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Improving Rabbiteye Blueberry Performance in a Calcareous Soil by Growing Plants in Pits Filled with Low-$CaCO_3$ Growth Media

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Abstract: Calcareous soils are not suitable for blueberry cultivation. Our aim was to improve the performance of blueberry plants in calcareous soils by using pits filled with growth media in combination with high levels of RNH_4^+ (proportion of N-NH_4^+ among the total applied N). Rabbiteye blueberry (*Vaccinium virgatum* Ait. cv. Ochlockonee) plants were grown in pits filled with a tuff/peat mixture (TP), a sandy soil (S) or a calcareous (CC), in full factorial combination with three levels of RNH_4^+: 33%, 66% or 100%. The two higher RNH_4^+ treatments decreased the pH of the low-$CaCO_3$ (S) and no-$CaCO_3$ (TP) media to $\leq$6.0 over 250 days of fertilization, but did not affect the pH of the CC soil over 650 days. Plant performance was superior in the TP and S media, as compared to the CC soil. The type of growth medium was the dominant factor accounting for the improved plant performance. The plants were sensitive to Mn deficiency in leaves during the spring period. The current results suggest that growing blueberry in pits filled with good aeration and low pH buffering capacity medium in combination with a high level of RNH_4^+ is a positive approach for its cultivation in calcareous soils.

Keywords: growth medium; ammonium nitrate; growth medium pH; nutrient concentration; chlorophyll concentration

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1. Introduction

World blueberry production has increased by about 56% over the last decade (2010–2019; [1]), due to the dramatic increase in demand for this healthy fruit. This expansion in production has led to greater interest in cultivating blueberries in regions with non-optimal soil conditions. Optimal soil conditions for blueberry cultivation are low pH (<5.5) [2,3], high aeration and high organic matter (OM) content [4]. It seems, therefore, that calcareous soils are not suitable enough for blueberry cultivation [3,5].

A soil's pH level can be controlled by the proportion of N-NH_4^+ among the total applied inorganic N, especially in growth systems where the fertigation technique (fertilization through the irrigation water) is applied [6,7]. Fertigation has been found to be more effective for blueberry growth, probably due to its plant shallow root system [8]. High rates of the RNH_4^+ in the fertigation solution were found to be effective in reducing the pH of the growth medium [3]. It was suggested that the acidification be via two main processes. The first one involves the ammonium being oxidized by the microorganisms, and two protons are released from one molecule of ammonium (Equation (1)). This process is carried out throughout the entire volume of the medium and is enhanced under conditions of well-aerations [9,10] and neutral and alkaline pH [11].

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \quad (1)$$

The other process involves the uptake of cationic ammonium by the roots, which is accompanied by the release of protons from the roots into the adjacent soil [12].

We have recently shown that the application of $CaCO_3$ to growth medium inhibited the growth of blueberry plants [13]. Concentrations of ≥5% $CaCO_3$ buffered the acidification induced by high rates of RNH_4^+ [3] almost completely.

Likewise, the vegetative growth, yield and the level of the mineral nutrition of blueberry plants are affected negatively in clayey soils [14], probably due to oxygen deficiencies consequent to the low permeability and diffusivity of the gases in these soils [15].

Growing plants in soilless systems is a common approach for coping with unsuitable soils [16]. Recently, we showed that a high level of RNH_4^+ can enhance the growth of southern highbush (*Vaccinium corymbosum* cv. Sunshine) blueberry plants in a soilless system [17]. The use of a soilless system in containers has some disadvantages compared to growing plants directly in the field soils. The roots' development in the restricted volume of the container is retarded over time [18]. The limited root growth also makes the plants more sensitive to irrigation and fertilization errors [16]. The degradation of organic components of the growth medium in the container reduces its quality over the long term. The container has no effective buffer zone between the medium and the surroundings, which leads to large fluctuations in the root zone temperature over the course of the day and over the different seasons of the year [19,20]. Although blueberry fruits are usually harvested manually, a gradual transition to mechanical harvesting is anticipated in view of the significant increase in global blueberry cultivation [1]. Because soilless container-based systems do not seem to be suitable for mechanical harvesting, alternative effective systems for cultivation of blueberry in the field soils are required.

Pits and trenches filled with various organic growth media are a feasible method for growing highbush blueberry in neutral or moderately calcic soils [5,21,22]. However, to the best of our knowledge, there is no published information regarding the performance and mineral nutrition of rabbiteye blueberry grown in pits containing growth media and subjected to acidification with a wide range of RNH_4^+ levels applied through the irrigation system. Accordingly, the present study aimed to improve the performance of blueberry plants in calcareous soils by using pits filled with growth media in combination with high levels of RNH_4^+.

2. Materials and Methods

2.1. Description of the Experiments

Eight-month-old rabbiteye blueberry (*Vaccinium virgatum* Ait. cv. Ochlockonee) plants, derived from tissue culture, were planted (26 November 2015) in pits in raised beds of calcareous, stony, shallow mountain soil inside a 40% pearl-colored shade-netting screen-house. The experiment was located in the Gush Etzion region in the Central Judean Mountains of Israel (600 m above sea level, 31°39′16.80″ N/35°07′8.88″ E). The raised beds were 1.2/0.8 m wide (bottom/top widths, respectively), 0.5 m high and 21 m long. Pits of a 64-L cube-shape (surface area of 0.16 m^2 and depth of 0.4 m) were dug manually in the rows of the raised beds. The distance between the centers of the neighboring pits in each planting row was 0.9 m and the distance between the centers of the neighboring rows was 2.0 m. The pits were filled with a tuff/peat mixture (TP; 60/40% *v*/*v*, Tuff Merom Golan Ltd., Afula, Israel), a sandy soil (S; Typic Haploxeralfs) or a calcareous, clayey soil (CC; Typic Xerofluvents).

Three RNH_4^+ treatments [$RNH_4^+ = 100 \times N\text{-}NH_4^+/(N\text{-}NH_4^+ + N\text{-}NO_3^-)$]: 33% (33-$RNH_4^+$), 66% (66-$RNH_4^+$) and 100% (100-$RNH_4^+$) were prepared by using different proportions of ammonium and nitrate sources in separate 2.5-m^3 tanks. Those tanks also contained the target concentrations of 80, 30, 100, 30 and 16 mg L^{-1} for N, P, K, Ca and Mg, respectively. The following salts were used to prepare the fertigation solutions: NH_4NO_3, $(NH_4)_2SO_4$, KNO_3, KH_2PO_4, K_2SO_4, $MgSO_4$ and $CaCl_2$. The concentrations of the micronutrients Fe, Mn, Zn, Cu and Mo were 1, 0.5, 0.17, 0.045 and 0.013 mg L^{-1} in chelation with EDTA, respectively. The electrical conductivity values of the solutions for the 33-RNH_4^+, 66-RNH_4^+ and 100-RNH_4^+ treatments were 1.0, 1.2 and 1.3 dS m^{-1}, respectively. The target solutions were applied through two irrigation drippers per plant (flow rate of 2.0 L h^{-1} for each dripper; Netafim Ltd., Tel Aviv, Israel). Drippers were attached to separate drip lines for each treatment in each block. Identical amounts of water were applied once a day to all treatments: 1–5 L per plant per day according to environmental conditions and plant development, based on the water-consumption curves of blueberry in this location [17].

Overall, there were nine treatments (three growth substrate treatments × three RNH_4^+ treatments), which were arranged in six randomized blocks. Each block was located on two adjacent raised beds and contained three plants per treatment. Measurements were taken until mid-November 2017 and based on the middle plant for each treatment in each block (n = 6).

