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Impact of Chelated or Inorganic Manganese and Zinc Applications in Closed Hydroponic Bean Crops on Growth, Yield, Photosynthesis, and Nutrient Uptake

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Abstract: In this study, we investigated the effect of individual and combined applications of manganese (Mn) and zinc (Zn) chelates on common bean grown in hydroponics (nutrient film technique—NFT) on physiological and agronomical responses. Inorganic sulphate forms of Mn and Zn were compared to their synthetic chelate forms, in the replenishment nutrient solution (RNS). Nutrient (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu) to water uptake ratios (termed uptake concentrations; UCs), growth, pods yield and quality, photosynthetic parameters and tissue nutrient status were evaluated in different cropping seasons (spring-summer and autumn-winter crops). Mean UCs of nutrients ranged as follows: 10.1–12.4 (N), 0.8–1.0 (P), 5.2–5.6 (K), 1.8–2.2 (Ca), 0.9–1.0 (Mg) mmol L^{-1}; 12.2–13.4 (Fe), 5.2–5.6 (Mn), 4.4–4.9 (Zn), 0.9–1.0 (Cu) µmol L^{-1}. Tissue macronutrient status remained unaffected in both seasons, however, Mn chelates in the RNS affected Fe within plants. Pod yield and quality, growth, photosynthesis and water uptake did not differ among treatments; however, seasonal variations are presented. Results suggest that the chelate forms of Mn and/or Zn in the refill solution for NFT-grown beans do not lead to any changes, adding superiority in the yield, photosynthesis, and nutritional status of the crops compared to their mineral forms.

Keywords: *Phaseolus vulgaris*; Mn; Zn; micronutrients; chelates; nutrient film technique-NFT; nutrient uptake concentrations

1. Introduction

Perhaps the most important components for economic success in the greenhouse industry are successfully addressing the water and nutrient needs of the crop. In the modern greenhouse industry, sustainable production mainly relies on hydroponic systems with the reuse of drainage water, since a close control in the supply of water and nutrients is impossible in soil-grown crops [1,2]. In such systems, the constitution and use of nutrient solution is a subject of permanent attention [3]. Although some 'universal' nutrient solutions (NS) are known by early investigators (e.g., [4]), crop specific NS has been of primary importance in the modern research concept of plant nutrition (e.g., [1,5–7]). Despite differences in NS formulations, all hydroponic systems supply inorganic nutrients exclusively via the irrigation water. To prepare NS containing all the essential nutrients, highly soluble inorganic salts are used to provide most of them (e.g., $CaNO_3 \times 4H_2O$, KNO_3, KH_2PO_4, $MgSO_4 \times 7H_2O$). However, the availability of most micronutrients, such as iron (Fe), manganese (Mn) and less zinc (Zn) and copper (Cu), strongly depends on the pH maintained in the root environment. The uptake of

the metallic micronutrients is more affected by the pH than by the supplied concentrations of these elements (e.g., increased availability by a decrease in pH value; [8]). In view of the above, chelated fertilizers were developed to increase micronutrient utilization efficiency and refer to inorganic nutrients bonded to an organic molecule (i.e., chelating agents), protecting them from oxidation and precipitation [9]. Ethylenediaminetetra acetic acid (EDTA), diethylenetriaminepenta acetic acid (DTPA) and ethylenediamine-di-o-hydroxyphenyl acetic acid (EDDHA) are the most common chelates used in soilless culture. Although boron can also form chelated complexes [10], their use in agriculture is unusual. Actually, only the metallic micronutrients, i.e., iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu), are used in chelated forms as fertilizers in agriculture. The use of iron chelate is a standard practice in greenhouse soilless crops because inorganic iron in nutrient solutions precipitates readily in the form of iron phosphate at pH levels up to 6, while at higher pH levels, an increasing amount of Fe precipitates as $Fe(OH)_3$ [11]. In contrast, micronutrients such as Mn and Zn are mainly supplied in their mineral forms (e.g., $MnSO_4 \times 4H_2O$ and $ZnSO_4 \times 7H_2O$, respectively) and, unlike Fe, fewer studies have been conducted on the supply of chelated Mn and Zn forms in soilless culture [12]. However, there is a notion that the mineral forms of these micronutrients in NS formulas, which are less expensive, are suitable for optimum plant growth [13,14]. In this context, there is still more information needed regarding the effects of fertilizer application on micronutrients in vegetables, since (i) optimum and toxic concentrations are very close [15], (ii) can be targeted serviced to food crops (biofortification, [16]) and (iii) nutrient interactions do not allow for the extrapolations of results to other production systems [17]. Subsequently, the utilization efficiency of chelated micronutrients applied individually or simultaneously in closed hydroponic conditions is of scientific interest and still has to be defined [12,18].

Commonly, metal micronutrients Fe, Zn, and Mn function similarly in plants and all are involved in chlorophyll synthesis and in the transfer of electrons and energy (e.g., electron transfer in metabolic processes like photosynthesis and respiration, [19,20]). Cu is also involved in physiological redox processes [21]. The accumulation of these micronutrients within plants generally follows the order of Mn > Fe > Zn > Cu [22]. The plants commonly take up the metal micronutrients from the root zone solution as divalent cations (Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}; [13]), however, the metals might be taken in metal-chelate complexes [23]. After uptake, metals are transported inside xylem by membrane transport proteins (ZIP and Nramp proteins), to be translocated from roots to shoots [24]. In soilless culture systems, a close relationship has been found between the concentrations of several micronutrients (e.g., Mn, Fe, Zn and Cu) in the external solution and the uptake by plants, however, this relationship may be easily disturbed by chelate complexes. For example, at higher pH values in the root environment, Fe is easily replaced in the chelate complex prior to uptake by other cations, such as Mn, Zn and Cu, affecting both the absorbance capacity of these elements and their mutual uptake ratio [1].

Maintaining an optimum nutrient level in the root zone solution requires an equilibrium between the concentrations of nutrients added to the closed system and the uptake by the crop. Thus, the procedure to add water and nutrients for a certain plant species based on the concept of mean uptake concentrations (UCs; being the ratio between the weights of nutrients per volume of water consumption) fits well in closed hydroponic systems [1,25]. However, there were some differences between the UCs under Northern Europe climatic (cool and humid) conditions [1,5,26] and the corresponding values for crops in Mediterranean climates (hot and dry) [3,6]. This is mainly related to higher transpiration rates, in the latter case due to higher radiation input [27]. Thus, determining the mean nutrient UCs for a particular crop at a certain growth stage adjusted to the prevailing climatic conditions is the first step to establish ionic compositions of NS supplied to closed hydroponic systems in Mediterranean greenhouses. Obviously, optimizing the mineral nutrition of bean crops according to this concept is not compatible with the use of a standard formula suggested in the literature.

This study aimed to investigate manganese (Mn) and zinc (Zn) chelated fertilizers applications, and considerations for their use in greenhouse closed hydroponic common bean crops (nutrient film technique, NFT). The study also aimed to determine nutrient to water uptake ratios (namely uptake

concentrations; UC) in bean crops, which constitutes a good basis for nutrients supply for closed hydroponic systems. In addition to a typical nutrient solution for hydroponic beans containing Mn and Zn in their mineral forms (i.e., control treatment), three more treatments were applied, differing in the supplied forms (synthetic chelates or inorganic) of Mn and Zn: (i) Mn-EDTA and Zn-SO_4, (ii) Zn-EDTA and Mn-SO_4, and (iii) Mn-EDTA and Zn-EDTA. Water uptake, plant nutrient status, gas exchange indices, chlorophylls, pod yield and nutritional quality were also determined.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Two experiments were conducted at the Agricultural Research Institute of Cyprus (latitude 34°94′ N, longitude 33°19′ E, altitude 35 m above sea level), in a multi span plastic film arched greenhouse under natural light conditions, from October 2018 to January 2019 (autumn-winter; AW), and from March to June 2019 (spring-summer; SS). Climatic data during the experimental period are reported in Table 1. Mean hourly solar radiation outside the greenhouse ranged between 1163 (AW crop) and 1975 (SS crop) kJ m^{-2}. The greenhouse was equipped with an automatic climate controller maintaining air temperature and relative air humidity between 14–28 °C (average 23.5 °C in AW crop and 25.1 °C in SS crop), and 60–80%, respectively. Mean values for the external climate and inside the greenhouse microclimate are reported in Table 1 [28].

Table 1. Daylight hourly values (standard errors in brackets) of solar radiation (SR, kJ m^{-2}) and ultraviolet radiation (UV, kJ m^{-2}); air temperature (T_o °C, outside greenhouse and T_i °C, inside greenhouse) and relative air humidity (Rh_o %, outside greenhouse and Rh_i %, inside greenhouse), in two cropping periods (autumn-winter; AW; spring-summer; SS).

