL was significantly different. In the first days of storage, weight gain was very low. This increase in weight was accelerated at the third harvest, especially in MR. The weight gain in the first days of storage, especially at the third harvest, can be due to the fact that plants were kept at warehousing bags with holes that allow for the exchange of carbon dioxide (CO_2), oxygen (O_2), and water vapor at the beginning of the storage period, which results in more available water in this period. However, the highest WL occurred at the first (1.07%) and the third (0.27%) harvest times in the MV species, and at the second harvest WL occurred in the MR species on the ninth day (1.26%). Among the three species, the lowest WL was related to MP (Figure 3). Among the harvesting times, the highest WL was observed at the second harvest time (0.11%), and the lowest WL belonged to the third harvest time. Among the HNS treatments, the highest rate of WL occurred in the N12P4K12; however, the WL rate was not significantly different from the HNS used. Nevertheless, at the first harvest time, the highest reduction in the MV species was found in the N12P4K12 and at the second harvest time treatment (0.61%). Furthermore, the highest reduction in MR species was observed in the N12P4K12 treatment (0.44%) (Figure 4).

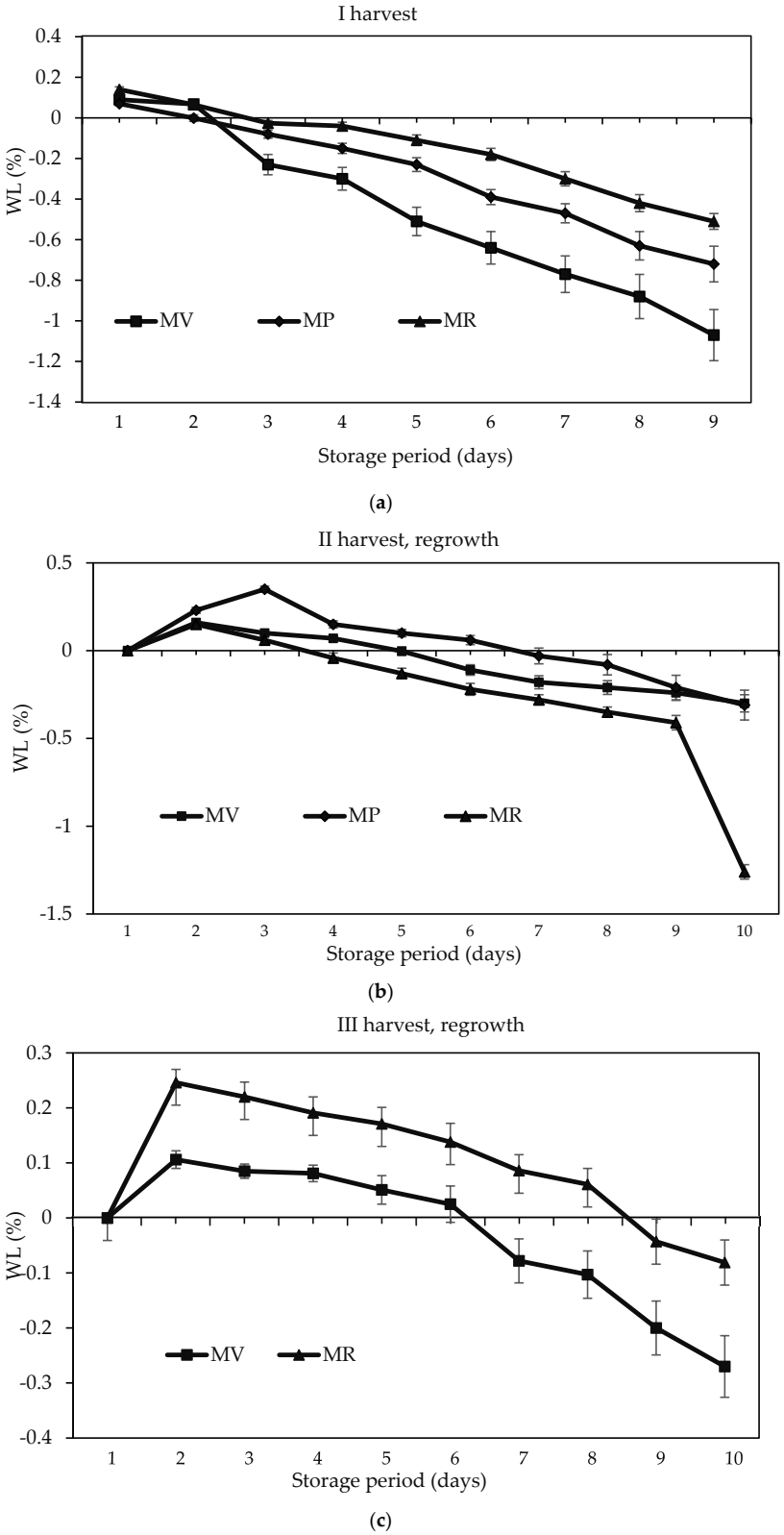
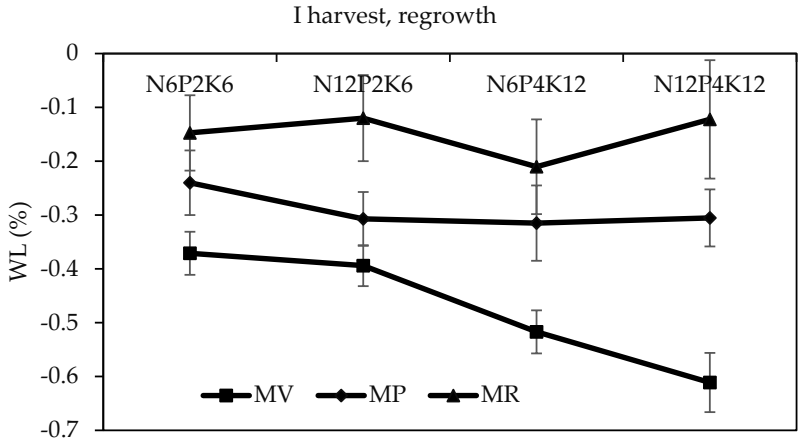
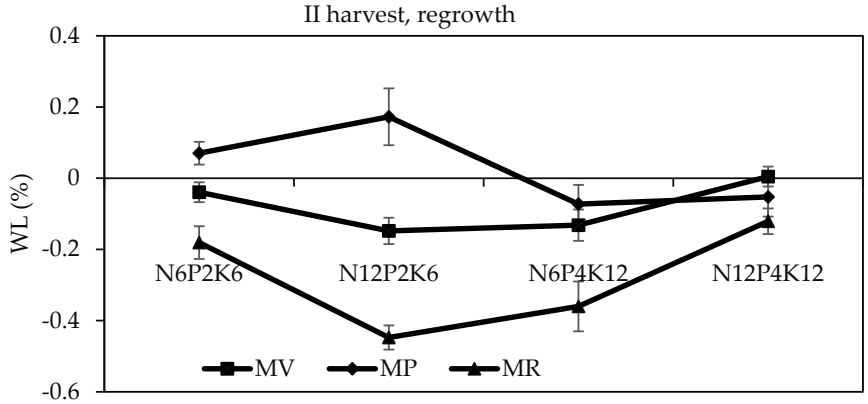


