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Testing the Effect of High pH and Low Nutrient Concentration on Four Leafy Vegetables in Hydroponics

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Abstract: Low nutrient and high pH of circulating water represent two of the main issues to overcome for a successful combination of aquaculture and hydroponics in aquaponics offering a sustainable and circular economy solution for vegetable production. The purpose of this study was to screen the tolerance of four herbs to high pH and low nutrient concentration in hydroponics, i.e., green and red basils, mint, and rocket salad, with a focus on plant yield and nutraceutical aspects. Results highlighted green basil as the most tolerant species to low nutrient and high pH conditions followed by mint. On the contrary, negative effects from high pH and low nutrient were reported on red basil and especially rocket salad, which strongly affect their marketability parameters. Rocket salad fresh biomass was more than halved under the combination of high pH and low nutrients. Results on green and red basil showed the importance of testing the tolerance to these agronomic conditions at both species and variety levels. Despite the reduction in biomass, leaf pigments were not influenced by high pH and low nutrients and therefore can be considered parameters of minor importance for the evaluation of these species. In conclusion, the tolerance of green basil and mint to high pH and low nutrients under hydroponic conditions has been highlighted. Further investigation coupled with fish farming will be able to reinforce the convenience of using these species for aquaponics.

Keywords: aquaponics; herbs; leaf pigments; plant nutrition; optimal pH; sustainability

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1. Introduction

Increasing world environmental issues related to population increase, climate change, soil degradation, water scarcity, and food security point to the necessity of low inputs, high resource efficiency, and closed loop production.

Hydroponic systems enable soilless plant growth using a mixture of water and nutrient solution. Among several advantages are a better control of plant nutrition, a more efficient use of space, the avoidance of herbicide and reduction of pesticide use, and fertilizer and water savings. Hydroponics also assures a more precise control of growth conditions such as lighting, humidity, and temperature, speeding up the growing period and increasing the yield regardless of weather conditions, particularly where suitable farming lands are not available [1–4]. The combination of aquaculture and hydroponics into the aquaponics seems a promising strategy to further reduce water and fertilizer waste despite the research in this sector is still inadequate to support the development of economically feasible operational systems [5,6]. Indeed, the use of fertilizers may have a strong impact on agricultural process sustainability [7] and a reduction in their use is strongly welcomed by authorities as well as convenient for producers reducing production costs. The aim of aquaponics is to recover nutrients released from aquaculture to decrease the addition of fertilizers to hydroponic systems, thereby increasing production efficiency and reducing environmental contaminations [8,9], which is a priority in European vegetable cultivation [10]. To achieve a self-sustaining integration between aquaculture and hydroponics, the control of water parameters is essential. They include pH, electrical conductivity (EC), temperature, dissolved oxygen, redox potential, total solids, salinity, and nutrient content [11]. A relevant point to be considered is the difference between aquaculture and hydroponic solutions. Ideal pH values in aquaculture range between 7.0 and 8.0 [12], favoring internal fish pH and nitrification in biofilters as well. These values depend on the cultivated species, fish density, and management system [13]. For hydroponics, a pH between 5.5 and 6.5 is instead the optimum for plant nutrient uptake, e.g., the optimal pH is between 6.0 and 6.8 for mint [14] while basil did not show yield reduction even at pH 4.0 [15]. The availability of potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg) is slightly decreased at a higher pH, but the availability of micronutrients as manganese (Mn), zinc (Zn), copper (Cu), and especially iron (Fe) is significantly reduced [16]. A major issue is alkalinity-induced leaf chlorosis for Fe deficiency due to its decreased uptake and/or availability [17]. Thus, nutrient reuse by plants must be optimized for improving plant yield and quality.

To integrate hydroponics and aquaculture, several strategies could be adopted to assure an adequate crop nutrient uptake: (i) combine the aquaculture water with high pH (>7.0) with foliar micronutrient application [18]; (ii) exploit the most suitable pH for nitrifying bacteria and fishes favoring the nutrient recovery through the bacterial nitrification [19]; (iii) use a mineralization unit to concentrate the nutrients of aquaculture water in decoupled systems [20]; and (iv) decrease the pH of solution before crop application in decoupled systems, considering an extra cost in production. However, the use of plants naturally tolerant to high pH and low nutrient concentrations might be a simple and successful agronomic strategy to be explored, as also highlighted by other authors [21]. The present work was carried out with the aim of screening the physiological response to high pH (i.e., >7.0) and low nutrient concentration (i.e., 25% standard hydroponic nutrient solution) of four herbs in hydroponics to assess their suitability for the agronomic conditions retrieved in commercial aquaponic systems, avoiding extra additions of nutrients and pH adjustments to maximize the environmentally friendly opportunity offered by this production system. Green and red basils, mint, and rocket salad were selected based on their diffusion in aquaponic systems as well as their low to medium nutritional needs [22].

2. Materials and Methods

2.1. Experimental Location and Growing Conditions

The experiment was conducted at the Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics, Pescia, Tuscany, Italy (lat. 43°54′ N, long. 10°42′ E), using four test species: two cultivars of sweet basil (*Ocimum basilicum*) the green one 'Tigullio' and the red one 'Red Rubin', a cultivar of mint (*Mentha piperita*), and a cultivar of rocket salad (*Eruca vesicaria*).

Seeds (120 per species) were sown in September 2019 on rockwool trays filled with vermiculite and substrate moisture was kept till the emergence of seedlings. After germination, the trays were irrigated with Hoagland's solution at 10% strength [23]. The plants were transplanted on the final hydroponic cultivation units at four-true leaf stage (about 3.5 weeks after sowing, i.e., October 2020).

Each hydroponic cultivation unit consisted of a black polyethylene pot (10 L) without drain holes with four plants placed in plastic tubes (50 mL) filled with rockwool and locked on the pot cap. The transplant from the trays to the corresponding pot was accomplished using plants with similar biomass and height (±5%). Each pot was filled with the same volume of nutrient solution, which was aerated by air pumps (250 L h^{-1}). Plants were kept in a growth chamber (23 ± 0.5 °C temperature, 70 ± 4.9% relative humidity, 10:14 h night:day photoperiod). Light was supplied with SOX-lamps (Philips®, Amsterdam, The Netherlands) providing 1050 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR), on average at the top of the canopy.