Period	SR	UV	T_o	Rh_o	T_i	Rh_i
			AW			
October	1375.2 (35.7)	47.5 (2.51)	17.5 (0.35)	63.4 (1.02)	27.5 (0.10)	70.4 (0.25)
November	1113.2 (59.46)	35.2 (1.87)	14.9 (0.42)	68.0 (1.96)	24.2 (0.07)	72.0 (0.23)
December	1089.9 (40.32)	34.4 (1.27)	14.5 (0.27)	70.5 (1.22)	21.5 (0.05)	69.2 (0.30)
January	1075.0 (810)	32.2 (1.05)	14.2 (0.34)	72.8 (1.50)	21.1 (0.07)	73.0 (0.35)
			SS			
March	1639.6 (58.52)	55.1 (1.96)	15.8 (0.20)	73.9 (0.79)	22.8 (0.06)	69.6 (0.20)
April	1884.0 (62.90)	67.1 (2.23)	18.4 (0.22)	69.1 (0.77)	24.4 (0.08)	67.5 (0.17)
May	2222.9 (61.95)	80.7 (2.24)	24.0 (0.22)	60.2 (0.71)	25.2 (0.04)	71.8 (0.15)
June	2156.2 (62.10)	70.3 (2.02)	27.1 (0.45)	62.3 (0.87)	27.6 (0.06)	78.5 (0.22)

Seeds of climbing common bean (*Phaseolus vulgaris* L. cv. Moraleda, Monsanto, the Netherlands) were sown on rockwool cubes (0.05 × 0.04 m), two weeks before transplanting. On 12 October 2018 (AW) and 27 March 2019 (SS), bean plantlets at the stage of two leaves were transferred to 20 individual NFT (nutrient film technique) growing gullies, each of which was a part of an independent hydroponic circuit (Figure 1). Two external gullies on each side were used to exclude border effects, and internal sixteen gullies were served as experimental units. Each gully ($L_{ength} \times W_{idth} \times H_{eight}$; 6.0 × 0.25 × 0.10 m) accommodated 48 plants, giving a final planting density of 10 plants m^{-2}. The fertigation system was based on a programmed addition of nutrients (the amounts of water and nutrients introduced into the system are equal to those absorbed by the plants). The fertilizer head unit thus prepared a replenishment nutrient solution for closed systems (RNS; [1]), in which nutrient concentrations were equal to the mean expected uptake concentrations (UC), originating from Savvas [14], and directed in replenishment tanks. Thereafter, RNS was automatically introduced, using suitable equipment into the nutrient solution collection tanks to replenish water and nutrients absorbed by the plants. Each drain tank (40 L) was connected to the corresponding replenishment tank (110 L; Figure 1). This was done via a floater, so that the volume of the nutrient solution in the closed system was kept constant. Mixing the

addition solution with the drainage solution and adjusting the pH (5.6) resulted in the preparation of the irrigation solution, which was transferred back to the culture at a rate of 0.1 m^3 h^{-1}. The system was continuously operated, enabling complete recycling of the nutrient solution, with zero discharge of fertigation effluents. Using a covered leak-proof system, the volume of water introduced to the system was considered to be equal to the crop water uptake. The design of NFT troughs and materials and the requirements for the management of recirculating NS is discussed by Neocleous and Savvas [29,30]. Bean plants were trained vertically using a plastic string to an overhead horizontal wire (2.2 m over plant rows). The string was attached to the base of the main stem and it was twisted around the central shoot. Pruning aimed to balance vegetation and fruiting, remove old leaves and improve ventilation and lighting conditions. Pest and diseases were controlled following good agricultural practices for greenhouse vegetable crops [31].

Figure 1. Common bean (*Phaseolus vulgaris* L. cv. Moraleda) grown in nutrient film technique (NFT) hydroponic circuits, under Mediterranean greenhouse conditions.

The RNS, used as control treatment, initially had the following composition: 4.8 mM K^+, 2.5 mM Ca^{2+}, 1.1 mM Mg^{2+}, 1.4 mM NH_4^+, 9.9 mM NO_3^-, 1.0 mM $H_2PO_4^-$, 1.0 mM SO_4^{2-}, 12 µM Fe as Fe-EDDHA, 5 µM Mn, 4 µM Zn, 0.6 µM Cu, 20 µM B, and 0.5 µM Mo. The EC and pH values of this NS were 1.6 dS m^{-1} and 5.6, respectively. At the reproductive stage, to maintain the target concentrations in the root environment, the nutrient solution composition was altered as follows: 5.6 mM K^+, 1.9 mM Ca^{2+}, 0.85 mM Mg^{2+}, 1.2 mM NH_4^+, 9.2 mM NO_3^-, 1.0 mM $H_2PO_4^-$, 0.8 mM SO_4^{2-}, 10 µM Fe as Fe-EDDHA, 5 µM Mn, 4 µM Zn, 0.6 µM Cu, 20 µM B, and 0.5 µM Mo. The EC and pH values of this NS were 1.5 dS m^{-1} and 5.6, respectively. Recommended compositions of nutrient solutions were based on those suggested by Savvas [14] for Mediterranean soilless common bean crops. The concentrations of all nutrients were identical in all cases. Treatments differed only in the forms of Mn and Zn in the replenishment NS, supplying Fe in all cases as Fe-EDDHA. Control treatment «Ct» corresponded to a replenishment NS with inorganic (i.e., sulphate) forms of Mn ($MnSO_4 \times H_2O$) and Zn ($ZnSO_4 \times 7H_2O$). Treatment «Mn» was a NS with Mn-EDTA and mineral Zn ($ZnSO_4 \times 7H_2O$). Treatment «Zn» was a NS with Zn-EDTA and mineral Mn ($MnSO_4 \times H_2O$). Treatment «All» was an NS with both chelated forms of Mn (Mn-EDTA) and Zn (Zn-EDTA). EDTA was previously used as the most satisfactory solution for Mn and Zn [12]. Experimental treatments were arranged in a randomized complete block design with four replications (one experimental unit per replicate).

2.2. Measurements and Analyses

NS samples from each experimental unit (recycled NS) and replenishment tank (nutrient solution added) were taken on 0, 30, 60 and 90 (days after transplanting—DAT), for measuring nutrient solution composition (i.e., K^+, Ca^{2+}, Mg^{2+}, NO_3^-, NH_4^+, $H_2PO_4^-$, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}), whereas plant material (i.e., leaves and pods) was collected 60 days after transplanting (DAT), to determine

tissue nutrient concentrations (i.e., N, P, K, Ca, Mg, Fe, Mn, Zn and Cu). The procedures described in Neocleous and Savvas [30] were followed to determine the N, P, K, Ca and Mg in solution and plant samples. The concentrations of selected micronutrients (Fe, Mn, Zn and Cu) in the subsamples of wet digested (a mixture of concentrated HNO_3 – HCl; 2:1 *v*/*v*) plant material, and in the NS, were determined by the atomic absorption spectrophotometry (Solaar M Series, Thermo Elemental, Cambridge, UK) procedure [32,33]. Thus, chemical composition of the added and recirculated NS was used to determine crop nutrient uptake (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu) per volume of water absorbed (mmol L^{-1} or μmol L^{-1}), as suggested previously [29,34]. Particularly, the mean uptake concentration (UC_x; mmol L^{-1} or μmol L^{-1}) of the x selected macro-or micronutrient was determined using the following equation provided in Neocleous and Savvas [29]:

$$UC_x = [W_s \times (C_{xin} - C_{xf}) + W_{add} \times C_{xadd}]/W_{add} \quad (1)$$

where, W_s was the amount of water in each hydroponic unit (L); W_{add} was the amount of water added to the closed system (L) in each time interval; ($C_{xin} - C_{xf}$) was the initial minus final concentration of the x nutrient in the recycled solution (mmol L^{-1} or μmol L^{-1}) in each time interval; C_{xadd} denotes the concentration of the nutrient x in the replenishment NS (mmol L^{-1} or μmol L^{-1}) in each treatment; Nitrogen UC corresponds to the sum of nitrate and ammonium UCs. This approach, based on the nutrient and water removal from the system, takes into account both plant nutrient uptake and losses (e.g., precipitation) from the system and estimated values are termed «apparent» UCs [8]. The results are presented on the basis of the development stages of bean crops (vegetative and reproductive phase), in order to be of practical significance and comparable with published recommendations.