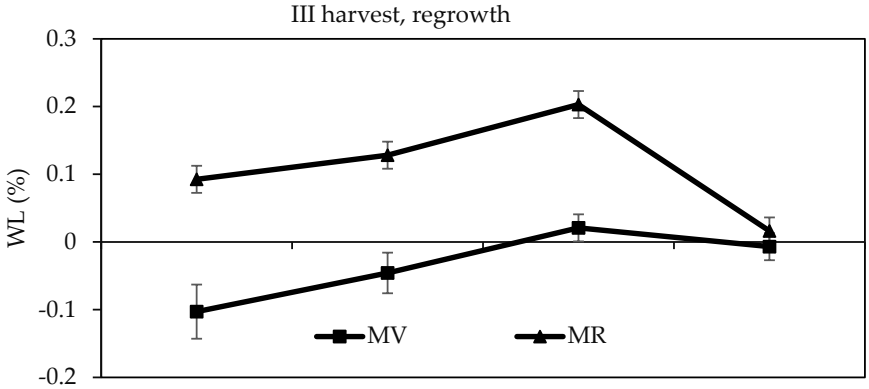
Figure 3. Influence of harvest time (first harvest: I harvest (**a**), second harvest: II harvest (**b**), regrowth and third harvest: III harvest (**c**), regrowth) on weight loss (WL) the headspace partial pressure of commercial mint (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR) daily during storage at 4 °C. Values are the mean of replicates ± SE.



(**a**)



(**b**)



(**c**)

Figure 4. Influence of hydroponic nutrient solutions (HNS) and harvest time (first harvest: I harvest (**a**), second harvest: II harvest (**b**), regrowth and third harvest: III harvest (**c**), regrowth) on weight loss (WL) the headspace partial pressure of commercial mint (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR) stored at 4 °C. N6: 6 mmol·L^{-1}; N12: 12 mmol L^{-1}; P2: 2 mmol L^{-1}; P4: 4 mmol L^{-1}; K6: 6 mmol L^{-1} and K12: 12 mmol L^{-1}. Values are the mean of replicates ± SE.