2.2. Experimental Design and Treatments

Each treatment consisted of three cultivation units (12 plants per treatment, i.e., 3 replicates of 4 plants each). Four treatments were applied for 21 days from transplanting: (1) standard nutrient solution and pH (control, SN-LpH); (2) standard nutrient solution with high pH (SN-HpH); (3) low-concentrated nutrient solution and standard pH (LN-LpH); and (4) low-concentrated nutrient solution and high pH (LN-HpH). For the first refill, Hoagland's solution at 100% with pH 5.5 prepared with tap water was used for the treatment 1, while Hoagland's solution at 100% with pH 7.5 prepared with tap water was used for the treatment 2. For treatments 3 and 4, low-concentrated nutrient solutions were prepared by adding 25% of salts used for solution 1 and 2 to the tap water and pH was adjusted at 5.5 for treatment 3 and 7.5 for treatment 4 (Table 1). The above nutrient solutions were also used for the subsequent replacements of the nutrient solution absorbed by plants during the cultivation. pH adjustments were made using 30% H_2SO_4 v/v or 10% NaOH w/v.

Table 1. Electrical conductivity (EC) and concentrations of macro and micronutrient in standard (SN) and low-concentrated (LN) nutrient solutions and in the tap water used for their preparation.

	EC	N-NO_3	N-NH_4	P	K	Ca	Mg	Na	S-SO_4	Cl	Fe	B	Cu	Zn	Mn	Mo
	dS m^{-1}					mmol L^{-1}							µmol L^{-1}			
Tap water	0.62	0.5	0.0	0.0	0.3	1.0	0.3	1.5	0.2	1.3	3.0	5.1	0.1	7.3	0.1	0.0
SN solution	2.14	14.0	1.0	1.00	6.0	4.0	2.0	1.5	2.0	1.3	45.0	45.1	1.0	7.3	10.0	1.0
LN solution	1.00	3.9	0.3	0.25	1.7	1.8	0.7	1.5	0.6	1.3	13.5	15.1	0.3	7.3	2.6	0.2

2.3. Nutrient Solution Menagement

pH, EC, and volume of nutrient solution in each cultivation unit were measured every two days. pH and EC were measured directly in the solution using specific electrodes. Figure 1 shows the actual values of pH and EC measured in the nutrient solutions of each treatment, averaged throughout the whole cultivation period and the replicates. The volume of nutrient solution adsorbed by plants was gravimetrically determined weighting the pots and calculating the differences between the initial (10 L) and the final volume (after 2 d). During the weighing, the plants, locked on the pot cap, were removed from the pot to avoid inaccuracy in the measure due to the plant growth. Thus, the evapotranspirated fraction was reintegrated up to restore the 10-L volume. The sum of the volumes replaced at each reintegration corresponded to the plant evapotranspiration (ET) during the whole cultivation period.

2.4. Plant Biometric Measurements and Determination of Nutrients

At the sampling (after 21 days), plant height and fresh weight (FW) of shoots and roots were determined separately for each plant per pot. Sub-samples of leaves (40 g per plant) were collected for the calculation of leaf area, measured using a leaf area meter (WinDIAS Image Analysis System, Delta-T Devices, Cambridge, UK). Fresh material was dried at 70 °C until constant dry weight was reached and weighted for the determination of the dry biomass (DW). Specific leaf area (SLA) was calculated as the ratio between leaf area and DW of sample used for the measure. Moreover, plant DW percentage (%DW) was calculated as follows: (FW/DW) × 100. Root/shoot ratio was calculated for each species and treatment as the ratio between root DW and shoot DW. Plant shoots were then reduced in powder using a grinder and used for the determination of mineral elements. Organic nitrogen (N-NH_4) was quantified through the Kjeldhal-Tecator method. Each sample (0.25 g) was digested with a Selenium Catalyst Tablet (VELP Scientifica, Usmate, MB, Italy) and 12 mL of H_2SO_4 at 370 °C till complete mineralization. Digested samples were analyzed using the VELP-UDK127 apparatus (VELP Scientifica, Usmate, MB, Italy), adding 50 mL of 40% NaOH w/v. The distillate was collected in a conical flask containing boric acid (4% w/V) and bromocresol green-methyl red color indicator. The content of organic N-NH_4 was determined by titration with 0.1 N HCl. Phosphorus content was determined, after nitric-perchloric acid digestion (1 h at 220 °C), through the ammonium molybdate colorimetric method using a spectrophotometer (Evolution™ 300 UV–Vis Spectrophotometer, Thermo Fisher Scientific Inc., Waltham, MA, USA). Digested samples were then used for the determination of K, Ca, Mg, Fe, Zn, and Mn through ICP-OES (Optima 7000, Perkin Elmer, Waltham, MA, USA). Nitrates (NO_3) were determined on dried powder through a colorimetric method [24] and then expressed on FW basis.