Physiological responses (leaf gas exchange indices i.e., net photosynthetic rate, stomatal conductance, transpiration and intercellular CO_2 concentration, and chlorophyll indices i.e., fluorescence and concentration) of bean crops were determined 60 DAT from triplicate measurements on three plants per experimental unit, using the instrumental methods reported in a previous work [35]. Harvesting commenced on 35 DAT and pods were harvested at commercial maturity (dark green pods 15 cm long), to determine fresh yield and quality parameters until crop termination (90 DAT). Total yield was measured in each experimental unit and on three labelled plants. For quality analyses, green pods samples (300 g) were immediately frozen, and stored at −30 °C in plastic bags. Pod nitrate content, reducing sugars (glucose and fructose), dry matter, ascorbic acid, total phenols, reducing antioxidant power (FRAP assay), radical scavenging activity (DPPH assay), and chlorophyll content were determined as previously described [36].

2.3. Statistical Analysis

A randomized complete block design with four replications (blocks) was used to study the effect of individual and combined manganese (Mn) and zinc (Zn) chelate applications on common beans. An analysis of variance (one-way ANOVA) was performed on selected data using the Statistical Analysis System (SAS ver. 9.2, Cary, NC, USA). A Duncan's multiple range test was performed for all parameters measured, when the ANOVA was significant at $p \leq 0.05$ level. Figures were drawn using GraphPad Prism (Version 5.0; GraphPad Software; San Diego, CA, USA).

Table 2. Macronutrient N, P, K, Ca and Mg (mmol L^{-1}) and micronutrient Fe, Mn, Zn and Cu (μmol L^{-1}) uptake concentrations (UCs) in different growth stages (Vegetative-V and Reproductive-R) of NFT-grown common bean plants submitted to nutrient solution (NS) treatments, differing in the supplied forms (chelates or inorganic) of manganese (Mn) and zinc (Zn) in two cropping seasons (spring-summer, SS crop; autumn-winter, AW crop). The treatments (NS) were: «Ct», NS with inorganic Mn and Zn; «Mn», NS with chelated Mn; «Zn», NS with chelated Zn; «All», NS with chelated Mn and Zn.

Chelates	**N**[†]		**P**		**K**		**Ca**		**Mg**		**Fe**		**Mn**		**Zn**		**Cu**	
SS crop	V	R	V	R	V	R	V	R	V	R	V	R	V	R	V	R	V	R
Ct	12.7d	11.1	0.82a	0.83a	5.29	5.62	2.33	1.98	0.93	0.89	13.8c	11.9a	5.22c	5.16	4.87	4.30	0.99	1.06
Mn	12.3ab	11.2	0.85b	0.86b	5.32	5.54	2.20	2.01	0.97	0.90	12.4b	12.1b	5.15b	5.15	4.79	4.36	0.93	1.05
Zn	12.2a	10.7	0.81a	0.82a	5.30	5.56	2.15	1.97	0.91	0.88	11.7a	12.2c	5.07a	5.18	4.92	4.42	0.99	1.03
All	12.4bc	11.3	0.87c	0.88c	5.28	5.40	2.23	2.00	0.95	0.91	14.1c	12.5d	5.18bc	5.16	4.86	4.35	0.98	1.06
Significance	*	NS	*	*	NS	NS	NS	NS	NS	NS	*	*	*	NS	NS	NS	NS	NS
Mean	12.4B	11.1A	0.84A	0.85A	5.30A	5.55B	2.21B	2.00A	0.94B	0.89A	13.0B	12.2A	5.15A	5.16A	4.86B	4.35A	0.97A	1.05B
AW crop																		
Ct	12.1	10.5b	1.04	0.98	5.19b	5.59	2.15	1.79	0.96a	0.84a	13.5	12.7	5.75	5.31	4.74	4.92	0.91	0.84ab
Mn	11.9	9.51a	0.99	1.00	5.05a	5.49	2.11	1.73	0.95a	0.85a	13.3	12.5	5.64	5.35	4.63	4.88	0.88	1.33c
Zn	11.9	10.0ab	1.01	1.02	5.24b	5.57	2.13	1.78	0.97ab	0.86ab	13.3	12.6	5.71	5.32	4.68	4.98	0.79	0.82a
All	12.0	10.3b	1.04	1.01	5.26b	5.85	2.10	1.89	1.05c	1.00b	13.5	12.7	5.63	5.27	4.77	4.87	0.85	0.82a
Significance	NS	*	NS	NS	*	NS	NS	NS	*	*	NS	NS	NS	NS	NS	NS	NS	*
Mean	12.0B	10.1A	1.03A	1.00A	5.19A	5.62B	2.12B	1.80A	0.98B	0.89A	13.4B	12.6A	5.64B	5.33A	4.71A	4.91B	0.86A	0.90A

[†] NO_3 + NH_4. NS and * indicate nonsignificant or significant differences at the 0.05 probability level. For each crop, means of different treatments (n = 4), for each characteristic within the same column, followed by different lower-case letters, are significantly different according to Duncan's multiple range test ($p \leq 5\%$). Overall means of vegetative and reproductive stage (n = 16) in each growth season (SS and AW), followed by different capital letters, show differences.

3. Results

3.1. Uptake Concentrations

An analysis of variance did not detect significant differences ($p < 0.05$) imposed by the tested chelate NS treatments on the mean uptake concentrations (UCs), in most of the cases studied, and any differences detected (e.g., P, Fe) were not clear and consistent (Table 2). Results also revealed that UCs obtained during the vegetative stage were higher than at the harvesting period for most of macro-and micronutrients, irrespective of the growing season (Table 2). During SS crop, estimated mean UCs were (vegetative-reproductive): 12.4–11.1 (N), 0.84–0.85 (P), 5.3–5.6 (K), 2.2–2.0 (Ca), 0.94–0.89 (Mg) mmol L^{-1}, 13.0–12.2 (Fe), 5.1–5.2 (Mn), 4.9–4.4 (Zn), 1.0–1.1 (Cu) μmol L^{-1}. Accordingly, those values were 12.0–10.1 (N), 1.03–1.00 (P), 5.2–5.6 (K), 2.12–1.80 (Ca), 0.98–0.89 (Mg) mmol L^{-1}, 13.4–12.6 (Fe), 5.6–5.3 (Mn), 4.7–4.9 (Zn), 0.86–0.90 (Cu) μmol L^{-1} during AW crop. Furthermore, from the data in Table 2, it was computed that the mean molar mutual uptake ratios of K:Ca:Mg and N:K resulted in comparable results in both growing seasons and averaged 0.63:0.26:0.11 and 2.3, respectively, during the vegetative stage, and 0.67:0.23:0.10 and 1.9, respectively, during the reproductive stage. Averaging data across treatments, growth stages and seasons UCs averaged 11.4 (N), 0.9 (P), 5.4 (K), 2.0 (Ca), 0.9 (Mg; mmol L^{-1}), 12.8 (Fe), 5.3 (Mn), 4.7 (Zn), and 0.9 (Cu; μmol L^{-1}).

3.2. Recycled Drainage Nutrients, EC, pH and Water Uptake

Similar to the results of UCs, applied chelate NS treatments did not produce strong differences with respect to the average nutrient concentrations, the electrical conductivity (EC) and pH in the recycled drainage solution (RDS) and plant water uptake. However, for commercial hydroponics, it is of practical significance to observe nutrient accumulation changes in RDS, with time indicating crop developmental stage (Figure 2). Generally, macronutrients accumulated at higher values in the RDS turning from the vegetative to reproductive stage (Figure 2A). However, P and Mn in the RDS tended to be stabilized at 1 mmol L^{-1} and 4 μmol L^{-1}, respectively. Micronuntrients Fe, Zn, and Cu exhibited a tendency to accumulate in the RDS, irrespectively of the growing season (Figure 2B). The electrical conductivity (EC) in the RDS ranged between 1.6–2.6 dS m^{-1} during most of the growing period, rising at relatively high values (>2.8 dS m^{-1}) only at final stages (Figure 2C). However, EC increase in RDS was greater in the spring-summer (SS) crop in comparison with autumn-winter (AW) crop at crop termination. With emphasis on the pH of the recirculating NS, values were always in the range between 5.5–6.3 (Figure 2C), with an average of 5.7 and 5.8 for the AW and SS crop, respectively. Furthermore, the uptake of water introduced to compensate for transpiration losses followed a similar pattern in both cropping seasons, summing the plant water requirements to 22.5 L $plant^{-1}$ and 56 L $plant^{-1}$, for the AW and SS crop, respectively (Figure 2D). Accordingly, the root zone solution temperature averaged between 23.2 to 24.9 °C in the two crops (Figure 2D).