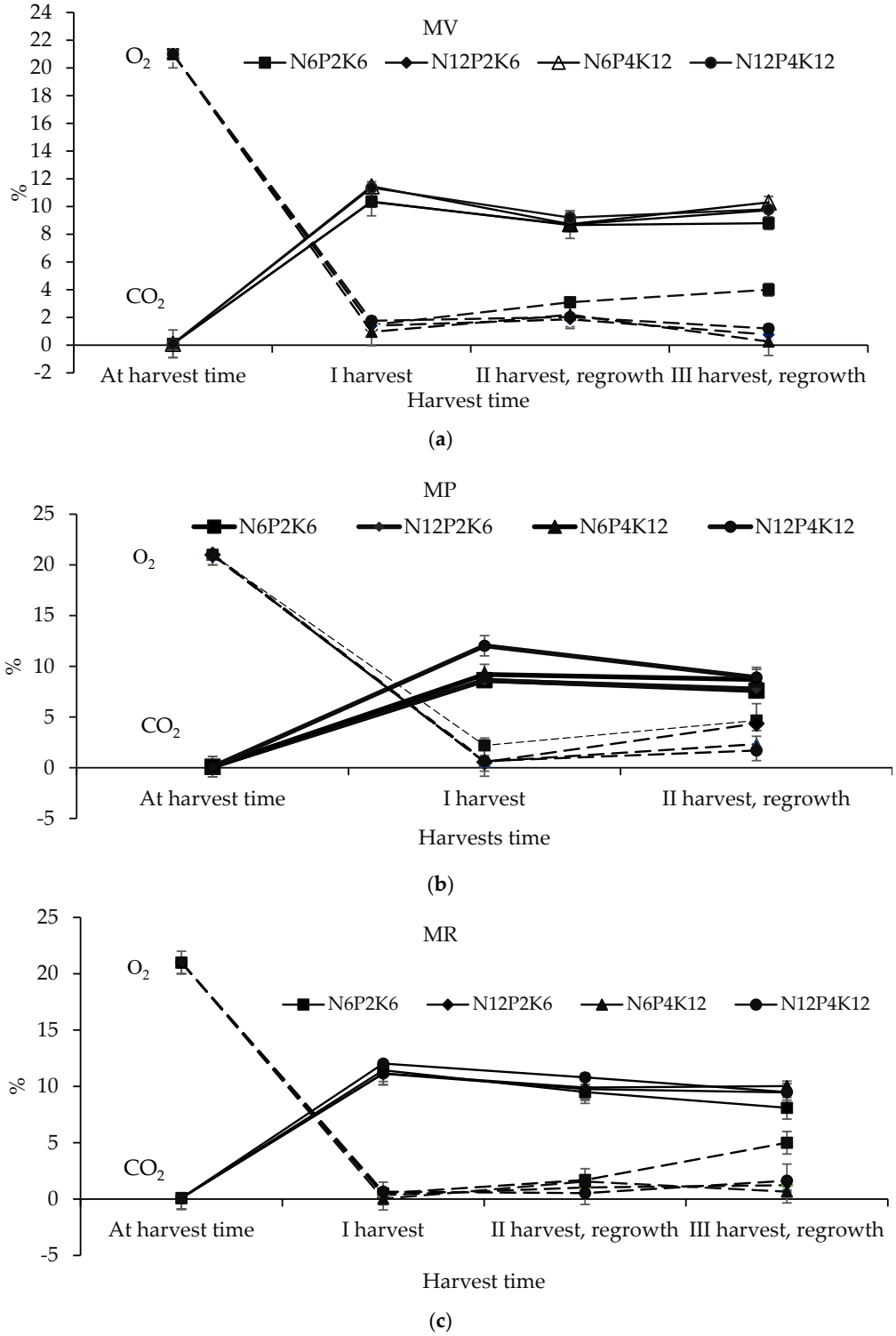


Figure 5. Influence of hydroponic nutrient solutions (HNS) and harvest time (first harvest: I harvest, second harvest: II harvest, regrowth and third harvest: III harvest, regrowth) on the headspace partial pressure of O_2 and CO_2 (%) of fresh-cut, commercial mint (*M. spicata* L. var. *viridis* (MV) (**a**); *M. piperita* L. (MP) (**b**); *M. spicata* L. var. *rubra* (MR) (**c**)© stored for 9 days at 4 °C. N6: 6 mmol L^{-1}; N12: 12 mmol L^{-1}; P2: 2 mmol L^{-1}; P4: 4 mmol L^{-1}; K6: 6 mmol L^{-1} and K12: 12 mmol L^{-1}. Values are the mean of replicates ± SE.

3.2.3. Respiration Rate and Headspace Analysis

Analyzing O_2 and CO_2 levels in the fresh-cut bags showed that HNS, species, and harvesting time significantly affected the parameters during the storage period (Table S2). In most tests, during the storage period, the O_2 rate usually decreased, and the rate of the produced CO_2 increased as a result of breathing. The initial concentrations of O_2 and CO_2 increased and decreased, respectively, at the same rate nine days after storage. As shown in Figure 5, after nine days of storage, the highest rate of CO_2 was obtained in the treatments with the highest concentrations of nutrients (N12P4K12), but the lowest rate was related to N6P2K6 and N12P2K6 treatments. In contrast, the N6P2K6 treatment resulted in the highest amount of O_2, while the lowest amount was associated with the N12P4K12 treatment (Figure 5). The highest rate of the produced CO_2 was related to the MR species and the lowest rate of produced CO_2 was recorded in the MP species (10.23 and 8.65%, respectively). In contrast, the highest rate of the produced O_2 was in the MP species and the least rate was related to the MR species (2.09 and 1.24%, respectively) at the first harvest time for the N6P2K6 treatment. The highest and lowest rates of CO_2 were obtained at the first and second harvest times, respectively (9.03 and 10.45%, respectively). On the other hand, the highest rate of O_2 was obtained at the second harvest time (2.25%), while the lowest rate was achieved at the first harvest time (0.91%) (Figure 5).

3.2.4. Mineral Ion Determinations

The results showed that HNS and species at different harvest times had a significant impact on the accumulation of NO_3^-, PO_4^{3-} and $CaCO_3$ in the tissue; however, the interaction between HNS and species was not significant (Table S2). The highest accumulation of NO_3^-, PO_4^{3-} and $CaCO_3$ was obtained at the third harvest time. The highest amount of NO_3^- was obtained in the N12P2K6 treatment on the first day of the third harvest time (2227.33 mg kg^{-1} LFW). Generally, the highest NO_3^- accumulation was observed at the third harvest, and the lowest accumulation rate was related to the first harvest time. Concerning the HNS, the highest accumulation of NO_3^- was obtained in N12P2K6 and the lowest accumulation values were observed in N6P2K6 and N6P4K12 treatments. Among the species, the highest NO_3^- accumulation in all harvesting times was in the MP species; however, MV and MR species did not significantly differ in NO_3^- accumulation (Table 5). There was a significant difference between the rates of PO_4^{3-} accumulation in the mint plant at different harvesting times. At all harvesting times, the highest accumulation of PO_4^{3-} was achieved in N6P4K12 and N12P4K12 treatments, and the lowest accumulation was obtained in N12P2K6. The results of the present experiment indicated that the high levels of P and K in the HNS led to an increase in PO_4^{3-} in the plant. Among the species, the highest accumulation of PO_4^{3-} was observed in MP at all harvesting times. Among the harvesting time, the highest accumulation of PO_4^{3-} was achieved at the third harvest time. The accumulation of $CaCO_3$ decreased by increasing the amount of K. According to the results, the highest accumulation of $CaCO_3$ was in N12P2K6 at all harvesting times; among the harvesting times, the highest accumulation was obtained at the third harvest. There was a significant difference among species in $CaCO_3$ at different harvest times, so the highest accumulation rate was observed in MP species.

Table 5. Influence hydroponic nutrient solutions (HNS) and harvest time (first harvest: I harvest, second harvest: II harvest, regrowth and third harvest: III harvest, regrowth) on the nutrients content (Nitrate (NO_3^-), phosphate (PO_4^{3-}) and calcium carbonate ($CaCO_3$) concentrations) of three commercial mint (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR) in the fresh-cut during its shelf life (at harvest (0) and nine days (9)) cultivated in a new growing system (NGS®). N6: 6 mmol L^{-1}; N12: 12 mmol·L^{-1}; P2: 2 mmol L^{-1}; P4: 4 mmol L^{-1}; K6: 6 mmol L^{-1} and K12: 12 mmol L^{-1}.