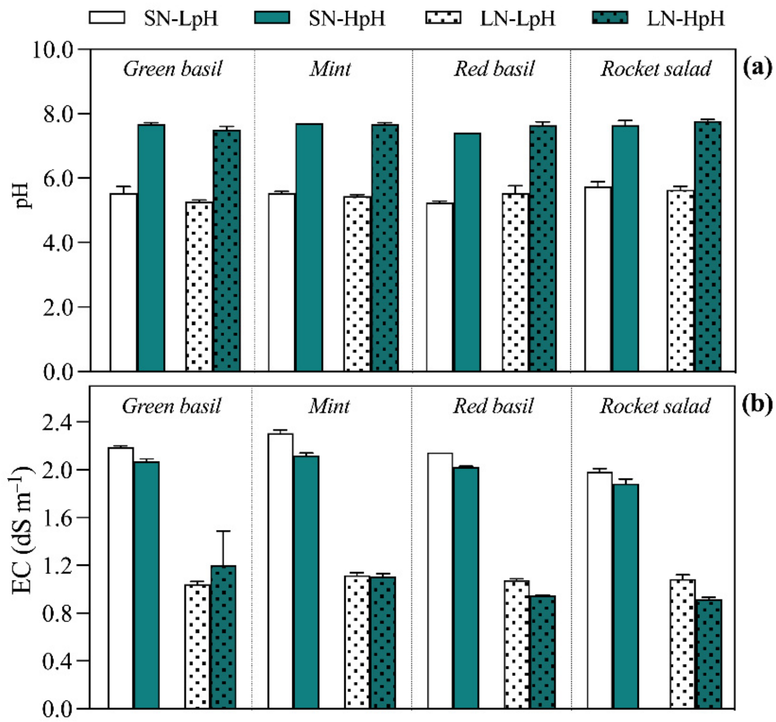


Figure 1. Average pH (**a**) and EC (**b**) of nutrient solutions measured during the trial. SN-LpH = standard nutrient concentration and standard pH (control); SN-HpH = standard nutrient concentration and high pH; LN-LpH = low nutrient concentration and standard pH; and LN-HpH = low nutrient concentration and high pH. Bars represent average values during all cultivation period + SD.

2.5. Pigment Analyses

For pigment analyses, leaf disks were collected from young and completely unfolded leaves (16 disks, 4 per plant, roughly 100 mg FW), in two technical replicates, and stored at −80 °C until analysis. Samples were extracted twice adding 5 mL of MeOH at −20 °C for 24 h. After 48 h, the supernatant was measured at 663, 648, and 470 nm. Chlorophyll *a* and *b* content and total carotenoids were calculated according to [25]. Total phenolic content was determined according to [26]. Greenness index was calculated as the ratio between total chlorophylls and carotenoids.

2.6. Statistics

The experiment was set up in a complete randomized block design, with three replicates of four plants per treatment. Normality and homogeneity of variance were evaluated with the Kolmogorov-Smirnov and Levene's tests, respectively. A two-way analysis of variance (ANOVA) was performed (with pH and nutrients as factors), followed by a Tukey's post-hoc test to detect significant differences between treatments ($p \leq 0.05$). A heatmap presenting the ratio of average values of treatment and control groups was also included to clearly display via colors the main differences between treatments. Graphs, the heatmap, and statistics were done with Prism 9 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Plant Biomass, Leaf Area and Water Uptake

High pH and low nutrient did not influence plant height in all tested species and the highest value was measured in green basil (data not shown). Shoot and root FW and DW were instead altered by pH and nutrient concentration in a species-dependent manner (Table 2). Green basil shoot and root FW and DW were not affected by pH or nutrient concentration, but the root %DW was decreased by high pH. An opposite behavior was observed in mint in which the root %DW was increased by high pH. High pH also had the same (i.e., opposite to one another) effect on the root/shoot ratio of these two species, while the low nutrient concentration increased this parameter in both species. The low nutrient concentration led to a significant decrease in the shoot FW of mint and the FW and DW of red basil and rocket salad, while only in the latter species the shoot FW was significantly negatively affected by high pH. Rocket salad was the most sensitive species to high pH (−42% shoot FW, on average) and low nutrient concentration (−54% on average) compared with the control treatment SN-LpH. Overall, pH and nutrient concentration caused more negative effects in red basil and rocket salad biomass parameters. In red basil, all treatments decreased the root FW and DW compared with the control conditions SN-LpH and, in particular, a significant interaction between the two treatments was observed for the root FW. The shoot %DW decreased only under LN treatments while high pH and low nutrients did not significantly influence the root %DW of this species. The root/shoot ratio revealed an opposite effect of high pH on this parameter under different fertigation regimes in red basil, i.e., a decrease under SN treatment and no effect under LN treatment. Opposite results were found in rocket salad where a higher shoot %DW was found under nutrient shortage conditions (+2.8% under LN-HpH than in control conditions). The root/shoot ratio was not altered by high pH and low nutrient concentration, thereby highlighting a proportional decrease of aerial and underground biomasses in this species compared with the others.

The LN treatment decreased plant leaf area in mint, red basil, and rocket salad while high pH had negative effects on this parameter only in rocket salad (Table 2). In particular, the highest decrease was measured in rocket salad under LN-HpH treatment (−65% than in the control treatment SN-LpH). The SLA was negatively influenced by high pH only in rocket salad, where the lowest value was again measured under LN-HpH condition (−41% than in control condition, data not shown). Rocket salad had the highest water consumption compared with the other plant species, particularly under control conditions (1681 ± 159.6 mL plant^{-1}). However, rocket salad plants supplemented with LN solution had a lower water uptake than under SN treatment showing the same trend observed for the leaf area. Green basil, mint, and red basil did not show any significant difference for this parameter.

Table 2. Growth parameters of green basil, mint, red basil, and rocket salad.