2.2. Characterization and Analysis of the Growth Media

The growth media were characterized using the following procedures: Samples of each growth medium were dried for 48 h at 45 °C and then crushed to pass through a 2-mm sieve. The textures of the CC and S media were determined using the hydrometer method [23]. The texture of the TP growth medium was not determined because particle-size distribution is not an acceptable method for characterizing peat. The particle-size distribution of the tuff fraction was 33% (w/w) 0–8 mm particles and 67% (w/w) 4–8 mm particles (personal communication, Jonathan Oserovitz, Tuff Merom Golan Ltd, Afula, Israel). Hygroscopic water content was determined after drying a 25-g sample of dry soil/medium at 105 °C for 48 h. Saturated water-holding capacity was determined according to a soil–water paste procedure [24]. Water content at field capacity (FC) was determined using saturated soil/medium samples placed on the same dry soil/medium and left to drain into the dry soil/medium for 48 h [25]. Air content at field capacity (AirFC) was calculated based on these data. The dry bulk densities of the soils/media were determined using the graduated cylinder method. $CaCO_3$ content was determined with a calcimeter [26]. The organic matter (OM) contents of the CC and S media were determined based on their weight loss in an oven that was kept at 540 °C for 12 h [27]. pH was measured in soil/medium–distilled water mixture extracts (1:2 w/v, respectively) using a pH meter (FC 200b, Hanna Instruments, Woonsocket, RI, USA). The cation-exchange capacity (CEC) of TP and S were determined by saturation with 1 mol L^{-1} NaOAc and displacement by 1 mol L^{-1} NH_4OAc after the salts were washed with 96% ethanol to an electrical conductivity of <10 µS m^{-1}. The CEC of CC was determined by saturation with 0.1 mol L^{-1} NaOAc + 0.4 mol L^{-1} NaCl (pH 8.2) and extraction by 0.25 mol L^{-1} $Mg(NO_3)_2$ (pH 7.5; [24]).

The clay and silt fractions were 5.8 and 8.0 times larger in the CC (respectively) than in the S (Table 1). This difference in soil texture led to a much higher water-holding capacity for CC, as compared to S at saturation, at field capacity and at the hygroscopic water content. Note that the water contents of TP at the field capacity and saturation points were very similar to those of CC. However, the bulk density of TP was much lower than that of CC and, therefore, its porosity was much higher (72%) than that of CC (57%) and S (46%).

The $CaCO_3$ content of CC was about 18.5 times greater than that of the S soil; whereas TP was free of $CaCO_3$. These differences were reflected in the pH values of these growth media. The OM content of CC was quite high for a soil from a semiarid, Mediterranean region and 10 times higher than the OM content of the S; whereas that of TP was very close to that of CC, due to the high OM content of peat. The CEC values of CC and TP were about six and four times greater than that of S, respectively.

Table 1. The major properties of the growth media [tuff/peat mixture (TP), sandy soil (S) and calcareous, clayey soil (CC)] used to fill the pits established in a field of calcareous, clayey soil.

	Soil Texture			WC [1]			AC [2]	BD [3]	Porosity	OM [4]	$CaCO_3$	pH	CEC [5]
	Sand	Silt	Clay	Air dry	Field capacity	Saturation	Field capacity						
Growth medium	$g\ kg^{-1}$			% w/w			% v/v	$g\ cm^{-3}$	%	$g\ kg^{-1}$			$mmol_c\ kg^{-1}$
Tuff/peat mixture (TP)				2.89	33.4	58.9	47.3	0.74	72	50 *	0.0	6.8	161
Sandy soil (S)	889	40	71	0.67	8.9	21.2	33.4	1.42	46	6.0	8.0	7.3	40
Calcareous clayey soil (CC)	269	320	411	6.66	34.8	66.2	17.0	1.15	57	64	149	7.8	247

[1] WC—Water content; [2] AC—air content; [3] BD—bulk density; [4] OM—organic matter content; [5] CEC—cation-exchange capacity; * 40% of the volume of the TP mixture was peat.

2.3. Plant Measurements and Analyses

2.3.1. Plant Size and Chlorophyll Concentrations

The chlorophyll concentrations in diagnostic leaves (the youngest fully developed leaf, usually the fourth to sixth leaf from the shoot tip) were measured every 50 days during the main growing season (May–November) with a SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies Inc., Aurora, IL, USA). The SPAD readings were calibrated (R^2 = 0.93, $p < 0.0001$) to the chlorophyll concentrations determined by the standard analytical method of dimethyl sulfoxide extraction [28]. The chlorophyll concentrations were determined by spectrophotometry at 648 and 665 nm (UV-2401PC, Shimadzu Scientific Instruments, Nakagyo-ku, Kyoto, Japan).

Every three months, a digital caliper (HUNTER 111419, MID Design, Tel Aviv, Israel) was used to measure the diameters of three major canes of each plant at a height of 5 cm above the growth-medium surface. The maximal height of each plant was also measured at that time. For all of these measurements, we used the middle plant in each treatment of each block (n = 6).

2.3.2. Mineral Concentrations

Diagnostic leaves (the youngest fully developed leaf, usually the fourth to sixth leaf from the shoot tip) were sampled three times during the growing season: on 16 May, 17 July and 8 November of the second year of growth (2017). These measurements were taken using the middle plant in each treatment of each block (n = 6). Each replicate at each sampling time consisted of 15 to 20 leaves. Sampled leaves were rinsed first in tap water and then twice in two different containers filled with deionized water. Then, the leaves were dried at 65 °C and ground to a fine powder. N, P and K were extracted by wet digestion with sulfuric acid and H_2O_2 [29]. The N and P concentrations were determined using a Gallery^{TM} Discrete Autoanalyzer (Thermo Fisher Scientific, Vantaa, Finland) and the K concentration was determined by flame photometry (Sherwood M410, Sherwood Scientific Ltd., Cambridge, UK). Ca, Mg, Fe, Mn and Zn concentrations were determined using an atomic absorption spectrophotometer (model AAnalyst 400, PerkinElmer, Waltham, MA, USA) after digesting the samples with nitric acid and perchlorate solutions.

2.4. pH of the Growth Media

Every two months, the pH was measured directly in the growth medium (in situ) using a pH meter (FC 200b, Hanna Instruments, Woonsocket, RI, USA). The measurements were taken at a depth of 10 cm, about 10 cm from the plant crown, which had been planted in the center of the pit.

2.5. Environmental Conditions

Environmental data concerning monthly average maximum and minimum air temperatures, monthly average relative humidity, monthly average solar radiation during the day (0800 to 1600 h) and rainfall as a function of time (Figure 1) were obtained from a weather station belonging to the Israel Meteorological Service. That station was located 3 km away from the experimental site (website of the Israel Meteorological Service, http://www.meteo.co.il/report/SingleStationReport, accessed on 12 January 2021).

The experimental site was located in a region with a typical Mediterranean climate, with wet mild winters (December–February) and dry, warm summers (June–August). The average minimum daily air temperature was about 5 °C in the winter and about 15 °C in the summer. The average maximum daily temperatures were 15 °C in the winter and 30 °C in the summer. The average relative humidity was between 40 and 80%. In general, the humidity was high during the winter and at the end of the summer and low during the spring. The monthly average solar radiation during the day (0800 to 1600 h) was highest in the summer (800 W m^{-2}) and lowest in the winter (300 W m^{-2}). During the course of this study, the rainy season lasted from November to March. Covering the screen-house with a pearl-colored shade net (40%) during the spring and summer might have created a microclimate in the screen-house that differed from the climate outside. A similar net covering has been found to reduce photosynthetically active radiation by 30–50% and to reduce the temperature by 3–5 °C, while increasing the minimal daily relative humidity by 3–10% [30].