Species	Storage (Days at 4 °C)	I Harvest			II Harvest, Regrowth			III Harvest, Regrowth		
		NO_3^- mg kg^{-1}	$CaCO_3$ mg g^{-1}	PO_4^{3-} mg g^{-1}	NO_3^- mg kg^{-1}	$CaCO_3$ mg g^{-1}	PO_4^{3-} mg g^{-1}	NO_3^- mg kg^{-1}	$CaCO_3$ mg g^{-1}	PO_4^{3-} mg g^{-1}
MV	0	251.5 ± 67.5 b	0.14 ± 0.02 c	0.40 ± 0.034 c	743.3 ± 161.3 c	0.36 ± 0.02 ab	0.52 ± 0.05 ab	1145.5 ± 205.9 b	0.49 ± 0.05 b	0.50 ± 0.05 b
	9	129.7 ± 33.6 b	1.08 ± 0.15 a	0.27 ± 0.04 d	735.0 ± 168.8 c	0.27 ± 0.02 b	0.57 ± 0.02 a	1077.3 ± 152.7 b	0.45 ± 0.06 b	0.64 ± 0.10 b
MP	0	503.6 ± 137.7 a	0.37 ± 0.05 c	0.67 ± 0.05 b	1120.8 ± 146.3 b	0.32 ± 0.04 b	0.45 ± 0.01 bc	2024.3 ± 435.1 a	1.92 ± 0.23 a	1.12 ± 0.14 a
	9	626.3 ± 88.0 a	0.64 ± 0.06 b	0.87 ± 0.08 a	1504.3 ± 110.6 a	0.43 ± 0.02 a	0.44 ± 0.04 cd	-	-	-
MR	0	135.8 ± 40.9 b	0.61 ± 0.06 b	0.39 ± 0.03 c	578.3 ± 89.9 c	0.45 ± 0.07 a	0.42 ± 0.03 de	1137.0 ± 173.6 b	0.50 ± 0.07 b	0.62 ± 0.06 b
	9	144.2 ± 35.1 b	0.44 ± 0.07 bc	0.47 ± 0.06 c	649.2 ± 93.7 c	0.30 ± 0.04 b	0.36 ± 0.04 e	1354.3 ± 204.3 b	0.51 ± 0.07 b	0.57 ± 0.08 b
HNS										
N6P2K6	0	106.7 ± 29.2 c	0.53 ± 0.07 bc	0.45 ± 0.06 cd	391.1 ± 49.0 e	0.38 ± 0.05 bc	0.39 ± 0.04 cb	814.4 ± 109.9 de	1.10 ± 0.24 bc	0.66 ± 0.16 bc
	9	303.8 ± 125.2 b	0.59 ± 0.08 b	0.46 ± 0.12 bcd	548.7 ± 147.5 de	0.31 ± 0.04 cd	0.35 ± 0.05 c	756.0 ± 58.1 e	0.67 ± 0.10 cd	0.37 ± 0.04 c
N12P2K6	0	663.9 ± 163.9 a	0.57 ± 0.05 b	0.36 ± 0.03 de	1343.3 ± 146.1 a	0.50 ± 0.08 a	0.41 ± 0.04 ab	2227.3 ± 261.4 a	1.02 ± 0.24 a	0.69 ± 0.09 ab
	9	497.8 ± 93.8 a	0.91 ± 0.18 a	0.31 ± 0.07 e	1477.8 ± 134.7 a	0.45 ± 0.04 ab	0.46 ± 0.03 cb	1479.3 ± 304.4 a	0.55 ± 0.07 ab	0.35 ± 0.10 bc
N6P4K12	0	102.9 ± 38.0 c	0.40 ± 0.07 bc	0.58 ± 0.07 bc	537.8 ± 120.0 e	0.30 ± 0.02 cd	0.38 ± 0.05 cb	641.4 ± 125.5 e	0.39 ± 0.06 cd	0.58 ± 0.08 bc
	9	102.0 ± 4.0 c	0.53 ± 0.07 bc	0.76 ± 0.11 a	764.4 ± 193.6 cd	0.30 ± 0.04 cd	0.52 ± 0.05 a	943.3 ± 263.1 dc	0.32 ± 0.05 cd	0.95 ± 0.11 a
N12P4K12	0	314.3± 57.9 b	0.34 ± 0.03 c	0.51 ± 0.07 bc	984.4 ± 136.8 bc	0.38 ± 0.04 cd	0.51 ± 0.05 a	1385.0 ± 89.6 bc	0.33 ± 0.03 d	0.74 ± 0.09 a
	9	296.0 ± 95.0 b	0.83 ± 0.19 a	0.59 ± 0.09 b	1060.4 ± 162.9 b	0.27 ± 0.02 d	0.48 ± 0.05 ab	1610.2 ± 126.2 b	0.44 ± 0.09 cd	0.60 ± 0.07 ab
Harvest time		298.5 ± 38.1 b	0.59 ± 0.04 b	0.50 ± 0.03 ab	888.5 ± 64.7 a	0.35 ± 0.02 c	0.44 ± 0.02 b	1272.5 ± 99.2 a	0.64 ± 0.07 b	0.64 ± 0.04 b

Means followed by different letters in the same column for the same factor are significantly different ($p \leq 0.05$) according to the LSD test.

3.2.5. Browning Potential (BP) and Soluble o-Quinone (So-Q) Content

Browning potential and soluble o-quinone content were evaluated over a period of nine days. BP and So-Q content were higher on the first day in comparison with the results obtained at the end of the storage time. The results showed that HNS, species, and harvest time had a significant impact on BP and So-Q content (Table S2). The highest BP was achieved at the third harvest time in N6P2K6 and MP species that did not differ from the MV species; however, the values in MR species were significantly lower than other two species at all harvest times. The amount of BP in N12P2K6 was lower than other HNS treatments but it did not significantly differ from N12P4K12 treatment. In addition, the highest content was obtained in N6P2K6 treatment, and it did not differ from N6P4K12 treatment (Table 6).

Table 6. Influence hydroponic nutrient solutions (HNS) and harvest time (first harvest: I harvest, second harvest: II harvest, regrowth and third harvest: III harvest, regrowth) on the enzymatic browning parameters of three commercial mint (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR) in the fresh-cut during its shelf life (at harvest (0) and nine days (9)) cultivated in a new growing system (NGS®). N6: 6 mmol L^{-1}; N12: 12 mmol L^{-1}; P2: 2 mmol L^{-1}; P4: 4 mmol L^{-1}; K6: 6 mmol L^{-1} and K12: 12 mmol L^{-1}.