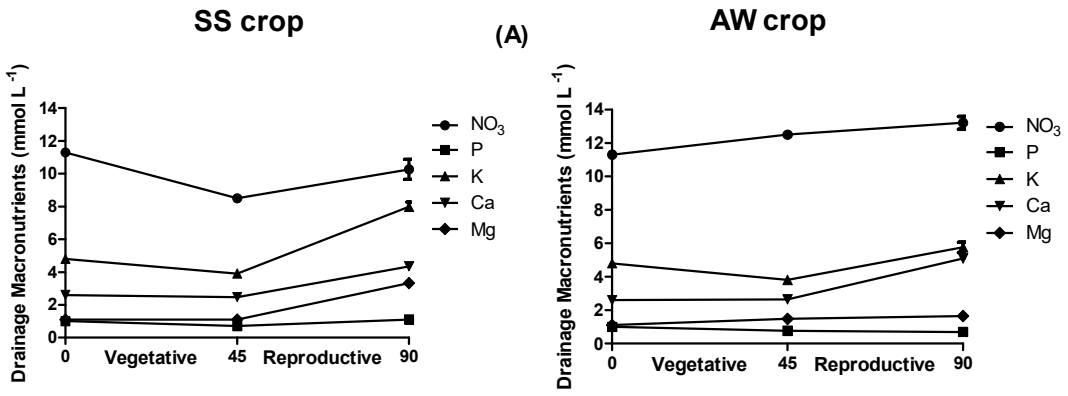

Figure 2. *Cont.*

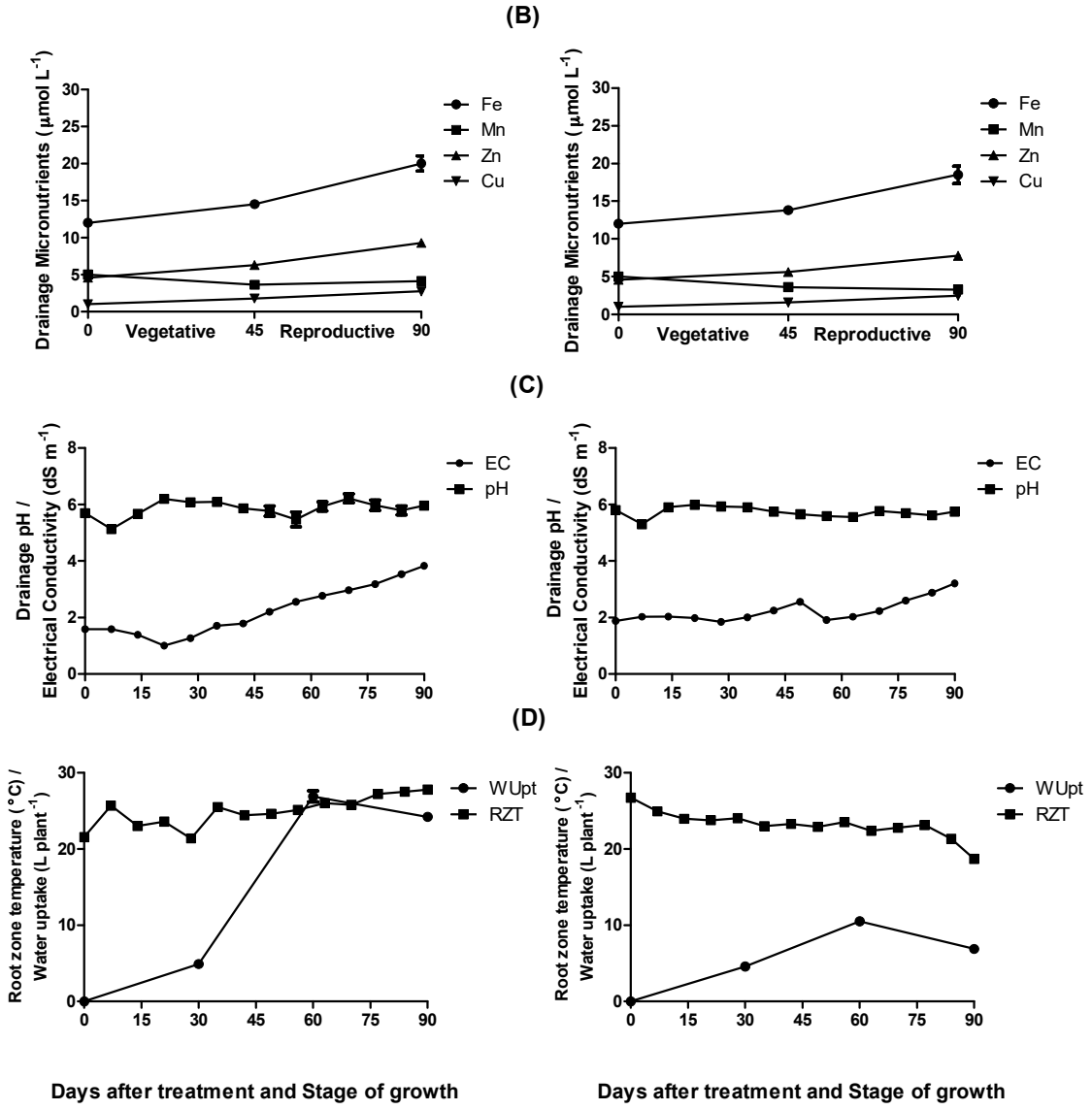

Figure 2. (**A**) Macro-(NO_3, P, K, Ca and Mg; mmol L^{-1}) and (**B**) micronutrient (Fe, Mn, Zn and Cu; µmol L^{-1}) concentrations, (**C**) electrical conductivity (EC; dS m^{-1}), pH and (**D**) root zone temperature (RZT; °C) in the recycled drainage solution and water uptake (L $plant^{-1}$) in two cropping seasons (spring-summer, SS crop; autumn-winter, AW crop) of hydroponic common bean, in relation with time indicating growth stages (Vegetative and Reproductive). Values are means calculated across treatments and vertical bars indicate ± standard errors of means (not shown when smaller than the symbol).

3.3. Physiological and Agronomical Responses

Common bean physiological (photosynthesis and related parameters), agronomical (yield and biomass production) and fresh pod quality parameters did not reveal any differences among the chelate NS applications of the current study. Pod yield averaged 972 g $plant^{-1}$ (9.7 kg m^{-2}), fresh biomass production averaged, 935 g $plant^{-1}$; and dry biomass production averaged 114 g $plant^{-1}$ (Figure 3A–C). The overall means for gas exchange parameters were as follows: chlorophyll fluorescence Fv/Fm ratio 0.80; net CO_2 assimilation, 15.8 µmol CO_2 m^{-2} s^{-1}; stomatal conductance, 0.41 mol H_2O m^{-2} s^{-1}; transpiration rates, 4.49 mmol H_2O m^{-2} s^{-1}; and intercellular CO_2 concentrations, 258 µmol CO_2 mol^{-1} air (Figure 3D–H). Moreover, green pods quality components and natural antioxidant compounds averaged as follows: nitrate content, 738 mg NO_3 kg^{-1} FW; vitC, 50.8 mg ascorbic acid kg^{-1} FW; total sugars, 10.2 mg glucose + fructose g^{-1} FW; dry matter 9.45% DW; reducing antioxidant

power-FRAP, 0.213 µmol ascorbic acid g^{-1} FW; radical scavenging activity-DPPH, 30.2 mg ascorbic acid equivalent $100g^{-1}$ FW; phenolics, 0.123 mg gallic acid g^{-1} FW; and total chlorophyll content, 0.027 mg g^{-1} FW (Figure 3I–P). Although comparisons between growing seasons were not an objective of this study, the results are presented as shown in Figure 3, to help understand the mechanisms of crop response.