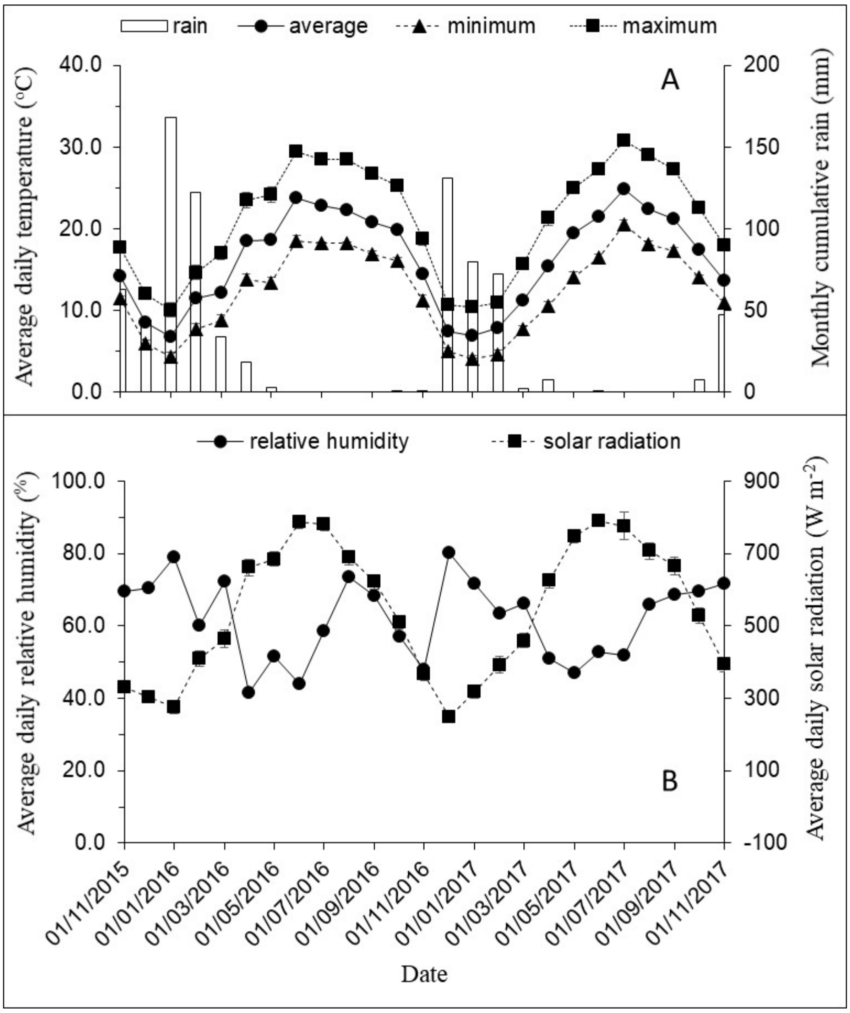

Figure 1. (**A**) Maximum, average and minimum daily air temperatures and monthly cumulative precipitation. (**B**) Average daily relative humidity and solar radiation during the daytime (0800 to 1600 h) based on daily measurements at the experiment site (website of the Israel Meteorology Services, http://www.meteo.co.il/report/SingleStationReport, accessed on 12 January 2021). SE values are based on daily measurements. For most of the dates, the SE values are smaller than the symbols.

2.6. Statistical Analysis

The effects of the growth medium, RNH_4^+ level, block and sampling time and their interactions on the pH of the growth medium, plant height, cane diameter and chlorophyll and mineral concentrations in diagnostic leaves were analyzed by four-way ANOVA using JMP 14 software [31]. Interactions between growth medium and RNH_4^+ treatment significantly affected the pH of the medium, plant height, cane diameter and the chlorophyll concentration. Therefore, those variables were analyzed separately for each growth medium using two-way ANOVA. In addition, the interaction of growth medium, RNH_4^+ level and measurement date significantly affected the medium's pH and, therefore, a second-order polynomial equation (Equation (2)) was used to examine the change in that parameter over time.

$$Y_t = a \times t^2 + b \times t + c \quad (2)$$

where Y_t is pH at time t, t is time (day) and a, b and c are constants that were determined by the NLIN procedure (JMP 14).

The interaction between growth medium and measurement date significantly affected plant height, cane diameter and chlorophyll concentration. Therefore, a best-fit linear line equation (Equation (3)) was used to describe the changes in these parameters over time. There was almost no change in cane diameter or plant height during the wintertime, from the measurement on Day 380 after fertigation began to that on Day 532 (5 December 2016 through 16 May 2017). Therefore, this period was not included in the linear-regression analysis. In addition, the best-fit linear equations were calculated for the changes in chlorophyll concentrations during the period of Day 196 through Day 380 in the first year, but not for the similar period during the second year, due to the extremely low chlorophyll concentrations in the new leaves that appeared in the spring of the second year.

$$Y_t = a \times t + b \quad (3)$$

where Y_t is the chlorophyll concentration, plant height or cane diameter at time t (mg m^{-2}), t is time (day) and a and b are constants that were determined by best-fit linear regression (JMP 14).

There was a significant effect of time of sampling (i.e., spring, summer or autumn) on the mineral concentrations in diagnostic leaves. Therefore, those concentrations were analyzed by two-way ANOVA for each sampling time. However, significant $RNH_4^+ \times$ growth medium interactive effects were observed for some of the concentrations of nutrients at each sampling time. Therefore, an additional analysis of the effects of the RNH_4^+ treatment was conducted, with two-way ANOVA performed for each growth medium.

3. Results

3.1. Effects of RNH_4^+ on the pH of the Different Growth Media

Growth medium and RNH_4^+ (p = 0.0003) had a significant interactive effect on the pH of the medium. Therefore, the effect of the RNH_4^+ treatments was examined separately for each growth medium (Table 2). In TP and S, the pH was significantly lower for 100-RNH_4^+ than for 33-RNH_4^+ and with an intermediate value observed for 66-RNH_4^+ (Table 2). The pH of CC was not affected by the RNH_4^+ treatments.

Similarly, $RNH_4^+ \times$ measurement date (p = 0.003), growth medium $\times$ measurement date (p < 0.0001) and $RNH_4^+ \times$ growth medium $\times$ measurement date (p = 0.0007) all had significant effects on the medium pH (Table 2). Accordingly, the effect of the growth media on pH is presented for each RNH_4^+ treatment separately (Figure 2). For all of the RNH_4^+ treatments, there was a significant decrease in the pH of the TP and S media over a period of 200–400 days (Figure 2). The pH of S was significantly lower than that of TP at most of the measurement points after Day 250, in the 100-RNH_4^+ and 66-RNH_4^+ treatments (Figure 2A,B). However, in the 33-RNH_4^+ treatment, the difference between the two media with regard to pH was non-significant at most of the measurement dates (Figure 2C). In all of the RNH_4^+ treatments, the pH levels in S and TP were significantly lower than those observed in CC, after approximately Day 250 from the start of fertigation. The pH of CC was barely affected by the RNH_4^+ treatments over the 650 days of the experiment (Figure 2).

The best-fitting lines for most of the treatments presented in Figure 2 were obtained using a second-order polynomial equation (Table 3). In response to 100-RNH_4^+, the lowest minimum pH values for the best-fitting lines were 4.25, 5.39 and 6.49 in S, TP and CC, respectively. In response to 33-RNH_4^+, the highest minimum pH values were 5.29, 6.27 and 6.94 in S, TP and CC, respectively. Consequently, we conclude that the smallest effect of the RNH_4^+ treatments on pH reduction was seen in CC, in the pH range of 6.49 to 6.94, and the largest effect was seen in S, in the pH range of 4.25 to 5.29. The minimum pH level was reached the most quickly in CC (379–394 days) and the most slowly in TP (454–574 days).

Table 2. The effects of the proportion of N-NH_4^+ among the total applied inorganic N (RNH_4^+) [$RNH_4^+ = 100 \times N\text{-}NH_4^+/(N\text{-}NH_4^+ + N\text{-}NO_3^-)$] and measurement date (26 November 2015 through 25 October 2017) on the average pH measured directly in the growth medium [tuff/peat mixture (TP), sandy soil (S) and calcareous, clayey soil (CC)].