		I Harvest		II Harvest, Regrowth		III Harvest, Regrowth	
Species	**Storage (Days at 4 °C)**	**BP (A_{340} LFW)**	**So-Q (A_{437} LFW)**	**BP (A_{340} LFW)**	**So-Q (A_{437} LFW)**	**BP (A_{340} LFW)**	**So-Q (A_{437} LFW)**
MV	0	29.3 ± 3.4 a	1.8 ± 0.16 ab	16.3 ± 2.5 b	2.5 ± 0.2 b	27.2 ± 4.5 a	2.9 ± 0.2 a
	9	23.9 ± 5.3 ab	1.7 ± 0.25 b	9.2 ± 0.6 c	2.1 ± 0.1 bc	28.9 ± 4.4 a	2.8 ± 0.1 a
MP	0	33.8 ± 3.1 a	2.2 ± 0.17 a	16.6 ± 1.6 b	1.9 ± 0.2 c	26.0 ± 3.4 a	3.1 ± 0.5 a
	9	24.4 ± 2.6 ab	2.3 ± 0.22 a	21.2 ± 2.0 a	1.4 ± 0.1 d		
MR	0	16.6 ± 1.3 bc	1.1 ± 0.15 b	10.8 ± 0.8 c	3.2 ± 0.2 a	9.8 ± 1.2 b	2.1 ± 0.3 b
	9	12.3 ± 1.7 c	1.6 ± 0.15 b	8.3 ± 0.5 c	2.4 ± 0.2 bc	26.2 ± 4.4 a	2.8 ± 0.3 a
HNS							
N6P2K6	0	31.2 ± 4.6 a	2.3 ± 0.23 a	19.1 ± 3.0 a	2.9 ± 0.3 a	22.0 ± 5.6 b	2.1 ± 0.2 b
	9	28.6 ± 6.8 ab	1.9 ± 0.21 ab	13.0 ± 2.2 ab	1.7 ± 0.2 c	38.9 ± 6.2 a	3.4 ± 0.2 a
N12P2K6	0	16.4 ± 1.7 c	1.5 ± 0.22 b	10.7 ± 1.0 b	2.6 ± 0.3 ab	17.1 ± 2.8 b	2.7 ± 0.4 ab
	9	15.1 ± 2.5 c	2.0 ± 0.28 ab	10.6 ± 2.5 b	1.8 ± 0.2 c	22.8 ± 5.7 b	2.7 ± 0.4 ab
N6P4K12	0	32.1 ± 3.8 a	1.7 ± 0.15 ab	16.2 ± 1.9 ab	2.2 ± 0.3 bc	28.6 ± 7.3 ab	3.2 ± 0.3 a
	9	18.6 ± 3.9 bc	1.8 ± 0.29 ab	14.7 ± 3.0 ab	2.0 ± 0.2 bc	26.4 ± 9.1 ab	2.5 ± 0.3 ab
N12P4K12	0	26.8 ± 3.4 abc	1.8 ± 0.20 ab	12.1 ± 1.2 b	2.4 ± 0.3 abc	12.6 ± 1.8 b	2.6 ± 0.2 ab
	9	18.3 ± 5.3 bc	1.7 ± 0.29 ab	13.4 ± 2.2 ab	2.2 ± 0.3 abc	22.3 ± 2.2 b	2.7 ± 0.2 ab
Harvest time		23.4 ± 1.6 a	1.8 ± 0.08 c	13.7 ± 0.8 b	2.2 ± 0.1 b	23.3 ± 2.0 a	2.7 ± 0.1 a

Means followed by different letters in the same column for the same factor are significantly different ($p \leq 0.05$) according to the LSD test.

The So-Q content was affected by HNS, species, and harvesting time. The So-Q content at the first day of storage was higher than the ninth day of storage. In addition, among different harvesting times, the highest content was achieved at the third harvest time. Among the HNS, the highest amount of So-Q was obtained in N6P2K6 treatment, and there was no significant difference between the species (Table 6). The results of the present experiment indicated that the amounts of So-Q at the first and third harvest times were the highest in MP species, while for MR species the highest content was found at the second harvest time. The results of harvest time and HNS interaction showed that the highest amount of So-Q was related to N6P2K6 treatment at all harvest times. Also, N6P4K12 treatment represented a high So-Q content in all harvest times.

3.2.6. Microbial Evaluation

The results showed that the interaction of species and harvest time were significant for TBC, MC and TYC (Table S2). At the first and second harvest time among MR species, the highest amount of TBC was recorded (5424.23 and 5133 CFU·g^{-1} LFW, respectively). Also, the highest MC was observed at the first harvest time of MP species (1333.30 CFU·g^{-1} LFW). Furthermore, our results indicated that the amount of TYC at the first harvest time in MP species had the highest rate (250.23 CFU·g^{-1} LFW) (Table 7). Our findings showed that the interaction of harvest time and HNS was significant for TYC. The results indicated that the highest and the lowest TYC were observed at the first and third harvest time, respectively. Also, at the first harvest time of N6P2K6 treatment (136.44 CFU·g^{-1} LFW), the second and third harvests of N12P2K6 treatment, the highest TYC were 94.67 and 68.33 CFU·g^{-1} LFW, respectively (Table 7).

Among the species, the highest MC belonged to the MP and the lowest rate was found in MV. The highest MC was obtained at the first harvest time while the lowest rate was found at the third harvest time. In general, the rate of microbial contamination in the NGS® was significantly higher at the first harvest time in comparison to the second and third harvest time.

Regarding TYC, at the first and second harvest time, the highest rate of contamination was observed in N6P2K6 and N12P4K12 in MP species. At the third harvest time, the highest TYC was obtained in the MV species in N12P2K6 treatment. Among the species at the first and second harvest time, the highest TYC was recorded for MP species. The highest TYC belonged to the MR species, and the lowest rate was obtained in MV species at all harvest times (Table 7). It should be noted that there was no sample of MP species at the third harvest time.

Table 7. Influence hydroponic nutrient solutions (HNS) and harvest time (first harvest: I harvest, second harvest: II harvest, regrowth and third harvest: III harvest, regrowth) on the total bacterial count (TBC), the mould count (MC) and yeast count (YC) (CFU·g^{-1} LFW) of three commercial mint (*M. spicata* L. var. *viridis* (MV); *M. piperita* L. (MP); *M. spicata* L. var. *rubra* (MR) cultivated in a new growing system (NGS®). N6: 6 mmol L^{-1}; N12: 12 mmol L^{-1}; P2: 2 mmol L^{-1}; P4: 4 mmol L^{-1}; K6: 6 mmol L^{-1} and K12: 12 mmol L^{-1}.