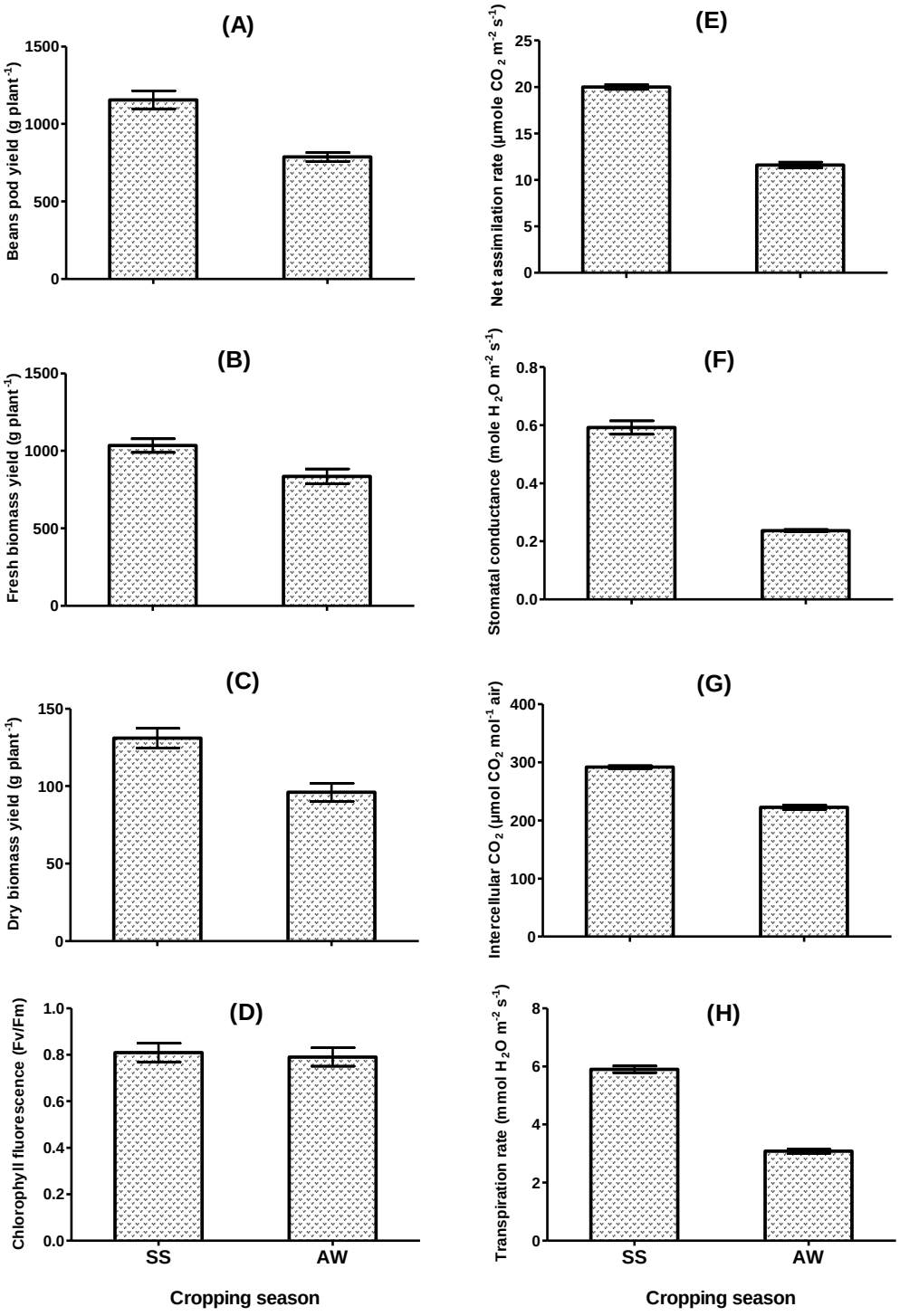

Figure 3. *Cont.*

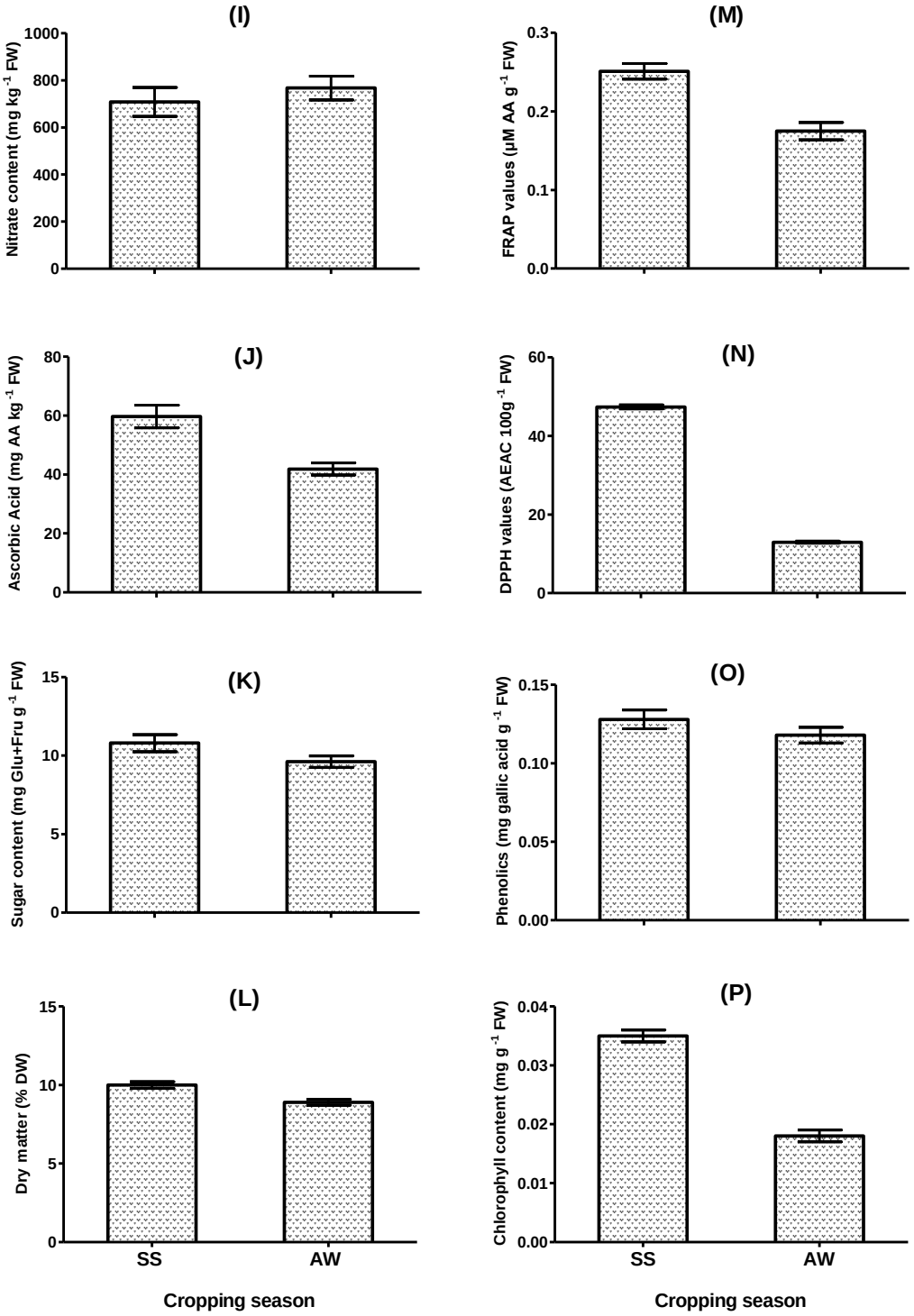

Figure 3. Evaluation of growth ((**A**) pod yield and (**B**,**C**) biomass production), (**D**) chlorophyll fluorescence, gas exchange parameters ((**E**) net CO_2 assimilation rate, (**F**) stomatal conductance, (**G**) intercellular CO_2 concentration and (**H**) transpiration), pod nutritional quality ((**I**) nitrate content, (**J**) ascorbic acid, (**K**) total sugars, (**L**) dry matter, antioxidant (**M**) FRAP and (**N**) DPPH values, (**O**) phenolics and (**P**) chlorophyll content) in two cropping seasons (spring-summer, SS crop; autumn-winter, AW crop) of hydroponic common bean. Values are means calculated across treatments and vertical bars indicate ± standard errors of means.

3.4. Tissue Nutrient Concentrations

Chelate NS treatments had no effect on macronutrient concentrations in the fully-grown young leaves of common bean. Particularly, N, P, K, Ca and Mg contents averaged 3.4, 0.48, 3.52, 2.76 and 0.30% DW (spring-summer crop), and 4.04, 0.51, 4.17, 3.75, and 0.45% DW (autumn-winter crop), respectively. However, chelate applications resulted in certain changes of micronutrients Fe and Zn in leaves and pods (Table 3). Higher values of Fe concentrations in leaves and fresh pods were observed in plants supplied with control NS (Ct), however, consistent differences were observed only compared to the plants supplied with chelate Mn (Table 3). Concentration changes for Zn concerned higher values in leaves and fresh pods of plants supplied with chelate Mn compared to the plants supplied with combined Mn and Zn chelates (i.e., all), while differences in relation to the control treatment Ct were negligible. In any case, micronutrient concentrations in leaves were higher than in fresh pods.

Table 3. Micronutrient concentrations as ppm (μg g^{-1} DW) in leaves and fresh pods of NFT-grown common bean plants, 60 days after treatment initiation submitted to the chelate forms of Mn and Zn in the nutrient solution (NS) in two cropping seasons (spring-summer, SS crop; autumn-winter, AW crop). The treatments (NS) were: «Ct», NS with inorganic Mn and Zn; «Mn», NS with chelated Mn; «Zn», NS with chelated Zn; «All», NS with chelated Mn and Zn.