		Shoot FW (g plant^{-1})	Root FW (g plant^{-1})	Shoot DW (g plant^{-1})	Root DW (g plant^{-1})	Shoot %DW	Root %DW	Root/Shoot	Leaf Area (cm^2 plant^{-1})	Plant ET (mL plant^{-1})
Green basil	SN-LpH	54.1 ± 10.32	20.7 ± 2.81	5.0 ± 1.30	0.94 ± 0.299	9.2 ± 1.26	4.5 ± 0.88	0.19 ± 0.035	938 ± 176.8	507 ± 98.7
	SN-HpH	52.9 ± 12.14	24.1 ± 8.59	4.5 ± 0.79	0.76 ± 0.085	8.6 ± 1.09	3.3 ± 0.72	0.17 ± 0.017	944 ± 208.2	531 ± 123.9
	LN-LpH	43.1 ± 4.60	24.3 ± 3.14	3.7 ± 0.60	0.89 ± 0.140	8.5 ± 0.50	3.7 ± 0.12	0.24 ± 0.001	802 ± 66.1	454 ± 56.2
	LN-HpH	47.8 ± 7.87	30.6 ± 4.01	4.7 ± 1.05	0.86 ± 0.110	9.8 ± 0.82	2.8 ± 0.52	0.18 ± 0.015	938 ± 281.3	493 ± 100.0
pH		ns	ns	ns	ns	ns	*	*	ns	ns
Nutrients		ns	ns	ns	ns	ns	ns	*	ns	ns
pH × Nutrients		ns	ns	ns	ns	ns	ns	ns	ns	ns
Mint	SN-LpH	55.0 ± 9.67	19.9 ± 4.55	6.1 ± 1.69	0.84 ± 0.101	11.0 ± 1.33	4.3 ± 0.65	0.14 ± 0.017	3587 ± 782.7	621 ± 189.0
	SN-HpH	46.8 ± 8.72	16.5 ± 1.14	4.7 ± 1.32	0.89 ± 0.265	10.0 ± 1.80	5.3 ± 1.22	0.19 ± 0.030	3087 ± 404.9	523 ± 70.4
	LN-LpH	43.6 ± 2.92	20.9 ± 3.93	4.7 ± 0.62	0.82 ± 0.134	10.7 ± 1.00	3.9 ± 0.27	0.17 ± 0.025	2862 ± 157.6	523 ± 22.7
	LN-HpH	35.2 ± 2.16	14.3 ± 2.83	3.9 ± 0.19	1.00 ± 0.123	11.0 ± 0.93	7.3 ± 2.34	0.26 ± 0.026	2249 ± 302.0	457 ± 29.0
pH		ns	ns	ns	ns	ns	*	**	ns	ns
Nutrients		*	ns	ns	ns	ns	ns	**	*	ns
pH× Nutrients		ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 2. *Cont.*

		Shoot FW (g plant^{-1})	Root FW (g plant^{-1})	Shoot DW (g plant^{-1})	Root DW (g plant^{-1})	Shoot %DW	Root %DW	Root/Shoot	Leaf Area (cm^2 plant^{-1})	Plant ET (mL plant^{-1})
Red basil	SN-LpH	24.1 ± 4.45	5.3 ± 0.50 ^{a}	1.6 ± 0.30	0.19 ± 0.049	6.8 ± 0.29	3.6 ± 0.56	0.12 ± 0.01 ^{a}	663 ± 148.3	451 ± 54.4
	SN-HpH	20.0 ± 3.16	3.5 ± 0.78 ^{b}	1.4 ± 0.23	0.11 ± 0.025	7.0 ± 0.28	3.3 ± 2.99	0.08 ± 0.00 ^{b}	566 ± 76.5	414 ± 52.9
	LN-LpH	18.1 ± 2.10	3.2 ± 0.65 ^{b}	1.2 ± 0.17	0.12 ± 0.015	6.4 ± 0.19	3.7 ± 0.29	0.10 ± 0.01 ^{ab}	444 ± 79.8	393 ± 27.6
	LN-HpH	15.6 ± 2.48	3.2 ± 0.06 ^{b}	1.0 ± 0.22	0.11 ± 0.006	6.6 ± 0.39	3.3 ± 0.22	0.11 ± 0.02 ^{ab}	482 ± 68.8	375 ± 19.2
pH		ns	*	ns	*	ns	ns	ns	ns	ns
Nutrients		*	**	*	*	*	ns	ns	*	ns
pH × Nutrients		ns	*	ns	ns	ns	ns	*	ns	ns
Rocket salad	SN-LpH	129.3 ± 6.48	26.9 ± 2.38	10.1 ± 1.48	1.63 ± 0.308	7.8 ± 0.91	6.0 ± 0.65	0.16 ± 0.012	2833 ± 227.7	1681 ± 159.6
	SN-HpH	94.1 ± 20.65	17.6 ± 1.87	7.7 ± 0.33	1.14 ± 0.167	8.5 ± 1.95	6.5 ± 0.31	0.15 ± 0.015	1960 ± 454.7	1209 ± 170.2
	LN-LpH	61.9 ± 16.12	15.5 ± 5.66	5.9 ± 1.57	0.96 ± 0.300	9.7 ± 1.93	6.3 ± 0.85	0.16 ± 0.010	1172 ± 340.7	1000 ± 271.5
	LN-HpH	56.2 ± 12.18	12.6 ± 2.78	6.0 ± 1.65	0.83 ± 0.151	10.6 ± 0.74	6.7 ± 0.61	0.14 ± 0.015	984 ± 180.0	996 ± 228.8
pH		*	*	ns	ns	ns	ns	ns	*	ns
Nutrients		***	**	**	**	*	ns	ns	***	**
pH × Nutrients		ns	ns	ns	ns	ns	ns	ns	ns	ns

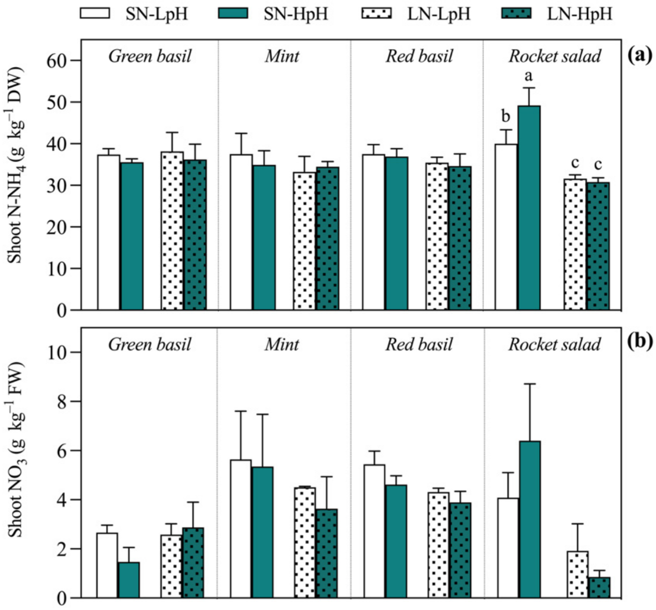
SN-LpH = standard nutrient concentration and standard pH (control); SN-HpH = standard nutrient concentration and high pH; LN-LpH = low nutrient concentration and standard pH; LN-HpH = low nutrient concentration and high pH; ET = evapotranspiration. Two-way ANOVA *p*-values and Tukey's post hoc results are reported in the table (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; ns = not significant). Data represent mean values of replicates ± SD. Lowercase letters indicate Tukey's post hoc results.