		I Harvest			II Harvest, Regrowth			III Harvest, Regrowth		
HNS	**Species**	**TBC**	**MC**	**TYC**	**TBC**	**MC**	**TYC**	**TBC**	**MC**	**TYC**
MV	N6P2K6	1360.7 ± 46.7 c	481 ± 53.1 cd	19.3 ± 0.6 c	1581 ± 70.6 b	513.3 ± 81.9 abcd	6 ± 1.1 bc	860.3 ± 40.9 ab	412 ± 115.9 a	4.6 ± 1.8 d
	N12P2K6	1418 ± 68.1 c	474 ± 57.8 d	24 ± 3.1 c	425 ± 8.3 b	733.3 ± 70.5 ab	4 ± 0.6 c	249.3 ± 71.5 cd	227.3 ± 34.7 a	0.0 ± d
	N6P4K12	1123.7 ± 28.4 c	580.3 ± 60.1 bcd	44.7 ± 6.7	697 ± 31.2 b	693.3 ± 70.5 ab	12 ± 2.3 bc	142.7 ± 24.8 d	238.7 ± 67.5 a	0.0 ± d
	N12P4K12	843 ± 63.4 c	482 ± 56.6 cd	31.3 ± 1.7 c	530 ± 88.1 b	746.7 ± 89.7 ab	8 ± 2 bc	262.7 ± 19.1 cd	248.3 ± 19.2	6 ± 1.1 d
MP	N6P2K6	1706 ± 13.1 c	1413.3 ± 420.5 a	346.7 ± 17.6 a	221 ± 65.6 b	384 ± 66.2 bcd	20 ± 3.1 bc			
	N12P2K6	316 ± 38.1 c	1133.3 ± 209.5 ab	240 ± 23.0 ab	196 ± 29.9 b	100 ± 22.5 d	18.7 ± 1.1 bc			
	N6P4K12	518 ± 62.4 c	1120 ± 105.8 abc	253.3 ± 40.5 ab	245 ± 44.3 b	176 ± 29.0 cd	112.7 ± 5.3 abc			
	N12P4K12	123 ± 24.9 c	1666.7 ± 357.9 a	160 ± 26.4 bc	279 ± 58.1 b	127 ± 14.6 d	177.3 ± 12.3 ab			
MR	N6P2K6	5816.7 ± 214.3 ab	420 ± 23.1 d	43.3 ± 8.8 c	8460 ± 1075.6 a	800 ± 190.5 ab	2 ± 1.1 c	891 ± 66.4 b	303 ± 8.9 a	
	N12P2K6	4243 ± 427.2 b	336 ± 72.7 d	15.3 ± 2.4 c	5223 ± 756.7 ab	673.3 ± 33.3 abc	261.3 ± 2 a	581.7 ± 63.3 cbd	319.3 ± 93.3 a	
	N6P4K12	4770 ± 840.1 ab	439.3 ± 100.9 d	16.7 ± 4.1 c	4290 ± 342.1 ab	388.3 ± 81.8 bcd	0.00 ± 00 c	1270.7 ± 115.5 a	214.7 ± 31.5 a	
	N12P4K12	6867 ± 124.7 a	362.7 ± 81.4 d	78.6 ± 10.7 c	2560 ± 296.9 ab	940 ± 161.6 a	24 ± 2.30 bc	639.3 ± 44.9 ab	1120.7 ± 21.9 a	110 ± 8.1 b
MV		1235.6 ± 77.5 b	1235.6 ± 77.5 b	504.3 ± 27.6 b	29.8 ± 3.3 b	808 ± 138.1 b	671.7 ± 41.8 a	7.5 ± 1.1 a	378.8 ± 87.1 b	281.6 ± 37.3 a
MP		281.8 ± 49.3 b	281.8 ± 49.3 b	1333.3 ± 144.8 a	250 ± 23.3 a	235 ± 23.8 b	196.8 ± 37.4 b	82.2 ± 20.4 a		
MR		5424.2 ± 367.9 a	5424.2 ± 367.9 a	389.5 ± 34.5 b	38.5 ± 8.4 b	5133 ± 711.5 a	700.4 ± 84.4 a	71.8 ± 29.2 a	845.7 ± 88.2 a	489.5 ± 112.6 a
N6P2K6		2449 ± 861.7 a	2449 ± 861.7 a	771.4 ± 22.0 a	136.4 ± 59.9 a	3421 ± 1312.6 a	565.8 ± 91.7 a	9.3 ± 2.9 a	875.7 ± 35.5 a	357.7 ± 57.1 a
N12P2K6		2014 ± 596.6 a	2014 ± 595.6 a	647.8 ± 139.7 a	93.1 ± 37.4 a	1948 ± 848.2 a	502.2 ± 103.6 a	94.7 ± 36.5 a	415.5 ± 85.7 a	273.3 ± 49.0 a
N6P4K12		2182 ± 707.1 a	2182 ± 707.1 a	713.2 ± 113.3 a	104.9 ± 39.2 a	1744 ± 647.6 a	419.2 ± 81.7 a	41.6 ± 19.3 a	706.7 ± 257.7 a	226.7 ± 33.7 a
N12P4K12		2611 ± 1069.8 a	2611 ± 1069.8 a	837.1 ± 233.9 a	90 ± 20.5 a	1123 ± 368.1 a	604.7 ± 130.3 a	69.9 ± 27.3 a	451 ± 87 a	684.5 ± 195.5 a

Means followed by different letters in the same column for the same factor are significantly different ($p \leq 0.05$) according to the LSD test.

4. Discussion

Effective nutrient management in SCSs is crucial to balance robust biomass growth and the production of high-quality essential raw materials, ensuring profitable outcomes. The results indicated that optimal HNS concentrations varied among mint species. Enhancements occurred in various growth traits, primarily attributed to the roles of HNS in plants, especially N supplementation. N is a vital element for plant growth and yield as it constitutes several metabolic compounds, including chlorophyll, cell walls, enzymes, hormones, nucleic acids, proteins, and vitamins [37]. These compounds play essential roles in cell division, cell enlargement, tissue and organ formation, among other critical processes. The improvement in parameters related to these compounds likely contributed to more growth in shoot fresh weight, subsequently leading to higher dry weight [38].

The results showed that in all species, DM was maximum at the third harvest and minimum at the second harvest. Also, in the third harvest, plants had better developed roots, grew enough branches, and had spent more energy on DM production, which agrees with similar findings in the literature [39]. The nutritional needs of plants differ across developmental stages, and numerous studies have demonstrated that alterations in HNS can significantly change plant growth and yield characteristics [25,26,28,29]. The explanation for the increase in DM in the third harvest may be the accumulation of NO_3^-, PO_4^{3-} and $CaCO_3$ in tissues at this stage in addition to the stages before. A positive correlation occurred between nutrient concentration and DM.