	Leaves				Pods			
Chelates	**Fe**	**Mn**	**Zn**	**Cu**	**Fe**	**Mn**	**Zn**	**Cu**
SS crop								
Ct	155d	68	92ab	5.54	62.8b	21.0	48.3ab	5.55b
Mn	119a	66	102b	5.60	51.8a	22.3	51.8b	4.96ab
Zn	139bc	74	88a	5.64	61.3ab	22.0	45.0ab	4.88ab
All	131ab	72	83a	5.87	62.0b	19.8	40.3a	4.10a
Significance	*	NS	*	NS	*	NS	*	*
AW crop								
Ct	214b	97	155ab	6.80	63.3b	25.3a	40.8a	5.03
Mn	174a	115	168b	6.55	48.3a	33.5c	50.3c	4.82
Zn	188ab	107	160ab	6.95	59.3b	26.3a	41.8ab	4.50
All	163a	95	136a	6.48	58.0ab	27.3ab	39.5a	4.21
Significance	*	NS	*	NS		*	*	NS

In each column for the same crop, means of different treatments (n = 4) followed by different lower-case letters are significantly different according to Duncan's multiple range test ($p \leq 5\%$). NS, and * indicate nonsignificant or significant differences at at the 0.05 probability level.

4. Discussion

From the results presented, it appears that a balanced supply of nutrients and continuously controlled pH in the NFT flowing nutrient solution ensured high pod yield of good quality and the high availability of nutrients in all NS treatments studied, resulting in negligible impacts on plants among chelated and unchelated supply forms of Mn and Zn. Furthermore, fine-tune adjustments of the refill NS composition were realized and show promises for a more efficient use of the hydroponic solution in Mediterranean bean crops. The need of plants for water and nutrients exhibit sharp changes related to plant species, growth stage, and climatic conditions (radiation, temperature, humidity) [8,9,37,38]. However, it has been documented that the nutrients-to-water uptake ratios fluctuate much less with time than their individual uptake [39]. The reason is photosynthesis and transpiration, which govern nutrient assimilation and water uptake, respectively, although independent processes are both facilitated by leaf stomata pores [37]. Thus, small fluctuations in the nutrient to water uptake ratios with time are expected as long as both processes i.e., assimilation and transpiration, change linearly with radiation [37]. In a similar work with zucchini [40], minimal variation in the UCs was ascribed to a similar effect of climatic factors to nutrient and water uptake. Such background is indeed present in the current study, since a similar net assimilation to transpiration ratio was observed in both seasons (Figure 3), although prevailing climatic conditions differed in some respects (radiation

input and mean air temperature; Table 1). Actually, mean nutrient UCs for the whole cropping period did not show large differences between spring-summer (SS) and autumn-winter (AW) growth seasons ranging from-to: 11.0–11.8 (N), 0.85–1.0 (P), 5.38–5.41 (K), 1.96–2.1 (Ca), 0.92–0.94 (Mg), mmol L^{-1} and 12.6–13.0 (Fe), 5.2–5.5 (Mn), 4.6–4.8 (Zn), 0.9–1.0 (Cu) μmol L^{-1}; corresponding well to the published results for soilless Mediterranean bean crops [6].

The nutrient needs of a plant change with age/size. The change from the vegetative to reproductive phase is important in fruiting vegetables, because leaves and fruits have different nutrient needs [41]. In addition, UCs change as the plant ages and the old leaves continue to transpire but with limited photosynthetic capacity. Particularly in common bean, N concentration in leaves is higher than in fruits. On the other hand, K is much higher in pods than in leaves. Thus, a critical factor for common bean nutrition in soilless culture is the N:K molar uptake ratio. De Kreij et al. [26] recommend a N:K molar ratio of 2.1. In the current, this ratio from 2.3 at the vegetative stage decreased to 1.9 with the fruit load. Similar to N, common bean accumulate high concentrations of Ca in leaves (average 32 mg g^{-1} DW), thus the K:Ca ratio of leaves is much lower than that of pods. Thus, our data impose that the K:Ca molar ratio in the replenishment NS should be increased from 2.4 at the vegetative stage to 2.9 at the start of harvesting. On the other hand, the higher accumulation of K in the root environment during the final stages in the spring-summer crop (Figure 2A) may stress the necessity to apply lower K concentrations than 5.6 mmol L^{-1} in the RNS at the reproductive stage of soilless beans under Mediterranean summer conditions. This is because K may be easily absorbed and transported within plants, through the transpiration stream at high water consumption cropping periods [19]. Regarding P and Mg concentrations inside plants and in the root zone solution, we can assume that applied K:Ca ratio did not hinder P and Mg acquisition by plants, further indicating a suitable K:Ca:Mg molar ratio and minor precipitation (e.g., Ca-phosphate) phenomena in the hydroponic system. The mean UCs of P and Mg fluctuated in a narrow range and averaged close to 1.0 mmol L^{-1}; however, micro adjustments of RNS (Table 2) may be of value at high production intensity. The UC of Fe tended to decrease with time, while that of Cu slightly increased. On the other hand, the UCs of Mn and Zn had no clear and consistent trend (Table 2). Taking into consideration that accumulation rates in the RDS and leaf nutrient concentrations were comparable with guided values for the micronuntrients Fe, Zn, and Cu [6,9], the estimated UCs of these macronutrients can be used to match precisely water and nutrient needs in soilless common bean crops. However, in the case of Mn, of which the accumulation in the RDS fluctuated at lower (average 4 μmol L^{-1}) than recommended levels (5 μmol L^{-1}; [6]), higher concentrations in the replenishment NS than those estimated in the present (Table 2), are suggested to retain target concentrations in the root environment. In any case, tissue (Table 3) and drainage nutrient concentrations (Figure 2A,B) can be reasonably compared with published results [6,9]. Overall, the above results are important in defining specific nutritional programs for Mediterranean common bean in closed soilless systems and reveal the need for frequent changes in NS composition to satisfy nutrient requirements at different morphological stages.

As mentioned elsewhere, the pH values of the recirculating NS were in the range between 5.5–6.3 during the entire plant growth, irrespectively of the growing season (Figure 2C). It is well known that pH controls micronutrients availability more than their absolute concentrations in hydroponics [3,8]. For example, solution pH over neutrality causes the precipitation of macronutrients Fe, Mn and Zn as hydroxides, whereas at a low pH, these micronutrients may precipitate as sulfides [18]. Thus, retaining pH values in most of the cropping period between 5.5–6.0 (Figure 2C), in combination with a continuously flowing NS (1.7 L per minute), was crucial to sustain high solubility of micronutrients (i.e., Mn and Zn) in all cases (chelates and inorganic applications), enabling plant roots with greater ability to absorb nutrients. It can be suggested that the continuously controlled pH of the hydroponic solution (average 5.8) and the growing system in the current study (NFT type system; [42]), might be one main reason of treatments similarities and negligible antagonistic nutrient uptake phenomena. With regard to electrical conductivity (EC), the increase of EC in RDS was greater in the spring-summer (SS) crop, in comparison with autumn-winter (AW) crop at crop termination (Figure 2C). This can

be explained due to the higher (2-fold increase) water uptake in the former (Figure 2D), resulting in higher content of residual salts (sodium chloride impurities) in the RDS, as shown previously [43]. Furthermore, it is well known that the daily water uptake is much higher in summer than in winter in areas, where global solar radiation is low at winter as in Mediterranean basin [31]. In particular, the total crop water requirements were about 225 and 560 mm for AW and SS crop, respectively (Figure 2D). The higher water uptake and photosynthetic activity in the SS crop also resulted in greater production of plant biomass and pod yield (1156 vs. 787 g plant^{-1}, respectively for SS and AW crops; Figure 3A). However, our data reveal that the ratio of product yield to water consumption (namely water use efficiency—WUE) exhibited higher values in the AW crop (35.6 kg m^{-3}) than SS crop (20.6 kg m^{-3}). Furthermore, comparing WUE data found in the present study with those found in conventional (soil grown) cropping greenhouses systems in Almería—Spain (i.e., WUE amounts 15 kg m^{-3} for autumn–winter common bean) [44], we may emphasize: (i) the significance of closed hydroponic bean crops in using natural resources like water in an efficient way in Mediterranean greenhouses and that (ii) WUE for the same hydroponic crop may have different time scales [45]. It was clear from the results that changes in global solar radiation and air temperature levels affected photosynthesis, yield and quality related parameters in Mediterranean bean crops (Figure 3). Actually, the higher photosynthetic capacity during SS compared to AW season resulted in fresh and dry biomass increases and secondary metabolites enhancement, most possible due to the higher availability of carbon-based substrates. Thus, absolute demand for nutrients from crops, which is principally determined by photosynthetic rates and dry weight accumulation, is expected to vary widely [38]. However, minimal variations of the mean UCs (Table 2) allow us to suggest that, when prevailing climatic conditions affect photosynthesis and transpiration (i.e., assimilated CO_2/transpired H_2O) in a similar way, nutrient to water uptake ratios (i.e., mass of nutrients/volume of water absorbed) exhibit small differences, which is of practical significance in commercial hydroponics.