3.2. Tissue Nutrient Content

The shoot N concentration was differently altered by pH and nutrient concentration levels in the tested plant species (Figure 2). The concentration of N-NH_4 (organic N) was higher under SN-HpH compared with the other treatments in rocket salad while no effect was observed in green basil, mint, and red basil (Figure 2a). Moreover, LN treatment decreased N-NH_4 always in rocket salad. The NO_3 concentration (Figure 2b) was slightly but significantly decreased by high pH in red basil while it was strongly reduced in red basil and rocket salad by LN treatment.

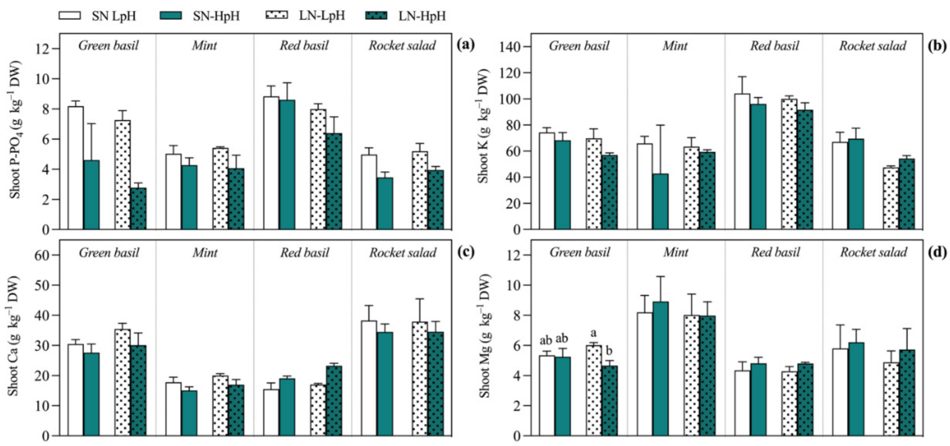
The high pH had a negative effect on the shoot P-PO_4 concentration in green basil, mint, and rocket salad while the low nutrient concentration decreased this parameter only in red basil (Figure 3a). Potassium was mostly altered in rocket salad in which the LN treatments decreased its concentration in shoot (−29% than control under LN-LpH treatment, Figure 3b). An effect on this element was also found in green basil where both the LN treatment and the high pH slightly, albeit significantly, decreased its concentration. Calcium was altered by both LN treatment and high pH in green basil, mint, and red basil while no difference was detected in rocket salad (Figure 3c). In particular, it generally slightly increased under LN treatments and decreased with high pH in green basil and mint, while increasing in red basil. Magnesium was decreased by high pH only in green basil, mostly under LN-HpH treatment (Figure 3d).

High pH and LN treatment generally had little or no effect on the analyzed micronutrients except for Mn (Figure 4). No significant changes were found for Fe and Zn (Figure 4a,c), while high pH decreased the Mn concentration in green basil, mint, and rocket salad (Figure 4b). Moreover, Mn concentration was also slightly, albeit significantly, decreased under LN treatments in green basil.



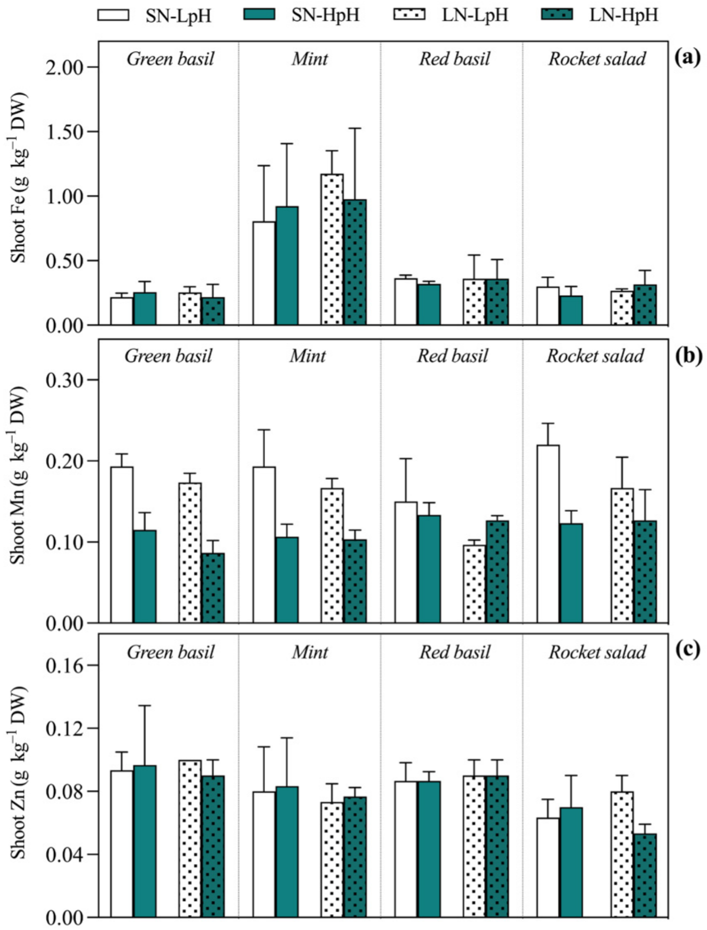
	Shoot N-NH4				Shoot NO3			
	GB	M	RB	RS	GB	M	RB	RS
pH	ns	ns	ns	*	ns	ns	*	ns
Nutrients	ns	ns	ns	***	ns	ns	**	**
pH × Nutrients	ns	ns	ns	*	ns	ns	ns	ns

Figure 2. N-NH_4 (organic N) on dry weight basis (**a**) and NO_3 on fresh weight basis (**b**) concentrations in shoots of green basil (GB), mint (M), red basil (RB), and rocket salad (RS). SN-LpH = standard nutrient concentration and standard pH (control); SN-HpH = standard nutrient concentration and high pH; LN-LpH = low nutrient concentration and standard pH; and LN-HpH = low nutrient concentration and high pH. Two-way ANOVA *p*-values and Tukey's post hoc results are displayed in the figure (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; ns = not significant). Bars represent average values + SD.