Treatment	pH
Variable	Significance: $F < 0.05$
RNH_4^+	<0.0001
Growth medium	<0.0001
Block	0.0008
Date	<0.0001
$RNH_4^+ \times$ Growth medium	0.0003
$RNH_4^+ \times$ Date	0.003
Growth medium × Date	<0.0001
$RNH_4^+ \times$ Growth medium × Date	0.0007
Separate analysis for each growth medium	
Tuff/Peat mixture (TP)	
RNH_4^+ (%)	**pH**
33	6.52 a
66	6.24 b
100	5.97 b
Variable	Significance: $F < 0.05$
RNH_4^+	<0.0001
Block	n.s.
Date	<0.0001
$RNH_4^+ \times$ Date	0.008
Sandy soil (S)	
RNH_4^+ (%)	**pH**
33	6.04 a
66	5.62 b
100	4.21 c
Variable	Significance: $F < 0.05$
RNH_4^+	<0.0001
Block	0.002
Date	<0.0001
$RNH_4^+ \times$ Date	0.0004
Calcareous clayey soil (CC)	
RNH_4^+ (%)	**pH**
33	7.15 a
66	6.95 a
100	7.10 a
Variable	Significance: $F < 0.05$
RNH_4^+	n.s.
Block	n.s.
Date	0.02
$RNH_4^+ \times$ Date	n.s.

Means followed by the same letter for each growth medium did not differ according to LSMeans Tukey's HSD test. $n = 6$; $p = 0.05$.

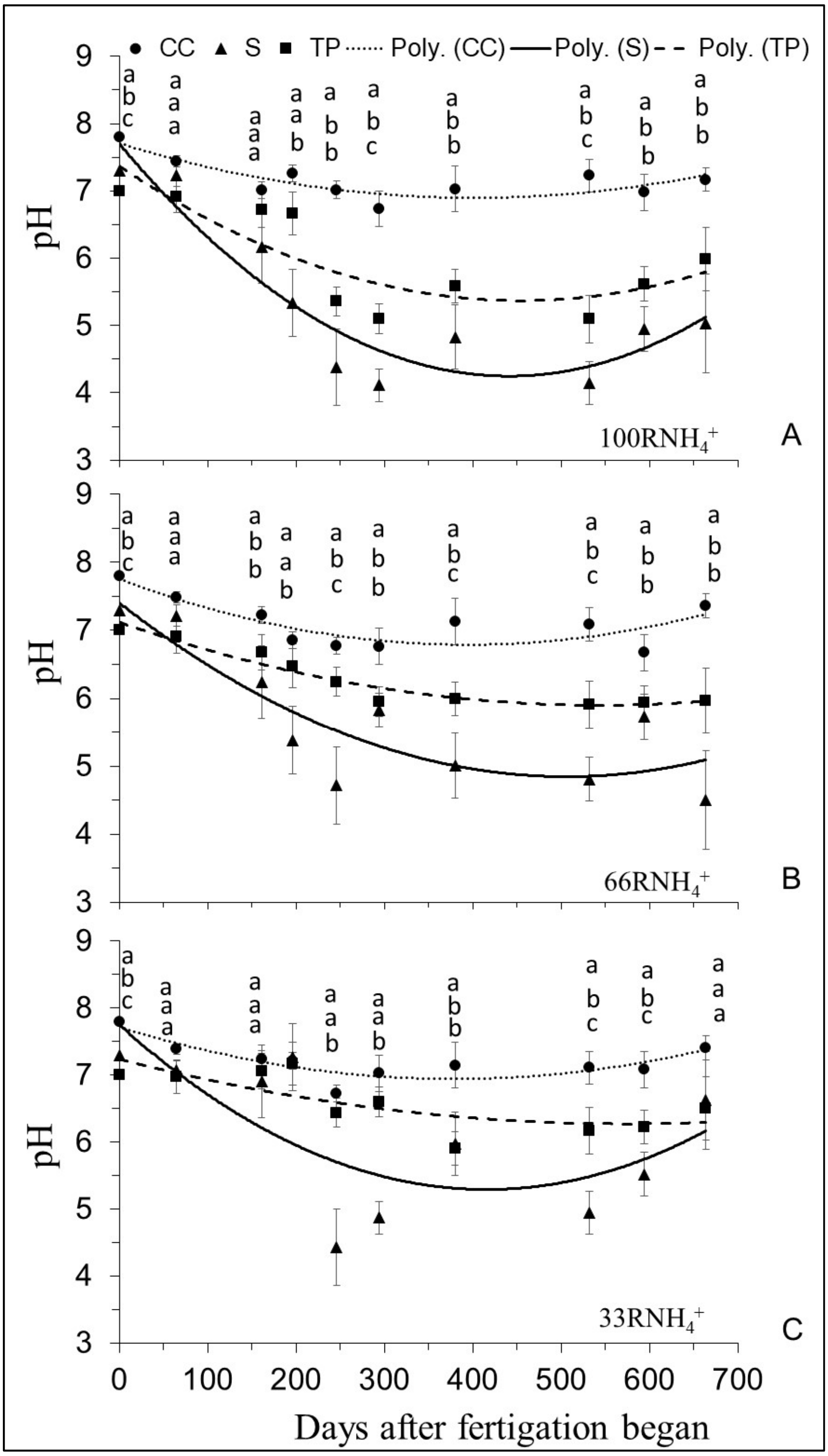

Figure 2. Effect of the growth medium [tuff/peat mixture (TP, squares), sandy soil (S, triangles) and calcareous, clayey soil (CC, circles)] on the pH measured directly in the growth medium over the course of the experiment (26 November 2015 to 8 November 2017) presented for treatments with three different proportions of N-NH_4^+ out of the total applied inorganic N [RNH_4^+, 100% (**A**), 66% (**B**) and 33% (**C**)]. Means for growth-medium treatments at each measurement point in each panel followed by the same letter do not differ according to LSMeans Tukey's HSD test; $n = 6$, $p = 0.05$. The best-fitting second-order polynomial equation for each treatment and their coefficients and statistical parameters are presented in Table 3.

Table 3. The-best fit parameters of Equation (2) for best-fit curves for pH as a function of time (data presented in Figure 2). Values in brackets are approximate standard errors; r^2 = coefficient of determination. a, b and c are constants that were determined by the NLIN procedure (JMP 14).

RNH_4^+	Medium	a	b	c	r^2	Time of Minimal pH	Minimum pH Value
%		pH day^{-2}	pH day^{-1}	pH		day	
33	S	1.42×10^{-5} (6.98×10^{-6})	−0.0118 (0.0049)	7.75 (0.69)	0.50	416	5.29
33	TP	2.93×10^{-6} (2.51×10^{-6})	−0.0034 (0.0018)	7.24 (0.25)	0.59	574	6.27
33	CC	5.40×10^{-6} (1.29×10^{-6})	−0.0041 (0.0009)	7.72 (0.13)	0.75	379	6.94
66	S	9.99×10^{-6} (4.62×10^{-6})	−0.0101 (0.0033)	7.40 (0.46)	0.74	506	4.84
66	TP	4.13×10^{-6} (9.51×10^{-7})	−0.0045 (0.0007)	7.12 (0.09)	0.94	543	5.90
66	CC	6.22×10^{-6} (1.83×10^{-6})	−0.0049 (0.0013)	7.76 (0.18)	0.69	394	6.79
100	S	1.78×10^{-5} (3.85×10^{-6})	−0.0157 (0.0027)	7.70 (0.38)	0.87	441	4.25
100	TP	9.72×10^{-6} (3.64×10^{-6})	−0.0088 (0.0026)	7.37 (0.36)	0.72	454	5.39
100	CC	9.96×10^{-6} (4.34×10^{-6})	−0.0076 (0.0031)	7.96 (0.43)	0.48	384	6.49

3.2. Combined Effects of Growth Medium and RNH_4^+ on Plant Performance

The growth medium and the RNH_4^+ treatments had a significant interactive ($p < 0.0001$) effect on chlorophyll concentration (Table 4). Accordingly, the effects of the RNH_4^+ levels on the chlorophyll concentrations are presented separately for each growth medium. In S and CC, chlorophyll concentrations were significantly higher in the 100-RNH_4^+ and 66-RNH_4^+ treatments than in the 33-RNH_4^+ treatment (Table 4). However, even the highest chlorophyll concentration in CC was lower than the lowest levels observed for S and TP. In TP, a trend of increasing concentration of chlorophyll in response to higher RNH_4^+ was observed, but that trend was not significant.

