



Combined Effects of N-NH₄ Level and a Nitrification Inhibitor on the Performance of Blueberry in High and Low pH-Buffer Media

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Received: 3 July 2024 / Accepted: 20 February 2025 / Published online: 15 March 2025
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Abstract

Purpose Application of nitrification inhibitor (NI) is considered as an efficient tool for optimization of N management. The main purpose of this study was to examine the combined effects of N-NH₄ level and NI on the performance of blueberry plants grown in low and high pH-buffered substrates.

Methods Three levels of N-NH₄ (20, 40 or 80 mg L⁻¹) were applied, with or without NI, to southern highbush blueberry plants (*Vaccinium corymbosum*, cv. Springhigh) grown in sandy substrate with or without CaCO₃ (5% w/w). The pH and the nutrient element composition of the leachate, the concentrations of nutrient elements in the leaves and branches, biomass production, chlorophyll concentrations and canopy size were measured over two years of cultivation.

Results An increase in the N-NH₄ level was accompanied by a decrease in the pH values of the leachates. The pH values of the leachate were higher in the CaCO₃ and NI treatments compared to those without CaCO₃ and without NI treatments. An increase in the level of N-NH₄ enhanced the biomass production in the substrate with CaCO₃, whereas the opposite effect was obtained in the treatment without CaCO₃. Addition of NI was associated with a decrease in biomass production compared to the treatment without NI in the treatments with and without CaCO₃. No deficiencies of the measured nutrient elements were identified in any treatment.

Conclusion Application of NI reduces the potential of contamination by NO₃ in high buffered-pH substrate, but it is not necessary in low pH-buffered substrate and it leads to a decrease of biomass production in low and high pH-buffered substrates.

Keywords Biomass production · Nutrient elements concentrations · Leachate composition · Sandy substrate · CaCO₃

Highlights

- Nitrification inhibitor (NI) delayed and moderated the pH drop caused by N-NH₄ application.
- High N-NH₄ level inhibited and enhanced biomass production in low and high pH-buffered substrates respectively.
- NI inhibited biomass production independently of the substrate pH-buffer capacity.

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1 Introduction

Nitrogen (N) is the element most consumed by plants, second only to carbon, and accounts for about 1–5% of the dry weight of plants (Hawkesford et al. 2012). Therefore, N is usually applied at relatively high concentrations, as compared to the other nutrient elements. However, excessive use of N can lead to environmental problems, including water and air pollution. Examples of such pollution include nitrate (NO₃) pollution of drinking-water reservoirs (McIsaac et al. 2001; Burns et al. 2009; Sebilo et al. 2013) and the emission of ammonia (NH₃) and nitrogen dioxide (N₂O), which pollute the air and exacerbate the greenhouse effect (Gruber and Galloway 2008), respectively. Therefore, the search for ways to minimize these environmental issues has become a major issue in N-fertilizer research (Kanter et al. 2020; Gu et al. 2023).

In agriculture, N is supplied through the application of various organic residues or as synthetic fertilizers containing

ammonium (NH_4), and/or nitrate (NO_3). A high NH_4 /Total N ratio can effectively decrease the soil pH (Silber and Bar-Tal 2019) through two main mechanisms: 1) the nitrification process in which two moles of protons are released per 1 mol of NH_4 (Bloom et al. 2005); and 2) the uptake of 1 mol of NH_4 by the plant, which results in the release of 1 mol of protons from the root (Hawkesford et al. 2012). In terms of acidification potential, the first mechanism is more effective than the second mechanism, whereas the second one can be more effective in the rhizosphere, especially in high buffer pH soils (Silber and Bar-Tal 2019). One of the main challenges in the application of NH_4 to pH-neutral and slightly alkaline soils is that, under those conditions, NH_4 undergoes very rapid oxidation (Molina 1985; Khalil et al. 2005; Tamir et al. 2013). Therefore, the majority of the N found in those soils is in the form of NO_3 . Consequently, the potential contamination of surface water and groundwater is higher for those soils than it is for acidic soils (Song et al. 2021).

Numerous studies have presented the great potential inherent in the use of nitrification inhibitors (NIs) to slow down the decline in NH_4 concentrations due to the formation of NO_3 in the soil and reduce potential environmental pollution (e.g., De and Cameron, 2002; Fanguiero et al. 2009; Tamir et al. 2013; Qiao et al. 2015; Barth et al. 2019; Norton and Ouyang 2019; Fu et al. 2020). NI application is considered to be an efficient way to reduce environmental water pollution by NO_3 . In addition, the application of NI together with NH_4 fertilizer enhances the proportion of N taken up by the plant as NH_4 (Liu et al. 2013; Qiao et al. 2015). Uptake of this form of N by the roots is accompanied by the release of protons to the rhizosphere (Hawkesford et al. 2012), which can enhance the availability of micronutrients in the rhizosphere (Sarkar and Wynjones 1982; Thomson et al. 1993). However, Dreyer et al (2020) have found that application of NI in calcareous soil reduced the uptake of Mn by wheat plant.