	Shoot P-PO4				Shoot K				Shoot Ca				Shoot Mg			
	GB	M	RB	RS	GB	M	RB	RS	GB	M	RB	RS	GB	M	RB	RS
pH	***	*	ns	***	*	ns	ns	ns	*	**	***	ns	**	ns	ns	ns
Nutrients	ns	ns	*	ns	*	ns	ns	***	*	*	**	ns	ns	ns	ns	ns
pH × Nutrients	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns

Figure 3. Shoot macronutrients of green basil (GB), mint (M), red basil (RB), and rocket salad (RS): P-PO_4 (**a**), K (**b**), Ca (**c**), and Mg (**d**). SN-LpH = standard nutrient concentration and standard pH (control); SN-HpH = standard nutrient concentration and high pH; LN-LpH = low nutrient concentration and standard pH; and LN-HpH = low nutrient concentration and high pH. Two-way ANOVA *p*-values and Tukey's post hoc results are displayed in the figure (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; ns = not significant). Bars represent average values + SD.



	Shoot Fe				Shoot Mn				Shoot Zn			
	GB	M	RB	RS	GB	M	RB	RS	GB	M	RB	RS
pH	ns	ns	ns	ns	***	***	ns	**	ns	ns	ns	ns
Nutrients	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
pH × Nutrients	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Figure 4. Shoot micronutrient content of green basil (GB), mint (M), red basil (RB), and rocket salad (RS): Fe (**a**), Mn (**b**), and Zn (**c**). SN-LpH = standard nutrient concentration and standard pH (control); SN-HpH = standard nutrient concentration and high pH; LN-LpH = low nutrient concentration and standard pH, and LN-HpH = low nutrient concentration and high pH. Two-way ANOVA *p*-values are displayed in the figure (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; ns = not significant). Bars represent average values + SD.

3.3. Leaf Pigments

Leaf pigments were generally little influenced by high pH and low nutrient concentration (Table 3). In green basil and rocket salad the pigment concentrations did not show significant differences between treatments. In mint, chlorophylls $a + b$ and carotenoids were lower under SN-HpH treatment, while LN treatment had a positive effect on phenols. Major effects were detected in red basil where all pigments were influenced by the interaction between pH and nutrient concentration. In particular, chlorophyll $a + b$ and carotenoids were higher under SN-HpH treatment than in the control treatment, thereby highlighting a positive effect of high pH on this parameter. A similar effect was retrieved in the total phenols in which, however, the difference was significant only between SN-HpH and LN-HpH. High pH and low nutrient concentration did not alter the chlorophyll a/b ratio in all tested species, while the greenness index was only higher in mint under LN treatments.

Table 3. Chlorophyll *a* + *b*, carotenoids, total phenols, chlorophyll *a*/*b*, and greenness of green basil, mint, red basil, and rocket salad.

		Chl *a* + *b* (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Total Phenols (A_{320} g^{-1} FW)	Chl *a*/Chl *b*	Greenness
Green basil	SN-LpH	1.5 ± 0.30	0.24 ± 0.03	6.4 ± 0.75	3.6 ± 0.25	6.2 ± 0.52
	SN-HpH	1.5 ± 0.19	0.25 ± 0.02	6.4 ± 0.57	3.8 ± 0.35	6.1 ± 0.47
	LN-LpH	1.4 ± 0.24	0.23 ± 0.03	6.1 ± 1.13	3.5 ± 0.25	6.2 ± 0.32
	LN-HpH	1.4 ± 0.11	0.24 ± 0.01	6.0 ± 0.33	3.8 ± 0.33	6.1 ± 0.71
pH		ns	ns	ns	ns	ns
Nutrients		ns	ns	ns	ns	ns
pH × Nutrients		ns	ns	ns	ns	ns
Mint	SN-LpH	2.3 ± 0.07 [a]	0.40 ± 0.02 [a]	10.5 ± 0.57	3.2 ± 0.16	5.7 ± 0.09
	SN-HpH	2.1 ± 0.21 [b]	0.37 ± 0.02 [b]	9.1 ± 1.18	3.2 ± 0.13	5.6 ± 0.33
	LN-LpH	2.4 ± 0.08 [a]	0.38 ± 0.02 [ab]	11.1 ± 0.16	3.3 ± 0.14	6.4 ± 0.42
	LN-HpH	2.5 ± 0.07 [a]	0.41 ± 0.01 [a]	11.4 ± 0.32	3.3 ± 0.04	6.1 ± 0.14
pH		ns	ns	ns	ns	ns
Nutrients		**	ns	**	ns	**
pH × Nutrients		*	*	ns	ns	ns
Red basil	SN-LpH	1.2 ± 0.31 [b]	0.24 ± 0.04 [b]	7.8 ± 1.65 [ab]	3.1 ± 0.27	5.2 ± 0.61
	SN-HpH	1.7 ± 0.21 [a]	0.30 ± 0.03 [a]	9.3 ± 0.79 [a]	3.1 ± 0.08	5.7 ± 0.50
	LN-LpH	1.5 ± 0.15 [ab]	0.27 ± 0.03 [ab]	8.2 ± 1.03 [ab]	3.2 ± 0.09	5.7 ± 0.31
	LN-HpH	1.4 ± 0.24 [ab]	0.23 ± 0.04 [b]	6.9 ± 1.28 [b]	3.2 ± 0.16	6.0 ± 0.22
pH		ns	ns	ns	ns	ns
Nutrients		ns	ns	*	ns	ns
pH × Nutrients		*	**	*	ns	ns
Rocket salad	SN-LpH	1.0 ± 0.25	0.16 ± 0.03	6.6 ± 0.69	3.3 ± 0.28	6.2 ± 0.20
	SN-HpH	1.1 ± 0.10	0.18 ± 0.03	8.0 ± 0.77	3.1 ± 0.47	6.5 ± 0.41
	LN-LpH	1.0 ± 0.28	0.18 ± 0.04	8.3 ± 2.48	3.0 ± 0.03	6.2 ± 0.54
	LN-HpH	1.1 ± 0.09	0.17 ± 0.02	7.4 ± 0.89	3.2 ± 0.16	6.1 ± 0.30
pH		ns	ns	ns	ns	ns
Nutrients		ns	ns	ns	ns	ns
pH × Nutrients		ns	ns	ns	ns	ns

SN-LpH = standard nutrient concentration and standard pH (control); SN-HpH = standard nutrient concentration and high pH; LN-LpH = low nutrient concentration and standard pH; and LN-HpH = low nutrient concentration and high pH. Two-way ANOVA *p*-values and Tukey's post hoc results are displayed in the table (* = $p \leq 0.05$; ** = $p \leq 0.01$; ns = not significant). Data represent average values ± SD. Lowercase letters indicate Tukey's post hoc results.