The growth-medium treatments and the date of measurement had a significant interactive effect ($p < 0.0001$) on chlorophyll concentration. Therefore, the effect of the growth medium on chlorophyll concentration over time was examined separately for each level of RNH_4^+ (Figure 3). In TP and S, chlorophyll concentrations increased over time to a peak value on Day 380 after the start of fertigation (5 December 2016) in response to all of the RNH_4^+ treatments (Figure 3A–C), but no significant differences between these two media were observed. In CC, the chlorophyll concentration declined from Day 196 after the start of fertigation in all of the RNH_4^+ treatments. Consequently, the chlorophyll concentrations were significantly higher in S and TP than in CC, starting from Day 294 after fertigation began. According to the regression lines, the rate of increase in the chlorophyll concentration over time (Coefficient a, Table 5) was similar for S and for TP (0.0055–0.0081 mg m^{-2} day^{-1}). As the RNH_4^+ level increased, the chlorophyll concentration increased over time in S, but not in TP.

The growth-medium and the RNH_4^+ treatments had a significant interactive effect ($p = 0.008$) on cane diameter at two years after planting (Table 4). Thus, the effects of the different levels of RNH_4^+ were examined separately for each growth medium. In CC, the cane diameter was smaller at 33-RNH_4^+ than at 66-RNH_4^+ and 100-RNH_4^+; whereas in TP and S, the effect of the RNH_4^+ treatments were not significant. However, the minimum cane diameter in TP was greater than the maximum cane diameter in S; whereas the minimum cane diameter in S was greater than the maximum cane diameter in CC.

Table 4. The effects of proportions of N-NH_4^+ among the total applied inorganic N (RNH_4^+) [RNH_4^+= 100×N-NH_4^+/(N-NH_4^++N-NO_3^-)] and the growth-medium treatments [tuff/peat mixture (TP), sandy soil (S) and calcareous clayey soil (CC)] on the chlorophyll concentration, cane diameter and plant height of rabbiteye blueberry (*Vaccinium virgatum* Ait., cv. Ochlockonee) during 2016 and 2017.

Treatment	**Chlorophyll Concentration**	**Canes Diameter**	**Height**
Variable	Significance: $F < 0.05$		
RNH_4^+	<0.0001	n.s.	0.04
Growth medium	<0.0001	<0.0001	<0.0001
Date	<0.0001	<0.0001	<0.0001
Block	n.s.	<0.0001	<0.0001
RNH_4^+ × Growth medium	<0.0001	0.008	<0.0001
RNH_4^+ × Date	n.s.	n.s.	n.s.
Growth medium × Date	<0.0001	<0.0001	<0.0001
RNH_4^+ × Growth medium × Date	n.s.	n.s.	n.s.
Separate Analysis for each growth medium			
Tuff/peat mixture (TP)			
RNH_4^+	**Chlorophyll Concentration**	**Canes Diameter**	**Height**
(%)	mg m^{-2}	mm	cm
33	3.90a	11.7 a	121.2 a
66	3.88a	10.9 a	118.1 ab
100	4.22a	11.3 a	109.8 b
Variable	Significance: $F < 0.05$		
RNH_4^+	n.s.	n.s.	0.04
Block	n.s.	<0.0001	<0.0001
Date	<0.0001	<0.0001	<0.0001
RNH_4^+ × Date	n.s.	n.s.	n.s.
Sandy soil (S)			
RNH_4^+	**Chlorophyll Concentration**	**Canes Diameter**	**Height**
(%)	mg m^{-2}	mm	cm
33	4.02 b	8.55 a	92.4 b
66	4.35 a	9.01 a	99.1 b
100	4.36 a	9.36 a	109.2 a
Variable	Significance: $F < 0.05$		
RNH_4^+	0.01	0.05	0.0006
Block	n.s.	<0.0001	<0.0001
Date	<0.0001	<0.0001	<0.0001
RNH_4^+ × Date	n.s.	n.s.	n.s.
Calcareous clayey soil (CC)			
RNH_4^+	**Chlorophyll Concentration**	**Canes Diameter**	**Height**
(%)	mg m^{-2}	mm	cm
33	2.07 b	7.3 b	74.6 b
66	3.64 a	8.0 a	87.8 a
100	3.10 a	8.1 a	79.5 b
Variable	Significance: $F < 0.05$		
RNH_4^+	<0.0001	0.039	0.0004
Block	0.04	0.018	<0.0001
Date	<.0001	<0.0001	n.s.
RNH_4^+ × Date	0.018	n.s.	n.s.

Means followed by the same letter for each growth medium did not differ according to LSMeans Tukey HSD test. $n = 6$; $p = 0.05$.

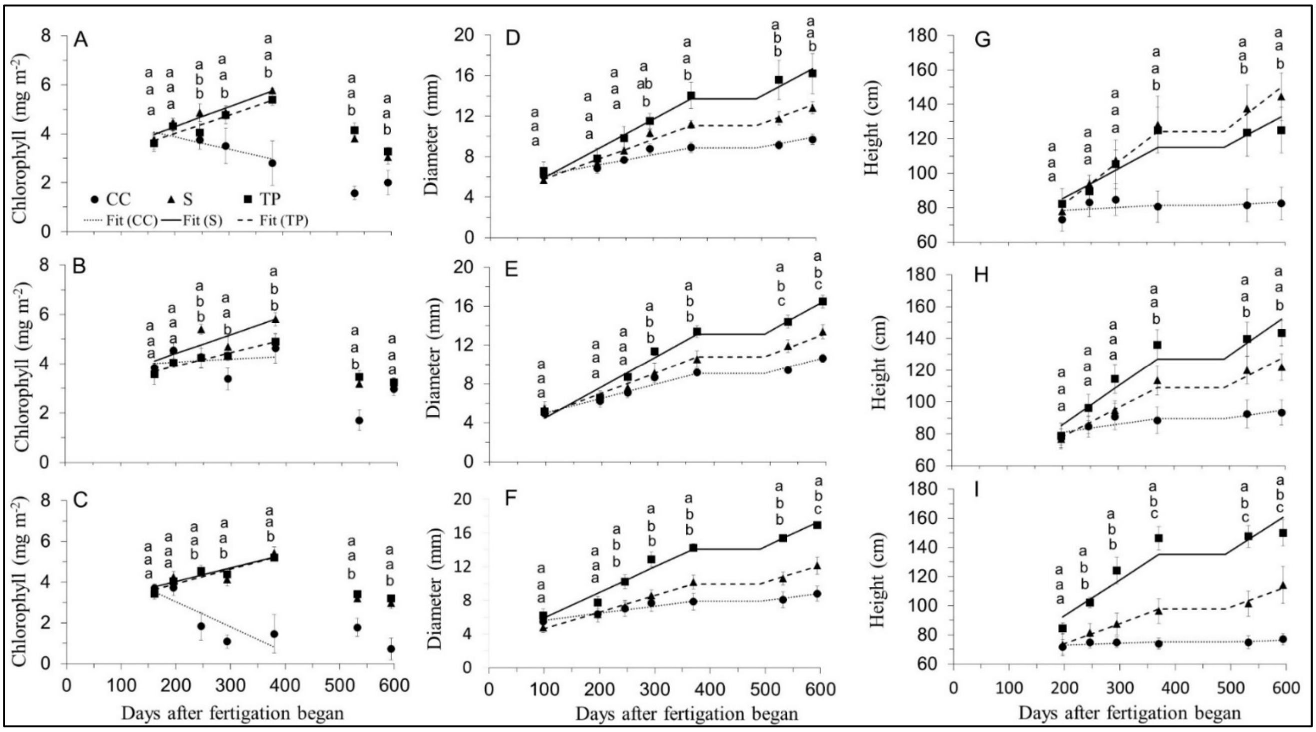

Figure 3. Effect of growth medium [tuff/peat mixture (TP, squares), sandy soil (S, triangles) and calcareous, clayey soil (CC, circles)] on chlorophyll concentration (**A**–**C**), cane diameter (**D**–**F**) and height (**G**–**I**) of rabbiteye blueberry plants (*Vaccinium virgatum* Ait., cv. Ochlockonee) over the course of the experiment (26 November 2015 through 8 November 2016). Plants were treated with three different proportions of N-NH_4^+ out of the total applied inorganic N [RNH_4^+, 100% (**A**,**D**,**G**), 66% (**B**,**E**,**H**) and 33% (**C**,**F**,**I**)]. Means for growth-medium treatments in each panel on each measurement day followed by the same letter do not differ according to LSMeans Tukey's HSD test; $n = 6$, $p = 0.05$. The best-fitting linear equations for each treatment and their coefficients and statistical parameters are presented in Table 5.