Blueberry can be a good model plant for examining the effect of NI on biomass production. The optimal soil conditions for blueberry growth are pH < 5.5, high organic content (Korcak 1989; Darnell and Hiss 2006), and very good drainage (Finn et al. 1993). In addition, numerous studies (Korcak 1988; Merhaut and Darnell 1996; Alt et al. 2017) have shown that blueberry plants prefer to take up NH_4 rather than NO_3 . Increasing doses of NH_4 as the main source of N fertilization of blueberry plants enhanced the plants performance and yield (Hirzel et al. 2024). However, it is still not agreed whether the effect of NH_4 on the production of blueberries is due to its effect on the plant's N-uptake or a result of the acidification of the soil rhizosphere, which enhances root development and/or the availability of iron (Fe) and manganese (Mn) (Tamir et al. 2021a, b, 2022, 2023). Messiga et al. (2021) reported that the application of NI did not improve the yield of blueberry plants in acidic soil. Osorio

et al. (2020) showed that the addition of a NI increased the chlorophyll concentration in blueberry leaves, but did not improve biomass production or increase the concentrations of micronutrients or N in the leaves. The effectiveness of the NI in acidic pH is expected to be lower compared to that which occurs in slight alkaline conditions, since nitrification processes are slowing down under low pH. To the best of our knowledge, no research has examined the combined effect of NI and N- NH_4 level on blueberry production in low and high pH substrates. Our research hypotheses are that in high pH-buffered substrate, high N- NH_4 level will produce high N- NO_3 concentration and it will increase the risk for environmental pollution. Application of NI can reduce this potential pollution, but may inhibit blueberry plant production and performance due to maintaining unfavorable alkaline pH. On the other hand, in low pH-buffered substrate, application of high level of NH_4 dose will lead to sharp acidification of the substrate and enhance the availability of phosphorus (P) and the microelements {mainly Iron (Fe) and manganese (Mn)} to the plants, but the sharp decrease of the pH will inhibit further nitrification. Therefore, the effect of application of NI to low pH-buffered substrate will be smaller than in high pH-buffered substrate.

Therefore, the main purpose of this study is to examine the combined effects of N- NH_4 fertilization and nitrification inhibitor (NI) on the production of blueberry plants grown in low or high pH-buffered substrates and the loss of N by excess nitrate leaching.

Our assumption is that the nutrient elements composition and the pH of the leachate from containers would be closely associated with the pH and composition of the substrate solution. Therefore, the nutrient elements composition and the pH of the leachate were measured as represents of the rhizosphere.

2 Materials and Methods

2.1 Description of the Experiments

The plants used in this study were 8-month-old, southern highbush blueberry (*Vaccinium corymbosum*, cv. Spring-high) plants produced from tissue culture. At the start of the study, the plants were well-established in terms of vegetative growth and root development, with roots that had developed in close interaction with the growth medium. They were transplanted (4 April 2020) into 25-L containers filled with a sandy substrate (grain size—20/30 mesh) free of CaCO_3 (Negev Minerals Ltd., Omer, Israel), which were kept in a net-house structure (18 m long, 18 m wide and 2.30 m high). That structure was located on the campus of the Agricultural Research Organization, The Volcani Center, Rishon LeZion, Israel (40 m above sea level). The net-house was covered by

white 30% and black 30% shade netting. The actual shade at the time of the experiment was 58% of the outside light radiation. Before the containers were filled, we put a 10-cm layer of coarse tuff (> 8 mm, Tuff Merom-Golan Ltd., Afula, Israel) at the bottom of each pot, in order to prevent water accumulation.

The experiment included two levels of CaCO_3 mixed into the sandy substrate: 0% (control, without CaCO_3) and 5% w/w; three levels of N-NH_4 (20, 40 or 80 mg L^{-1}), and two NI treatments, with and without NI (0 and 1% w/w of the ratio NI/ total applied N). Thus, the concentration of NI in the 1% treatment was 0.2, 0.4 and 0.8 mg L^{-1} , for the three levels of N-NH_4 : 20, 40 or 80 mg L^{-1} , respectively. The design of the experimental treatments is full factorial. The NI tested in this work was 3,4-dimethylpyrazole phosphate (DMPP). The DMPP concentration chosen for use in this study is in accordance with the recommendation (Benckiser et al. 2013), and based on a preliminary experiment, and in light of the fact that high concentrations of the DMPP can lead to phyto-toxicity (Rodrigues et al. 2018). The 5% w/w level of CaCO_3 was obtained by mixing the sandy substrate with CaCO_3 powder (< 2 mm; CaCO_3 > 90%, 30% defined as the active fraction, Negev Industrial Minerals Ltd., Omer, Israel) prior to the filling of the pots. The use of CaCO_3 amendment to maintain slight alkaline pH values was chosen because of the high pH-buffer capacity of this geological mineral (Bloom et al. 2005). The dose of CaCO_3 chosen for use in this study is based on our previous study (Tamir et al. 2021b).