3.4. Overall Effects

A summary of the effects of treatments on the main investigated yield and quality parameters is reported as a heatmap in Figure 5. Results highlighted different plant responses in terms of biomass, nutritional elements, and leaf pigments, which depended on plant species and variety. Recognizing crop yield as a key factor, or the first discriminating factor from an economic point of view, green basil was the most suitable species among the four tested herbs. When instead considering nutritional properties, green and red basils seem to the least affected by suboptimal growing conditions. Reviewing the main measured performance indicators, the shoot biomass was the most negatively affected parameter in all plants, ranking worst before the uptake of some nutrients, especially NO_3, P-PO_4, Mn, and Zn. Leaf pigments were less affected, instead showing in all species values that were very similar to the control treatment or even higher as in the case of red basil. Overall, the low nutrient concentration mainly influenced plant growth while high pH had major detrimental effects on the absorption of some nutrients like P and Mn, but positive effects on the accumulation of Ca in red basil. The high pH induced the worse effects on shoot biomass of rocket salad while the low nutrient availability affected mint, red basil, and rocket salad. The negative effect of high pH and low nutrient concentration was reflected also on the lower root biomass in red basil and rocket salad.

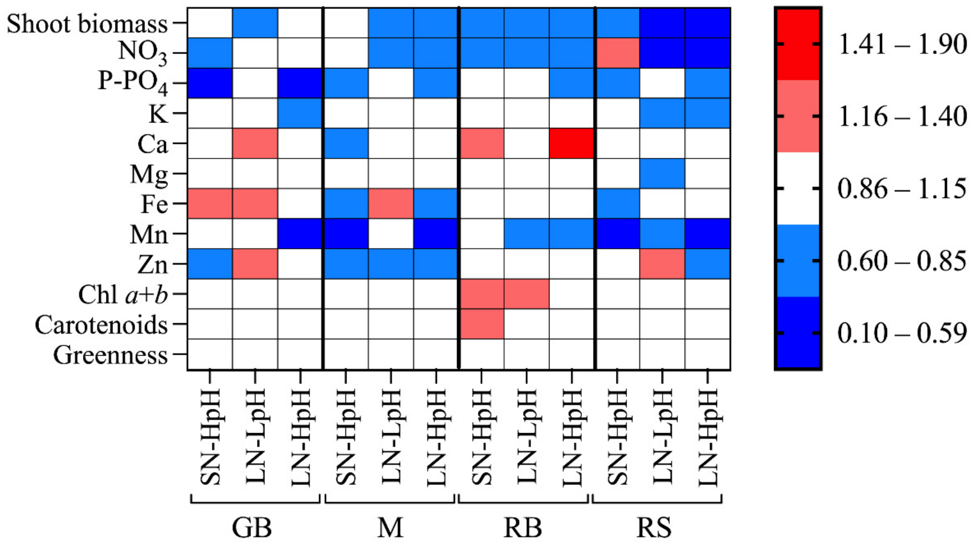


Figure 5. Heatmaps with yield and quality parameters of green basil (GB), mint (M), red basil (RB), and rocket salad (RS). Data are presented as the ratio of average values of treatment and control groups (SN-LpH). SN-HpH = standard nutrient concentration and high pH; LN-LpH = low nutrient concentration and standard pH; and LN-HpH = low nutrient concentration and high pH.

4. Discussion

Standard nutrient solutions for hydroponics are usually prepared with parameters that exceed the safety thresholds for fishes in aquaculture, for example N-NO_3 concentration (>5–7 mmol L^{-1}) and pH level (<6.5–8.5) [27]. In the present work, the worst growing conditions for plants (i.e., the LN-HpH treatment) would be, in general, the most favorable for fish life, with pH ranging between 7.4 and 7.8, and a low concentration of most of the micro and macronutrients. By definition, aquaponics requires that more than 50% of nutrients used by plants for their optimal growth are derived by fish waste [12] thus limiting the addition of extra nutrients in on-demand coupled systems. However, an addition of fertilizers can be essential to obtain comparable yields between aquaponic and hydroponic systems [28]. A possible solution can therefore consist in identifying cultivated species suitable for the agronomic characteristics of these production systems, i.e., tolerant to high pH and low nutrient concentration in the root zone. As example, N-NO_3 concentration must be below specific concentration, e.g., about 36 mmol L^{-1} for juvenile tilapia culture [29] or about 10 mmol L^{-1} for potted knifejaw [30]. Thus, for some fishes as tilapia even the N-NO_3 concentration in our SN treatment would be more than appropriate if nutrient adjustments fulfil the aquaponic principles. On the contrary, less attention has been paid to the importance of pH in nutrient solution for plant yield and nutraceutical aspects. Other authors investigated as an example the application on basils of aquaculture water after a pH adjustment at 5.8 [31].