The interactive effect of the growth medium and the measurement date on cane diameter was significant ($p < 0.0001$). Therefore, the effect of the growth-medium treatments on cane diameter over time was analyzed separately for each level of RNH_4^+ (Figure 3D–F). The initial cane diameters were similar across all of the treatments, but the rates of growth were different (Figure 3 and Table 5). For each RNH_4^+ level, cane diameter increased most quickly in TP, more slowly in S and slowest of all in CC. The average coefficient-rate values for all of the RNH_4^+ levels in TP, S and CC were 0.030, 0.020 and 0.011-mm day^{-1}, respectively.

The growth medium and the RNH_4^+ treatments had a significant interactive ($p < 0.0001$) effect on plant height at two years after planting (Table 4). Therefore, the results showing the effects of the different levels of RNH_4^+ on plant height are presented separately for each growth medium (Table 4). The minimum plant heights in S and TP were higher than the maximum plant height in CC. In TP, plant height decreased as the RNH_4^+ level increased. An opposite trend was observed in S. In CC, no clear trend was observed in relation to the RNH_4^+ level.

Growth medium and measurement date had a significant interactive ($p < 0.0001$) effect on plant height. Accordingly, the effects of the different growth media on plant height over time were examined separately for each level of RNH_4^+ (Figure 3G–I). In TP, the increase in height over time was significantly greater for 33-RNH_4^+ than for 100-RNH_4^+; whereas the opposite effect was observed in S (Table 5). In CC, the smallest and largest increases in plant height were obtained for 33-RNH_4^+ and 66-RNH_4^+ (0.013 and 0.050 cm day^{-1}, respectively), respectively. The minimum rates of increase in plant height in S and TP were higher than the maximum rate in CC (Table 5).

Table 5. The best-fit parameters of Equation (3) for best-fit curves of chlorophyll concentration, cane diameter and plant height as functions of time (data shown in Figure 3); r^2 = coefficient of determination. a and b are constants that were determined by best-fit linear regression (JMP 14).

Chlorophyll—Figure 3A–C						
					Prob. of $F < 0.05$	
RNH_4^+	**Medium**	**a**	**b**	**r^2**	**a**	**b**
%		mg m^{-2} day^{-1}	mg m^{-2}			
33	S	0.0066	2.72	0.76	0.05	0.017
33	TP	0.0071	2.49	0.88	0.02	0.009
33	CC	−0.0123	5.51	0.71	n.s.	0.02
66	S	0.0077	2.88	0.70	n.s.	0.03
66	TP	0.0055	2.81	0.94	0.006	0.0009
66	CC	0.0012	3.82	0.04	n.s.	0.03
100	S	0.0081	2.66	0.93	0.008	0.005
100	TP	0.0075	2.51	0.89	0.017	0.009
100	CC	−0.0050	4.86	0.64	n.s.	0.003
Cane Diameter—Figure 3D–F						
					Prob. of $F < 0.05$	
RNH_4^+	**Medium**	**a**	**b**	**r^2**	**a**	**b**
%		mm day^{-1}	mm			
33	S	0.0198	2.643	0.68	<0.0001	0.0004
33	TP	0.0303	2.886	0.89	<0.0001	0.0003
33	CC	0.0084	4.798	0.18	0.005	<0.0001
66	S	0.0220	2.635	0.70	<0.0001	0.003
66	TP	0.0319	1.334	0.88	<0.0001	0.03
66	CC	0.0151	3.503	0.83	<0.0001	<0.0001
100	S	0.0195	3.859	0.89	<0.0001	<0.0001
100	TP	0.0286	3.113	0.67	<0.0001	0.02
100	CC	0.0099	5.214	0.58	<0.0001	<0.0001
Height—Figure 3G–I						
					Prob. of $F < 0.05$	
RNH_4^+	**Medium**	**a**	**b**	**r^2**	**a**	**b**
%		cm day^{-1}	cm			
33	S	0.139	46.15	0.31	0.0004	0.0006
33	TP	0.247	43.71	0.72	<0.0001	0.0009
33	CC	0.013	70.35	0.02	n.s.	<0.0001
66	S	0.178	42.98	0.57	<0.0001	0.0002
66	TP	0.241	37.90	0.55	<0.0001	0.006
66	CC	0.050	71.08	0.07	n.s.	<0.0001
100	S	0.247	32.89	0.61	<0.0001	0.04
100	TP	0.172	51.21	0.36	0.002	0.007
100	CC	0.017	75.08	0.01	n.s.	<0.0001

The results of these analyses indicate that the type of growth medium in the pits was the dominant factor affecting plant performance, in terms of chlorophyll concentration, cane diameter and plant height; whereas the RNH_4^+ treatments had only a minor effect.

3.3. Combined Effect of Growth Medium and RNH_4^+ on Mineral Concentrations in the Leaves

Overall, the concentrations of all examined elements were higher in the spring than in the summer and autumn (Table 6). The concentrations of N, P and Fe were lower in the autumn than in the summer. The concentrations of Ca, Mg, Mn and Zn were higher in the summer than in the autumn. In light of the significant effect of time of sampling on most of the nutrient concentrations (except for K, Table 6), we present the results showing the effects of the levels of RNH_4^+ and type of growth medium on nutrient concentrations for each sampling date (Table 6).

At all sampling times (spring, summer and autumn), the concentrations of the major elements (N, P, K and Ca) in the leaves were higher in CC than in TP, with intermediate levels observed in S (Table 6). In the spring, the concentration of Mn was significantly higher in TP and S than in CC. However, during the summer and autumn, the concentration of Mn was higher in CC than in TP or S.

In the spring, none of the concentrations of the measured elements were significantly affected by the RNH_4^+ treatments. Nevertheless, during the summer, the concentration of K was significantly higher in the 33-RNH_4^+ than in the two higher RNH_4^+ treatments (Table 6).

Growth medium and RNH_4^+ had significant interactive effects on the concentrations of Mg (p = 0.0008) and Fe (p < 0.0001) in the summer, as well as the concentrations of N (p = 0.0007), P (p = 0.002), K (p < 0.0001), Ca (p = 0.018) and Mg (p = 0.003, Table 6) in the autumn. Therefore, the effects of RNH_4^+ level on the concentrations of these elements were examined separately for each growth medium (Table 7). In CC, during the summer, the concentration of Mg decreased as the level of RNH_4^+ increased; whereas in S and TP, the concentration of Mg was not affected by the RNH_4^+ treatments. In the same summer, the concentration of Fe increased as the RNH_4^+ increased in TP and S; whereas in CC, the concentration of Fe was not affected by the RNH_4^+ treatments. In the autumn, the concentrations of N, P, K, Ca and Mg decreased as the level of the RNH_4^+ increased in CC; whereas in TP and S, the concentrations of the same nutrients were not affected by the RNH_4^+ treatments.