Plants can decipher nutrient doses and adjust biomass partitioning accordingly [40]. Growth and yield are strongly influenced by genotype and environmental factors [41]. According to the available literature, these two variables are negatively affected by high and low nutrient concentrations [42,43]. Thus, the lowest LFW per plant and per m^2 was achieved in the most concentrated HNS, resulting from osmotic stress. LFW increased by the heightened presence of N; however, high concentrations of P and K decreased the LFW and yield due to osmotic stress. In the second harvest, none of the plants survived the treatment with the highest HNS concentration. In contrast, the highest LFW was observed in the least concentrated HNS. This result is in line with the findings of Roosta [44] who reported that spraying K solution increased the percentage of DM in mint. However, the current result is inconsistent with the increase in plant fresh weight. At all harvesting times, N12P4K6 and N12P4K12 resulted in the lowest fresh yields and the highest percentage of DM. By using N12P4K12, which had the highest concentration of nutrients, the lowest fresh yield was obtained. Therefore, HNS-dependent partitioning of biomass may differ among plant species in NGS® in the greenhouse. Decreasing in yield by increasing the concentration of nutrients due to osmotic stress has previously been reported in leafy vegetables [2,11,12,19].

Furthermore, osmotic stress at high concentrations appears to reduce LDG. The amount of N did not induce a significant difference in LDG. Previous research has shown that osmotic stress occurs due to high-concentrations of K and P, and reduces the LDG, which is consistent with the results of the current experiment [45]. Also, differences in LDG among various species may emanate from their genetic potential [46].

Colour is one of the most important traits characterizing the quality of vegetables since its change during storage affects marketing. Colour indirectly indicates freshness and microbial deterioration/contamination in stored plant products [47]. The values of L^*, a^*, and b^* significantly decreased by increasing the HNS concentration, especially by increasing the N concentration. In treatments with the N concentration of 6 mmol·L^{-1}, compared to the two other HNS with the N value of 12 mmol·L^{-1}, the values of colour parameters were higher in all harvest times and species. One of the possible reasons for the decrease in b^* in N12P2K6 and N12P4K12 treatments is the chlorophyll decomposition due to high concentration of salt and osmotic stress that leads to decreased absorption of nutrients, as well as plant metabolites biosynthesis. Similar results were observed in other leafy plants such as lettuce, where higher nutrient concentrations induced a decrease in L^* and b^* [48].

A vast majority of mints is sold as fresh-cut leaves or cuttings, where preservation of freshness, texture, shelf life, and aroma are essential [49]. WL is a physiological event in fresh-cut products and in fresh aromatic and medicinal herbs. It can be decelerated by controlling temperature and humidity and using suitable and efficient packaging [50,51]. Plastic film performance was significantly maintained in all species. The permeability characteristics of the film on the bags might have induced a low respiratory rate. In the current study, WL was very low at the beginning of storage. The highest WL occurred in bags prepared from the mint of the first harvest time. This accelerated WL in the first harvest time may be due to the crispness and wateriness of the tissues.

The responses of species were studied in terms of respiration rate and headspace analysis. Data analysis showed that the HNS did not affect the rate of CO_2 and O_2 production in any species, which is consistent with the findings of Luna et al. [11] on several varieties of lettuce. One of the reasons for justifying and explaining the reduced respiration rate in the third harvest in MP species compared to the first harvest time is that MP species had a higher DM, which means they had less water and bioactivity. This hypothesis has already been reported by Scuderi et al. [52] who highlighted that the reduced respiration of lettuce was related to the increased amount of DM and less water in plant tissues. The highest respiration rate was observed in response to the N12P4K12 treatment, with the highest amount of NS, which agrees with other reports that showed higher respiration rates in response to higher NS and EC concentrations [52].

The NO_3^- content in edible fruits and vegetables is an important quality characteristic, and the EU encourages good agricultural practices to reduce NO_3^- contents in leafy herbal plants [53]. The NO_3^- content in the three species during the first harvest time was very low, well below the maximum NO_3^- content allowed for vegetables by the EU (Commission Regulation No. 1258/2011). The highest accumulation of NO_3^- occurred in the treatment with the highest amount of N supplementation and the lowest amounts of P and K. Several authors showed that P and K deficiency reduced the activity of nitrate reductase enzyme and caused an increase in the NO_3^- content [54,55]. Therefore, it seems that in treatments with lower P and K, the activity of nitrate reductase enzyme decreased, which may induce an increase in NO_3^- [56], as observed by Miceli et al. [47] on Swiss Chard plants. Nevertheless, a significant challenge with leafy herbal plants, such as mint, is the substantial accumulation of NO_3^- in their tissues, often surpassing 2500 mg NO_3^- kg^{-1}. European Commission regulations No1881/2006 and 1258/2011 have set NO_3^- thresholds for herbal plants at 1000–2500 mg NO_3^- kg^{-1} [57]. Another item that is affected by the accumulation rate of NO_3^- in plant tissues is shelf life. The rate of NO_3^- accumulation in the first day was highest, which is consistent with the results of Miceli et al. [47] (Table 5). Some plants reduce NO_3^- to ammonium ions in the aerial parts, which is mainly due to the different processes of NO_3^- reductase in different parts of the plants. NO_3^- reductase is known as a regulatory enzyme that limits the amount of plant NO_3^-. The activity of this enzyme shows a large variation in various plant species, depending on HNS and environmental conditions. One of the main reasons accounting for this variation could be related to differences in genetic origins of plant species or maybe indirectly with the concentrations of Mo and Fe in plants [1,58–60].

NO_3^- in the plant cell cytosol is converted to nitrite by the NO_3^- reductase enzyme. Then, NO_3^- is reduced to ammonium or ammonia by the nitrite reductase enzyme. While these compounds enter the structure of organic compounds, the resultant NADPH and NADH act as electron donors for the activity of the nitrate reductase enzyme. Therefore, the synthesis of the nitrate reductase enzyme is controlled by NO_3^- presence, and this ion induces the relevant enzymatic synthesis pathway [58]. Thus, any factor that leads to a decrease in the activity of nitrate reductase enzyme in plant cells is potentially associated with the accumulation of NO_3^- in plant shoots. In a relevant experiment, Atkin and Cummins et al. [61] showed that the increase in temperature reduces the activity of the nitrate reductase enzyme. Therefore, it seems that in the present experiment, due to the high temperature at the third harvest, the activity of nitrate reductase enzyme decreased, thus

increasing foliar NO_3^- accumulation. In addition, the previous study which was conducted in summer at high temperatures showed a higher accumulation of NO_3^-. Similarly, in our results, the highest accumulation occurred in the third harvest that coincided with high temperatures [56,62,63].