Results highlighted different plant responses to high pH and low nutrients in terms of biomass, nutritional elements, and leaf pigments, which depended on plant species and varieties (Figure 5). A species-dependent effect on plant biomass parameters using the aquaculture water has already been reported on other leafy vegetables in which the marketable yield was differently affected [21,32,33]. An important reduction in nutrient concentration in the root zone can affect crop yield in most of cultivated species [34] even if in deep liquid cultures nutrients are generally highly available to the crops and can be controlled with a high precision [1]. Indeed, mint plants growing in a deep liquid system showed reduced biomass and leaf area when exposed to macronutrient deficiency [35], but an addition of extra fertilizers to aquaculture water has been shown to avoid yield penalties in this species [28] and in rocket salad [36,37]. However, while mint was less affected by our experimental conditions, the shoot fresh biomass of rocket salad under the combination of LN-HpH was more than halved and the leaf area was severely decreased. This strong biomass penalty was also associated to the highest biomass increment during the cultivation period highlighting a highest productivity and, likely, a higher susceptibility of this species compared with the others. Among all nutrients, LN treatments strongly decreased the uptake of N in rocket salad, as also found by Yang et al. [38], as well as K, both fundamental elements with structural and physiological functions in plants [34]. Stathopoulou et al. [37] reported that an extra addition of K and Fe in aquaponic systems improved rocket salad biomass production without negative effect on fishes. In the present work, plants underwent nutrient concentrations in the root zone equal to 2.1 dS m^{-1} in SN treatments and 1.0 dS m^{-1} in LN treatments. Other authors reported the optimal range of EC for both yield and nutritional parameters of rocket salad at 1.5–1.8 dS m^{-1} while a halving of fresh biomass at 1.2 dS m^{-1} [38] supporting our results obtained under LN treatment.

Among the tested species, green basil did not show a significant reduction in the harvestable organs displaying tolerance to low nutrient concentration and high pH in the root zone. Indeed, it is one of the most widely cultivated species in commercial aquaponic and hydroponic production sites [1,39]. Fewer studies have been carried out to our knowledge on the effect of nutrient solution pH on produced yield of rocket salad and other leafy vegetables grown hydroponically [40]. Negative effects on both shoot and root biomasses have been reported under alkalinity stress in these species [41]. In our experimental conditions, only rocket salad showed a reduced yield as a function of pH variations. Therefore, it can be argued that a proper fertilizer addition, in compliance with aquaponics requirements, would be sufficient for the successful production of red basil and mint irrigated with aquaculture water, as highlighted in previous works [28], while pH level would be of minor relevance.

Interestingly, plant water uptake of mint and red basil did not follow the same trend of leaf area, which is a factor driving plant evapotranspiration under abiotic stress [42]. Indeed, crop evapotranspiration may influence plant nutrient uptake in hydroponic systems. The use of a mineralization unit between the hydroponics and aquaculture compartments in decoupled systems has been shown to increase the nutrient available to the plants in presence of high crop transpiration rates [20]. In previous works, we found that the plant water uptake and root biomass had a higher degree of correlation compared with leaf area in deep liquid culture [43]. Root morphological adaptation and performance can indeed play a major role to overcome limitations or abiotic stresses which may occur in the root zone of soilless-grown plants [40]. As a matter of fact, high pH and low nutrients have a positive effect on the root to shoot ratio in green basil, supporting an active plant response to the unfavorable nutrient availability [44]. On the contrary, red basil and rocket salad passively suffered the adverse growing conditions showing a reduced root and shoot biomass, and mint showed a higher root to shoot ratio, but this was not sufficient in facing the detrimental effect of LN treatment on the shoot FW. Root response to environmental changes is indeed species-specific and even more complex under soilless conditions. Therefore, further studies are needed, especially assessing the effects of pH in the root zone of soilless-grown plants [40].

The nutrient shortage decreased the NO_3 concentration in rocket salad while the high pH decreased this parameter in red basil thereby improving their value as food. The NO_3 can in fact be harmful for human beings due to its conversion into nitrites, which can cause several diseases, particularly if consumed fresh [45]. Similarly, the use of water derived from aquaculture did not increase in other studies the NO_3 concentration in other leafy vegetables, such as lettuce and kale [46]. In aquaculture wastewater, N is usually excreted by fish as ammonia which is then converted in NO_3 by nitrifying bacteria requiring high pH for optimal conditions [47]. Therefore, it appears crucial to increase the knowledge on plant response to high pH under hydroponic conditions [40]. Nitrogen and P efficiency have been shown to increase in aquaponics compared with hydroponics in basil and other horticultural crops [48]. In our experimental conditions, organic N was negatively affected by low nutrients while increased under SN-HpH treatment only in rocket salad. The high pH generally decreased the P content. Phosphorus starvation has been reported to reduce P concentration shots especially if coupled with N and K deficiency [49]. Indeed, a decrease in P availability with an increase in pH in aquaponic nutrient solutions has been already highlighted [50]. The high pH also decreased Ca in green basil and mint probably due to the formation of insoluble calcium phosphates [50] while an unclear effect was observed in red basil. The nutrient shortage instead increased Ca concentration in green basil, mint, and red basil likely due to a lower competition of this element with K. However, the higher K intake is generally coupled with a lower Ca and Mg uptake [51], while in the present study Mg was unaffected by nutrient concentration. Similarly, Fe and Zn were unaffected by neither high pH nor low nutrient while it is generally accepted that the above conditions can severely limit the availability of micronutrients in hydroponics; in particular, pH higher than 7.0 can induce Fe, Zn, and Mn deficiencies [52]. A strong decrease in Mn was instead induced in green basil, mint, and rocket salad under high pH, although the detected values were not below the sufficiency thresholds for herbs [53].

Leaf pigments were less affected by treatments as also observed by other authors at the first harvest of *Taraxacum officinale* grown hydroponically with increasing pH of the nutrient solution [54]. All pigments increased under high pH in red basil. However, such an increase was significantly different only under optimal nutrient concentration compared with the control treatment, thereby highlighting an interaction between factors. This trend was possibly linked to the complex composition of pigments in dark-color leaves. Thus, leaf pigments did not prove to be a suitable parameter to assess possible negative effects of aquaculture water on leafy vegetable biomass in aquaponics.

5. Conclusions

Among the tested species, results highlighted that green basil is the most tolerant herb to the high pH and low nutrient concentration of nutrient solution under hydroponics, followed by mint. The strong penalty in biomass parameters found in red basil and especially rocket salad highlighted the possible unsuitability of these leafy vegetables for the direct application of water derived from aquaculture without opportune adjustments. Further investigation using fishes are required for a suitable use of green basil and mint for aquaponic purposes.

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