Table 6. The effects of the proportion of N-NH_4^+ among the total applied inorganic N (RNH_4^+) [RNH_4^+ = 100×N-NH_4^+/(N-NH_4^++N-NO_3^-)] and the growth medium [tuff/peat mixture (TP), sandy soil (S) and calcareous, clayey soil (CC)] on nutrient concentrations in the diagnostic leaves of rabbiteye blueberry plants (*Vaccinium virgatum* Ait., cv. Ochlockonee) in the spring (16 May 2017), summer (17 July 2017) and autumn (8 November 2017).

Variable	N	P	K	Ca	Mg	Fe	Mn	Zn
		g kg^{-1} dry weight				mg kg^{-1} dry weight		
Date								
Spring	19.3 a	1.67 a	5.16 a	7.97 a	1.67 a	508 a	35.0 a	35.8 a
Summer	17.4 b	1.45 b	4.80 a	2.05 b	0.46 b	125 b	27.6 b	19.3 b
Autumn	12.9 c	1.12 c	5.12 a	2.59 b	0.54 b	47 b	29.8 b	27.9 ab
RNH_4^+ (%)								
33	17.1 a	1.46 a	5.76 a	4.48 a	0.93 a	220 a	26.8 b	23.9 a
66	16.2 a	1.39 a	4.66 b	3.83 a	0.82 a	388 a	33.8 a	23.8 a
100	15.9 a	1.39 a	4.57 b	4.16 a	0.94 a	219 a	31.9 ab	25.0 a
Growth medium								
Tuff/Peat mixture (TP)	15.0 b	1.24 c	4.20 b	3.81 b	0.79 b	254 a	29.0 a	26.9 a
Sandy soil (S)	16.5 a	1.39 b	4.54 b	3.44 b	0.88 ab	261 a	33.0 a	24.6 a
Calcareous clayey soil (CC)	17.8 a	1.61 a	6.25 a	5.23 a	1.00 a	311 a	30.5 a	21.3 a
Variable				Significance: $F < 0.05$				
RNH_4^+	n.s.	n.s.	<0.0001	n.s.	n.s.	0.035	0.019	n.s.
Medium	<0.0001	<0.0001	<0.0001	<0.0001	0.01	n.s.	n.s.	n.s.
Block	n.s.	n.s.	n.s.	n.s.	n.s.	0.039	n.s.	n.s.
Date	<0.0001	<0.0001	n.s.	<0.0001	<0.0001	<0.0001	0.014	<0.0001
RNH_4^+ × Medium	0.001	0.003	<0.0001	0.004	0.0004	n.s.	n.s.	n.s.
RNH_4^+ × Date	n.s.	n.s.	0.017	n.s.	0.04	0.006	n.s.	n.s.
Medium × Date	n.s.	n.s.	0.0002	n.s.	n.s.	n.s.	<0.0001	n.s.
RNH_4^+ × Medium×Date	n.s.	n.s.	0.002	n.s.	n.s.	n.s.	n.s.	n.s.

Table 6. *Cont.*

Variable	N	P	K	Ca	Mg	Fe	Mn	Zn
				Spring (16 May 2017)				
RNH_4^+ (%)								
33	19.7 a	1.62 a	5.41 a	8.36 a	1.67 a	462 a	29.3 a	32.6 a
66	19.8 a	1.76 a	5.50 a	7.69 a	1.58 a	734 a	39.0 a	35.5 a
100	18.7 a	1.70 a	4.79 a	8.20 a	1.83 a	469 a	40.5 a	32.2 a
Growth medium								
Tuff/Peat mixture (TP)	17.2 b	1.50 b	4.40 b	7.23 b	1.42 b	656 a	36.8 a	33.5 a
Sandy soil (S)	19.7 ab	1.74 ab	5.32 ab	6.84 b	1.66 ab	523 a	48.9 a	33.8 a
Calcareous clayey soil (CC)	20.9 a	1.83 a	5.97 a	10.20 a	1.81 a	488 a	22.9 b	33.0 a
Variable				Significance: $F < 0.05$				
RNH_4^+	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Growth medium	0.01	0.013	0.008	0.001	0.008	n.s.	0.0002	n.s.
Block	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.04
$RNH_4^+ \times$ Growth medium	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
				Summer (17 July 2017)				
RNH_4^+ (%)								
33	18.3 a	1.57 a	5.56 a	2.40 a			25.3 a	24.8 a
66	16.6 a	1.35 a	4.33 b	1.86 a			29.0 a	12.6 a
100	17.2 a	1.45 a	4.53 b	1.93 a			29.2 a	19.8 a
Growth medium								
Tuff/Peat mixture (TP)	15.6 b	1.26 b	4.14 b	1.57 b			25.0 a	14.9 a
Sandy soil (S)	17.5 ab	1.46 ab	4.24 b	1.50 b			24.9 a	32.7 a
Calcareous clayey soil (CC)	18.9 a	1.65 a	6.03 a	3.12 a			33.5 a	9.57 a
Variable				Significance: $F < 0.05$				
RNH_4^+	n.s.	n.s.	0.005	n.s.			n.s.	n.s.
Growth medium	0.006	0.015	<0.0001	<0.0001			n.s.	n.s.
Block	n.s.	n.s.	n.s.	n.s.			n.s.	n.s.
$RNH_4^+ \times$ Growth medium	n.s.	n.s.	n.s.	n.s.	0.0008	<0.0001	n.s.	n.s.
				Autumn (8 November 2017)				
RNH_4^+ (%)								
33						41.9 a	25.3 b	36.7 a
66						55.8 a	34.1 a	27.8 a
100						42.5 a	31.3 ab	26.3 a
Growth medium								
Tuff/Peat mixture (TP)						53.3 a	31.0 ab	42.6 a
Sandy soil (S)						38.3 a	25.9 b	22.9 a
Calcareous clayey soil (BS)						48.6 a	33.9 a	25.3 a
Variable				Significance: $F < 0.05$				
RNH_4^+						n.s.	0.016	n.s.
Growth medium						n.s.	0.027	n.s.
Block						n.s.	0.001	n.s.
$RNH_4^+ \times$ Growth medium	0.0007	0.002	<0.0001	0.018	0.003	n.s.	n.s.	n.s.

Means in the column followed by the same letter did not differ according to LSMeans Tukey HSD test. $n = 6$; $p = 0.05$.

Table 7. Analysis of the effects of each growth medium [tuff/peat mixture (TP), sandy soil (S) and calcareous, clayey soil (CC)] and the proportion of N-NH_4^+ among the total applied inorganic N (RNH_4^+) [RNH_4^+ = 100 × N-NH_4^+/(N-NH_4^++N-NO_3^-)] on the nutrient concentrations in the diagnostic leaves of rabbiteye blueberry plants (*Vaccinium virgatum* Ait., cv. Ochlockonee). The interactions between the treatments significantly affected Mg and Fe levels at the summer sampling time (17 July 2017; probability of F = 0.0008 and <0.0001, respectively, Table 6), as well as the levels of N, P, K, Ca and Mg at the autumn sampling time (8 November 2017; probability of F = 0.0007, 0.002, <0.0001, 0.018 and 0.003, respectively, Table 6).