Chelates are compounds that stabilize Fe^{3+} ion and other positively charged metal ions (Zn^{+2}, Mn^{+2} and Cu^{+2} [9]. The fate of the chelating agents during nutrient uptake by the root cells is not clear; e.g., iron may be released from the chelator, when it is reduced from Fe^{3+} to Fe^{2+} from reducing cofactor NADPH in roots and the chelator may then diffuse back into the nutrient solution and react with other metal ions [1,19]. In another aspect, there is a notion that excess Mn in the nutrient solution reduces the uptake of Fe. This study showed that in soilless common bean cops, the addition of chelated Mn reduced the concentrations of Fe in leaves and fresh pods. It seems that the increased stability/solubility of Mn in the solution as chelate compound reduced the translocation of Fe in young leaves (Table 3), rather than its uptake (Table 2). This can be explained by the fact that these micronutrients have common metal transporters (e.g., ZIP proteins), which are capable of transporting a variety of cations, including Fe, Mn and Zn inside the plants [24,46]. Another possible explanation is that xylem-uploading transporters and intracellular chelators (e.g., citrate), appearing to route the root-to-shoot mobility of a metal, also have common substrates [18,23]. For example, increasing Mn concentrations in the nutrient solution inhibited the Fe translocation in rice [22] and the translocation of Fe from the roots to shoots of tomato plants decreased as the Mn supply increased, although Fe was retained in high levels in the roots [8]. However, the result was not clear and consistent in the case of Zn, probably because Zn has a high mobility within plants. In any case, no significant effect of micronutrients supply forms on biomass production and yield was found. Therefore, since growth or yield is considered to be a better criterion to evaluate nutrient interactions in crops, no interaction can be derived [17]. In addition, primary (e.g., photosynthesis) and secondary (e.g., antioxidant molecules) metabolism remained unaffected with applied treatments, suggesting that metal micronutrients involved in these metabolic functions were, in all cases, at appropriate levels. Indeed, plant analysis revealed that leaf concentrations in all cases were in the sufficiency range reported in the literature [9] and only few subtle effects were observed. To sum up, individual or combined chelate applications of Mn and Zn did not add superiority compared to their mineral forms in the nutritional status of the crop under current experimental conditions. It is not irrelevant that Giaquinto et al. [9] reported that it

is more possible to use less stable Fe chelate forms (EDTA and DTPA) than expensive Fe-EDDHA in hydroponic systems with regular pH control. Last but not least, the uptake ratios of Zn and Mn amounted to 37 and 41% (mol/mol) of the Fe uptake, indicating the relative addition rates of these micronutrients at the corresponding ratio in the refill NS, to satisfy plant nutrient requirements for optimal growth. However, calibration ratio modifications may be needed in common bean cultivars, with strong genotypic differences in the acquisition and use of these micronutrients.

5. Conclusions

This study indicated that a balanced supply of nutrients and continuously monitored pH in the hydroponic flowing nutrient solution eliminated differences among treatments and ensured a high pod yield of good quality and the high availability of nutrients in all nutrient solution treatments studied. The supply of Mn and/or Zn chelates did not show superiority on selected physiological and agronomical parameters in relation to their inorganic forms, suggesting that both forms may be well supplied in Mediterranean hydroponic common bean crops. In any case, fine-tune adjustments of the refill nutrient solution composition were realized and show promises for a more efficient use of the hydroponic solution in Mediterranean common bean crops. Overall, the uptake concentrations (UCs) of the bean crops were 11.4 (N), 0.9 (P), 5.4 (K), 2.0 (Ca), 0.9 (Mg; mmol L^{-1}), 12.8 (Fe), 5.3 (Mn), 4.7 (Zn), and 0.9 (Cu; μmol L^{-1}).

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List of Abbreviations and Symbols

Abbreviations	
AW	autumn-winter
DAT	days after transplanting
DTPA	diethylenetriaminepenta acetic acid
DW	dry weight
EC	electrical conductivity
EDDHA	ethylenediamine-di-o-hydroxyphenyl acetic acid
EDTA	ethylenediaminetetra acetic acid
FW	fresh weight
NADPH	nicotinamide adenine dinucleotide phosphate
NFT	nutrient film technique
NS	nutrient solution
pH	hydrogen exponent
RDS	recycled drainage solution
RNS	replenishment nutrient solution
SS	spring-summer
UCs	uptake concentrations
WUE	water use efficiency
Symbols	
Ca	calcium
Cu	copper
C_{xadd}	the concentration of the nutrient x in the replenishment NS (mmol L^{-1} or μmol L^{-1}) in each treatment
$(C_{xin} - C_{xf})$	the initial minus final concentration of the x nutrient in the recycled solution (mmol L^{-1} or μmol L^{-1}) in each time interval

Fe	iron
K	potassium
Mg	magnesium
Mn	manganese
N	nitrogen
P	phosphorus
Rh_i	relative air humidity inside greenhouse (%)
Rh_o	relative air humidity outside greenhouse (%)
SR	solar radiation (kJ m^{-2})
T_i	air temperature inside greenhouse (°C)
T_o	air temperature outside greenhouse (°C)
UV	ultraviolet radiation (kJ m^{-2})
W_{add}	the amount of water added to the closed system (L) in each time interval
W_s	the amount of water in each hydroponic unit (L)
Zn	zinc

References

1. Sonneveld, C.; Voogt, W. *Plant Nutrition of Greenhouse Crops*; Springer: Dordrecht, The Netherlands, 2009. [CrossRef]
2. Savvas, D.; Gruda, N. Application of Soilless Culture Technologies in the Modern Greenhouse Industry—A Review. *Eur. J. Hortic. Sci.* **2018**, *83*, 280–293. [CrossRef]
3. Raviv, M.; Lieth, J.H. *Significance of Soilless Culture in Agriculture*; Elsevier BV: Amsterdam, The Netherlands, 2008; pp. 1–11.
4. Hoagland, D.R.; Arnon, D.I. The Water-Culture Method for Growing Plants without Soil. In *Circular (California Agricultural Experiment Station)*; University of California: Davis, CA, USA, 1950.
5. Sonneveld, C.; Straver, N. *Nutrient Solutions for Vegetables and Flowers Grown in Water or Substrates*; Research Station for Floriculture and Glasshouse Vegetables: Aalsmeer, The Netherlands; Naaldwijk, The Netherlands, 1994; Volume 8.
6. Savvas, D.; Gianquinto, G.P.; Tüzel, Y.; Gruda, N. Soilless Culture. In *Good Agricultural Practices for Greenhouse Vegetable Crops. Principles for Mediterranean Climate Areas*; FAO, Ed.; Food and Agriculture Organization of the United Nations: Rome, Italy, 2013; pp. 303–354.
7. Savvas, D.; Neocleous, D. Developments in Soilless/Hydroponic Cultivation of Vegetables. In *Achieving Sustainable Cultivation of Vegetables (Burleigh Dodds Series in Agricultural Science)*; Hochmuth, G., Ed.; Burleigh Dodds Science Publishing: Cambridge, UK, 2019; pp. 1–12, ISBN 978-1-78676-236-8.
8. Adams, P. Nutritional Control in Hydroponics. In *Hydroponic Production of Vegetables and Ornamental*; Savvas, D., Passam, H., Eds.; Embryo Publications: Athens, Greece, 2002; pp. 211–261, ISBN 960-8002-12-5.
9. Gianquinto, G.; Munoz, P.; Pardossi, A.; Ramazzotti, S.; Savvas, D. Soil Fertility and Plant Nutrition. In *Good Agricultural Practices for Greenhouse Vegetable Crops. Principles for Mediterranean Climate Areas*; Plant Production and Protection Paper 217; FAO: Rome, Italy, 2013; pp. 215–270.
10. Liu, Q.D.; Mudadu, M.S.; Thummel, R.; Tao, Y.; Wang, S. From Blue to Red: Syntheses, Structures, Electronic and Electroluminescent Properties of Tunable Luminescent N,N Chelate Boron Complexes. *Adv. Funct. Mater.* **2005**, *15*, 143–154. [CrossRef]
11. De Rijck, G.; Schrevens, E. Cationic speciation in nutrient solutions as a function of pH. *J. Plant Nutr.* **1998**, *21*, 861–870. [CrossRef]
12. López-Rayo, S.; Correas, C.; Lucena, J.J. Novel chelating agents as manganese and zinc fertilisers: Characterisation, theoretical speciation and stability in solution. *Chem. Speciat. Bioavailab.* **2012**, *24*, 147–158. [CrossRef]
13. Jones, J.B., Jr. *Hydroponics, A Practical Guide for the Soilless Grower*; St. Lucie Press: Boca Raton, FL, USA, 1997; ISBN 9780849331671.
14. Savvas, D. *Soilless Culture. Hydroponics—Substrates*; Agrotypos Publishing: Athens, Greece, 2012; ISBN 978-960-7667-44-1. (In Greek)
15. Wang, Z.-H.; Li, S.-X.; Malhi, S. Effects of fertilization and other agronomic measures on nutritional quality of crops. *J. Sci. Food Agric.* **2007**, *88*, 7–23. [CrossRef]