Under management conditions with low N intake, plants reduce the activity of nitrate reductase enzyme, but with exposure to NO_3^- ions, the rate of activity of this enzyme increases [64]. The concentration of chemical fertilizers is known as one of the factors affecting NO_3^- accumulation. In vegetables, this accumulation often depends on the amount and type of nutrients around the roots, which is closely related to the amount and timing of chemical fertilizer consumption [65].

The HNS with the highest P concentration determined the highest PO_4^{3-} accumulation in plants. In a study by Fallovo et al. [56] on lettuce plants, the increase in nutrient supplementation increased the accumulation of PO_4^{3-}, which is consistent with the results of the current study.

$CaCO_3$ uptake appears to be directly related to high N concentrations, while it is inversely related to high K and P concentrations. The rate of $CaCO_3$ accumulation significantly decreased during the shelf life for all three harvests. In a study on mint plants, spraying K reduced the concentration of $CaCO_3$ in both leaves and roots [44], which is consistent with the results of our experiment.

The highest amount of BP occurred in response to treatments with the lowest amount of N, while the lowest BP was observed when using the highest N concentration. Applications with lower N concentrations proved optimal for preserving phenols, maintaining high levels of antioxidant and free radical scavenging activity throughout storage, albeit potentially at a reduced yield. The observed decrease in BP in fresh-cut mints with the lowest amount of N in the present experiment could be attributed to the slowing down of phenolic oxidation, thus positively influencing their accumulation [66]. The shelf life and quality of freshly harvested mint are influenced by HNS treatments and harvesting time. Additionally, the genetic background plays a role in the response of BP and So-Q content in mint. Qualitative reduction in the appearance of fresh-cut products is one of the main factors negatively affecting consumer preference and vegetable marketing [67]. Apart from yellowing, the tissue BP is one of the most important factors limiting the shelf life and marketability of processed products. Both $CaCO_3$ and K are effective elements in preventing the loss of firmness. $CaCO_3$ is one of the most essential and effective nutrients in increasing and maintaining the quality of cut plant tissues. Calcium accumulation in plant tissues strengthens polymeric bonds between the middle lamella of the cell pectocellulosic membrane while improving the network strength of cell walls and thus increasing the mechanical strength of tissues [68]. In our study, the lowest BP rate was found in the treatment with the highest K while the highest BP rate was observed in response to the highest N concentration and the lowest P and K concentration. Olivos et al. [69] reported the effective roles of P, K, and $CaCO_3$ in increasing the shelf life during the storage time. K plays a key role in the firmness of plant tissue. Thus, maintaining the balance in this element is essential [27]. Calcium together with K plays an essential role in permeability, dehydration, maintenance of inflammatory pressure, cell function and in general plant quality, storage properties, and marketing. Calcium-induced stiffness is related to its effect on cell wall components that maintain the membrane and cell wall, but K induced stiffness is related to changes in cell hydrostatic pressure [27,69,70].

Browning occurs due to the disruption of cellular integrity and the subsequent release of polyphenol oxidases, which facilitate the oxidation of phenolic compounds into quinones. The quinones mentioned above demonstrate a significant level of reactivity. They frequently endure in engaging in chemical reactions with each other, as well as with proteins. This ultimately leads to the production of brown pigments in plants. Its control has been extensively studied and reported in several plant species [70,71].

Given the utilization of mints in the food and pharmaceutical industries, it is imperative that they exhibit low microbial contamination and appear free from pathogenic germs. Addi-

tionally, they must comply with the legal requirements outlined in Commission Regulation (EC) No 2073/2005. In SCS, microbial contamination is usually lower than in TCS. In previous research on this topic, researchers reported that post-harvest microbial contamination in NFT [52] and NGS® [72] could be reduced. Also, in the present study, the contamination rate was significantly lower compared to the data on microbial contamination reported in the literature for horticultural crops cultivated in TCS. In a study by Selma et al. [72] on comparing the microbial contamination levels of *Lactuca sativa* L. cultivated in soils and NGS®, the results confirmed that contaminations in the soil system were much higher than the NGS®. In this study, it was reported that the SCS appeared as the most suitable system in terms of minimizing microbial contamination. The findings of our study also showed that the NGS® system could be very effective in reducing microbial contamination in mints. In fact, cultivation through NGS® allows the farmer to obtain a cleaner and hygienically safer product than older SCSs and traditionally soil-grown systems.

5. Conclusions

The current study systematically examined the dynamic effects of various nutrient concentrations on the growth, yield, physiological aspects, and shelf life of commercial mint at different harvesting times. It can be concluded that the yield and quality traits of mint grown in NGS® were significantly affected by the HNS and the harvesting times. NGS® is recommended for its ability to control of plant nutrition in order to manage the quality characteristics of the raw materials for fresh-cut products. The findings revealed that an increase in nitrogen concentration in HNS had a negative effect on specific quality parameters, such as higher NO_3^- content. Notably, the highest NO_3^- accumulation occurred at the third harvest, while the lowest was observed at the first harvest. Although the study indicated negligible post-harvest changes due to variations in HNS, these observations are contingent upon species differences. Optimal results, including the highest DM, were achieved using the highest concentration of HNS. N6P2K6 and N12P2K6 treatments in NGS® demonstrated potential for enhancing marketable fresh yield and fresh-cut product quality. Among the species, MP exhibited the highest DM, while MV and MR showed the highest yield. Furthermore, bacterial contamination decreased at the second and third harvests in NGS®, which is attributed to the shorter growth period and potential increased resistance of plants to bacteria. Adjusting the HNS in the growing medium according to climatic conditions and plant species is crucial for obtaining a uniform product with high yield and quality in NGS®. The study underscores the importance of tailoring hydroponic nutrient solutions to specific plant species and environmental conditions for achieving optimal yields and quality in commercial mint cultivation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14030610/s1, Table S1: three-way ANOVA of dry matter (DM), leaf fresh weight per plant (LFW), leaf daily growth (LDG), yield per square meter, L^*, a^* and b^* as affected by hydroponic nutrient solutions (HNS), mint species and harvest time; Table S2: three-way ANOVA of counts (CFU g^{-1}) of different groups of microorganisms and nutrient content as affected by hydroponic nutrient solutions (HNS), mint species and harvest time.

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