Summer (17 July 2017)					
	Mg	**Fe**			
	g kg^{-1} dry weight	mg kg^{-1} dry weight			
Tuff/Peat mixture (TP)					
RNH_4^+ (%)					
33	0.33 a	78.8 a			
66	0.43 a	84.8 a			
100	0.39 a	117 a			
Variable	Significance: F < 0.05				
RNH_4^+ (%)	n.s.	n.s			
Sandy soil (S)					
RNH_4^+ (%)					
33	0.41 a	119 b			
66	0.51 a	101 b			
100	0.40 a	302 a			
Variable	Significance: F < 0.05				
RNH_4^+ (%)	n.s.	0.025			
Calcareous clayey soil (CC)					
RNH_4^+ (%)					
33	0.77 a	128 a			
66	0.55 ab	166 a			
100	0.38 b	108 a			
Variable	Significance: F < 0.05				
RNH_4^+ (%)	0.009	n.s.			
Autumn (8 November 2017)					
	N	**P**	**K**	**Ca**	**Mg**
	g kg^{-1} dry weight				
Tuff/Peat mixture (TP)					
RNH_4^+ (%)					
33	11.1 b	0.90 a	4.23 a	2.11 a	0.49 a
66	13.0 a	0.98 a	4.53 a	2.71 a	0.52 a
100	12.6 a	0.97 a	3.71 a	2.34 a	0.52 a
Variable	Significance: F < 0.05				
RNH_4^+ (%)	0.001	n.s.	n.s.	n.s.	n.s.
Sandy soil (S)					
RNH_4^+ (%)					
33	12.1 a	0.95 a	4.41 a	2.16 a	0.43 b
66	13.0 a	1.03 a	3.91 a	2.60 a	0.47 ab
100	12.6 a	0.96 a	3.82 a	2.13 a	0.53 a
Variable	Significance: F < 0.05				
RNH_4^+ (%)	n.s.	n.s.	n.s.	n.s.	0.045
Calcareous clayey soil (CC)					
RNH_4^+ (%)					
33	17.4 a	1.82 a	10.5 a	4.05 a	0.91 a
66	12.8 b	1.30 b	4.88 b	2.35 b	0.50 b
100	12.2 b	1.18 b	5.87 b	3.02 ab	0.53 ab
Variable	Significance: F < 0.05				
RNH_4^+ (%)	0.013	0.018	<0.0001	0.05	0.02

Means in the column followed by the same letter did not differ according to LSMeans Tukey HSD test. n = 6; p = 0.05.

4. Discussion

We found that the type of growth medium was the major factor influencing the performance of blueberry plants, probably due to the marked differences in the $CaCO_3$ contents and physical properties of the different media (Table 1). The recommended soil characteristics for optimal cultivation of blueberry plants are low pH, high aeration and high OM content [2–4]. The ability of RNH_4^+ treatments to decrease the pH differed considerably in the tested growth media (Table 2 and Figure 2). The most significant effect was observed in the low- and no-$CaCO_3$ media (S and TP, respectively, Table 1). $CaCO_3$ is considered to be the soil component with the highest capacity for buffering acidification processes [32]. The results of the current study are in agreement with our previous ones [3], that the pH of a sandy soil that contained $\geq$50 g $CaCO_3$ kg^{-1} soil was unaffected by long-term application of 100% RNH_4^+. On the other hand, in the same soil with very low $CaCO_3$ content ($\leq$10 g $CaCO_3$ kg^{-1} soil), the long-term application of 33% RNH_4^+ lowered the pH to a level of <5.5, which is suitable for blueberry growth [3]. Another soil factor that may affect the pH-buffering capacity of the soil is CEC [32], which was higher in CC than in S medium (Table 1). The high levels of $CaCO_3$ and CEC of CC medium buffered the expected acidification following the application of higher rates of ammonium (Figure 2). On the other hand, the relative low levels of $CaCO_3$ and CEC of S medium were not sufficient to buffer that pH reduction, even at the lowest RNH_4^+ level. The effect of RNH_4^+ level on pH reduction in TP medium was greater than that observed in CC and smaller than that observed in S, due to its CEC value (Table 1). The difference among the growth media was also expressed by time until the pH was lowered to a steady state level. The minimum pH was reached after 386, 454 and 524 days after beginning of fertigation, at CC, S and TP media, respectively, in accordance with their $CaCO_3$ and CEC levels (Table 3).

The acidification of S and TP to pH $\leq$6.0 was associated with enhanced plant performance, in parameters of chlorophyll concentration, cane diameter and plant height, compared to CC (Table 4 and Figure 3). These results are in agreement with our previous ones done in soilless systems that demonstrated the reduction in plant performance of rabbiteye (cv. Titan) and southern highbush (cv. Sunshine) blueberry plants, as the pH was higher than a threshold value of 5.5 [3,17].

It is interesting to note that although the pH of the CC soil was not reduced even by the highest level of RNH_4^+, the RNH_4^+ did have a positive influence on plant performance, especially chlorophyll concentration, which was lower at 33-RNH_4^+ than at higher RNH_4^+ levels (Table 4). The positive effect of a high RNH_4^+ levels on plant performance in CC was probably a result of the acidification of the rhizosphere by direct ammonium uptake, with no significant effect on the pH reduction in the bulk soil, as suggested by Kafkafi and Ganmore Neumann [33].

TP had a significantly higher OM content than S (50 and 6 g kg^{-1}, respectively, Table 1). Despite this large difference, the performance of blueberry plants in these two media was quite comparable. These results indicate that OM content does not have a direct positive effect on the vegetative performance of these plants, but probably affects that performance indirectly through the aeration of the medium (AirFC; Table 1). Zhang [34] reported that increased OM content corresponded to increased soil porosity, which is positively correlated with aeration processes [15].

The concentrations of most of the examined elements in leaves were in the range of the levels recommended by Retamales and Hancock [35] for rabbiteye blueberry cultivars. The concentrations of major elements were significantly higher in the restricted-growth plants in CC than in the plants with larger canopies grown in TP and S (Table 6), probably due to a "dilution effect" in the larger plants, as suggested by Scagel [36] and Turner et al. [37] for other calcifuge plants. This explanation is also supported by low concentrations of most of the elements during the summer; the time when the increase in plant biomass was predominant (June–September, 200 to 300 days after transplanting, Figure 3). This dilution effect is also supported by the fact that the highest concentrations of the major nutrients were seen during the autumn in the plants that were grown in CC and fertigated with 33-RNH_4^+, as compared to the plants grown in the same medium with 66-RNH_4^+ or 100-RNH_4^+ (Table 7). In the 33-RNH_4^+ treatment, the pH was highest and the performance of plants was lowest (Tables 2 and 3, respectively). The only nutrient that might be a limiting factor in the current work was Mn, which was lower than the recommended value (>25 mg kg^{-1}, [35]) for rabbiteye blueberry cultivars, in CC at the spring time (Table 6). These results are in agreement with the finding that the Mn concentration during the spring was elevated by the acidification process and was positively related to the improved plant growth at the end of the growth season [3,17]. Note that although the content of organic matter and CEC value of the S medium are much lower than those of the TP, the concentrations of the elements in the leaves of the plants grown in these media were not different. We suggest that this is probably because of the relative small volume of the substrate in the pit combined with frequent fertigation that masked the expected effects of the chemical properties of the growth media on nutrients uptake by the plants.

The type of growth medium had a strong effect on the performance of blueberry due to pH-buffering capacity ($CaCO_3$ content and CEC) and the level of aeration (AirFC). However, the pH-buffering capacity was high and the aeration level was low in the same growth medium (CC), making it impossible to estimate their individual specific effect.

To the best of our knowledge, this is the first study to evaluate the feasibility of using pits to grow blueberry plants directly in calcareous soils. Examining this approach is justified because growing plants in growth medium in open pits in direct contact with the surrounding soil is essentially different from growing them in isolated containers. Due to their large buffer zone, plants grown in open pits are less sensitive to negative environmental effects, such as temperature fluctuation or an excess or lack of water and/or nutrients. On the other hand, over the long term, there is a possibility that the roots could grow out of the growth medium-filled pit and into the surrounding calcareous soil. Therefore, the current study results should be further validated for longer duration and more mature fruit-bearing plants.

5. Conclusions

Blueberry plants are sensitive to Mn deficiency in their leaves during the spring blooming period that is related to the pH of the growth medium. The current study suggests that growing plants in pits filled with suitable growth medium is a possible technique for the cultivation of rabbiteye blueberry in calcareous field soils. The properties of the growth media that contributed to the better performance of plants were good aeration and low buffering capacity against pH reduction. It seems that the dominant factor accounting for improved plant performance was the type of growth medium in the pits, and the RNH_4^+ treatment had only a minor effect. However, a high level of RNH_4^+ can be more effective for decreasing the pH of media with low buffering capacities, whereas, an extremely low level of RNH_4^+ is expected to raise the pH of media with low buffering capacities, resulting in the deficiency of Mn and the poor performance of blueberry plants.

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