16. Monika, G.; Natasha, S.; Saloni, S.; Payal, K.; Aman, K.; Venkatesh, C.; Priya, A. Biofortified Crops Generated by Breeding, Agronomy, and Transgenic Approaches Are Improving Lives of Millions of People around the World. *Front. Nutr.* **2018**, *5*, 12.
17. Rietra, R.; Heinen, M.; Dimkpa, C.O.; Bindraban, P.S. Effects of Nutrient Antagonism and Synergism on Yield and Fertilizer Use Efficiency. *Commun. Soil Sci. Plant Anal.* **2017**, *48*, 1895–1920. [CrossRef]
18. Sambo, P.; Nicoletto, C.; Giro, A.; Pii, Y.; Valentinuzzi, F.; Mimmo, T.; Lugli, P.; Orzes, G.; Mazzetto, F.; Astolfi, S.; et al. Hydroponic Solutions for Soilless Production Systems: Issues and Opportunities in a Smart Agriculture Perspective. *Front. Plant Sci.* **2019**, *10*, 923. [CrossRef]
19. Lazar, T.; Taiz, L.; Zeiger, E. Plant physiology. 3rd ed. *Ann. Bot.* **2003**, *91*, 750–751. [CrossRef]
20. Kobraee, S. Effect of zinc, iron and manganese fertilization on concentrations of these metals in the stem and leaves of soybean and on the chlorophyll content in leaves during the reproductive development stages. *J. Elem.* **2016**, *21*, 395–412. [CrossRef]
21. Welch, R.M.; Shuman, L. Micronutrient Nutrition of Plants. *Crit. Rev. Plant Sci.* **1995**, *14*, 49–82. [CrossRef]
22. Fageria, N.; Baligar, V.; Clark, R. Micronutrients in Crop Production. *Adv. Agron.* **2002**, *77*, 185–268. [CrossRef]
23. Clemens, S.; Palmgren, M.; Krämer, U. A long way ahead: Understanding and engineering plant metal accumulation. *Trends Plant Sci.* **2002**, *7*, 309–315. [CrossRef]
24. Guerinot, M.L. The ZIP Family of Metal Transporters. *Biochim. Biophys. Acta (BBA) Biomembr.* **2000**, *1–2*, 190–198. [CrossRef]
25. Savvas, D. SW-Soil and Water: Automated Replenishment of Recycled Greenhouse Effluents with Individual Nutrients in Hydroponics by Means of Two Alternative Models. *Biosyst. Eng.* **2002**, *83*, 225–236. [CrossRef]
26. De Kreij, C.; Voogt, W.; Baas, R. *Nutrient Solutions and Water Quality for Soilless Culture*; Research Station for Floriculture and Glasshouse Vegetables (PBG): Naaldwijk, The Netherlands, 1999; p. 196, ISSN 1385-3015.
27. Ropokis, A.; Ntatsi, G.; Kittas, C.; Katsoulas, N.; Savvas, D. Impact of Cultivar and Grafting on Nutrient and Water Uptake by Sweet Pepper (Capsicum annuum L.) Grown Hydroponically Under Mediterranean Climatic Conditions. *Front. Plant Sci.* **2018**, *9*, 9. [CrossRef]
28. Neocleous, D.; Nikolaou, G. Antioxidant Seasonal Changes in Soilless Greenhouse Sweet Peppers. *Agronomy* **2019**, *9*, 730. [CrossRef]
29. Neocleous, D.; Savvas, D. Effect of different macronutrient cation ratios on macronutrient and water uptake by melon (Cucumis melo) grown in recirculating nutrient solution. *J. Plant Nutr. Soil Sci.* **2015**, *178*, 320–332. [CrossRef]
30. Neocleous, D.; Savvas, D. NaCl accumulation and macronutrient uptake by a melon crop in a closed hydroponic system in relation to water uptake. *Agric. Water Manag.* **2016**, *165*, 22–32. [CrossRef]
31. FAO. *Good Agricultural Practices for Greenhouse Vegetable Crops*; FAO Plant Production and Protection Paper; Food and Agriculture Organization of the United Nations: Rome, Italy, 2013; Volume 217, E-ISBN 978-92-5-107650-7.
32. Kalra, Y.P. *Handbook of Reference Methods for Plant Analysis*; CRC Press: Boca Raton, FL, USA, 1998; ISBN 1-57444-124-8.
33. Estefan, G.; Sommer, R.; Ryan, J. *Methods of Soil, Plant and Water Analysis: A Manual for the West Asia and North Africa Region: Third Edition*; International Center for Agricultural Research in the Dry Areas: Beirut, Lebanon, 2013; (ICARDA).
34. Pardossi, A.; Falossi, F.; Malorgio, F.; Incrocci, L.; Bellocchi, G. Empirical Models of Macronutrient Uptake in Melon Plants Grown in Recirculating Nutrient Solution Culture. *J. Plant Nutr.* **2005**, *27*, 1261–1280. [CrossRef]
35. Neocleous, D.; Savvas, D. The effects of phosphorus supply limitation on photosynthesis, biomass production, nutritional quality, and mineral nutrition in lettuce grown in a recirculating nutrient solution. *Sci. Hortic.* **2019**, *252*, 379–387. [CrossRef]
36. Neocleous, D.; Ntatsi, G. Seasonal variations of antioxidants and other agronomic features in soilless production of selected fresh aromatic herbs. *Sci. Hortic.* **2018**, *234*, 290–299. [CrossRef]
37. Sonneveld, C. Composition of Nutrient Solution. In *Hydroponic Production of Vegetables and Ornamentals*; Savvas, D., Passam, H.C., Eds.; Embryo Publications: Athens, Greece, 2002; pp. 179–210.
38. Bar-Yosef, B. Fertigation Management and Crops Response to Solution Recycling in Semi-Closed Greenhouses. In *Soilless Culture*; Elsevier BV: Amsterdam, The Netherlands, 2008; pp. 341–424.
39. Savvas, D.; Lenz, F. Nährstoffaufnahme von Aubergine (*Solanum Melongena* L.) in Hydrokultur. *Gartenbauwissenschaft* **1995**, *60*, 29–33.

40. Neocleous, D.; Savvas, D. Simulating NaCl accumulation in a closed hydroponic crop of zucchini: Impact on macronutrient uptake, growth, yield, and photosynthesis. *J. Plant Nutr. Soil Sci.* **2017**, *180*, 283–293. [CrossRef]
41. Silber, A.; Bar-Tal, A. Nutrition of Substrate-Grown Plants. In *Soilless Culture. Theory and Practice*; Raviv, M., Lieth, J.M., Eds.; Elsevier: Amsterdam, The Netherlands, 2008; pp. 291–328, ISBN 978-0-444-52975-6.
42. Neocleous, D.; Savvas, D. Modelling Ca2+ accumulation in soilless zucchini crops: Physiological and agronomical responses. *Agric. Water Manag.* **2018**, *203*, 197–206. [CrossRef]
43. Savvas, D.; Gizas, G.; Karras, G.; Lydakis-Simantiris, N.; Salahas, G.; Papadimitriou, M.; Tsouka, N. Interactions between Silicon and NaCl-Salinity in a Soilless Culture of Roses in Greenhouse. *Europ. J. Hort. Sci.* **2007**, *72*, 73–79.
44. Gallardo, M.; Thompson, R.B.; Fernández, M.D. Water Requirements and Irrigation Management in Mediterranean Greenhouses: The Case of the Southeast Coast of Spain. In *Good Agricultural Practices for Greenhouse Vegetable Crops*; Plant Production and Protection Paper 217; FAO: Rome, Italy, 2013; pp. 109–136.
45. Hatfield, J.L.; Dold, C. Water-Use Efficiency: Advances and Challenges in a Changing Climate. *Front. Plant Sci.* **2019**, *10*, 103. [CrossRef]
46. Korshunova, Y.O.; Eide, D.; Clark, W.G.; Guerinot, M.L.; Pakrasi, H.B. The IRT1 protein from Arabidopsis thaliana is a metal transporter with a broad substrate range. *Plant Mol. Biol.* **1999**, *40*, 37–44. [CrossRef]