The N-NH_4 and NI treatments, as well as the other nutrient elements, were applied using fertigation solutions (fertilization via irrigation system) from 1.5 m^3 tanks containing the target composition of all of the essential nutrient elements. The fertigation solutions were delivered using electric pumps (MPU 2010–561, Flowjet, Cleaning Equipment Ltd., Kendal, UK) with a flow rate of 12 L h^{-1} , which were connected to a drip-irrigation system (discharge of 4.0 L h^{-1} per container; Netafim Ltd., Tel Aviv, Israel). Irrigation was applied by once an hour pulse during daylight hours, through the spring–autumn growth period (March–November). The volume of water applied per pulse of irrigation was identical throughout the day and was determined according to a daily leaching fraction of about 15%. The measured drainage volumes are shown in Table S1. The concentrations of P, potassium (K) and magnesium (Mg) in the fertigation solution were identical for all treatments: 30, 100 and 30 mg L^{-1} , respectively. $(\text{NH}_4)_2\text{SO}_4$, H_3PO_4 , KH_2PO_4 , K_2SO_4 , MgSO_4 and a stock of micronutrients chelated with EDTA were used to bring the micronutrient solutions to concentrations of 1.5 mg L^{-1} Fe, 0.75 mg L^{-1} Mn, 0.3 mg L^{-1} Zinc (Zn), 0.09 mg L^{-1} copper (Cu) and 0.025 mg L^{-1} molybdenum (Mo) (ICL Fertilizers Ltd., Haifa, Israel). The concentration of Mn was brought to 1.5 mg L^{-1} using a specific chelate

of Mn with EDTA (ICL Fertilizers Ltd., Haifa, Israel). The concentration of calcium (Ca) in the tap water was about 30–40 mg L^{-1} , which is sufficient for optimal growth of blueberry, based on our previous studies (Tamir et al. 2020; Tamir et al. 2023), therefore, we did not apply any additional Ca. All treatments were replicated eight times, with one pot for each replication, and the pots were arranged randomly in the net-house.

2.2 Plant Measurements and Analyses

2.2.1 Chlorophyll Concentrations, Foliage Coverage and Biomass Production

The concentrations of chlorophyll in diagnostic leaves (i.e., the youngest fully developed leaf, usually the fourth to sixth leaf from the shoot apical meristem) were measured periodically during the growing season with a SPAD device (502 Plus chlorophyll meter; Spectrum Technologies Inc., Aurora, IL, USA). The SPAD readings were calibrated ($R^2 = 0.90$, p -value < 0.0001) to the chlorophyll concentrations that were determined by dimethyl sulfoxide (DMSO) extraction (Barnes et al. 1992) and spectrophotometer measurements (UV-2401PC; Shimadzu Scientific Instruments, Nakagyo, Kyoto, Japan).

The foliage coverage was measured periodically during the growing season, using a non-destructive method (Patrignani and Ochsner 2015). The foliage of each plant was photographed from a uniform height (1.3 m from the edge of the pot) using a cellular device, after which the plant cover was calculated using the software Canopeo (Mathworks, Inc., Natick, MA, USA).

The accumulation of plant biomass over the growing season (March–December in Year 1 and August in Year 2) was evaluated over 2 years. All plants were pruned to the same height of 40 cm above the sandy substrate surface on two dates: 7 December 2020 (8 months from the date of transplanting) and 20 August 2021 (1 year and 6 months from the date of transplanting). After pruning, the leaves and branches from each plant were separated, weighed to determine their fresh weight and placed in an oven to dry at 65 °C for 72 h. After that drying procedure, the plant parts were weighed again to determine their dry weights.

2.2.2 Nutrient Elements Concentrations in the Plant Organs

Diagnostic leaves (the youngest fully developed leaf, usually the fourth to sixth leaf from the shoot tip) were sampled at three different times during the growing seasons, according to plant development: in late June (26 June 2021, about 2 months after spring sprouting), late August (28 August 2020, middle of the growing season) and early December

(8 December 2020, end of the growing season). At each sampling event, eight replicates (one replicate = one plant in one pot) were sampled from each treatment. Each replicate consisted of 15 to 20 diagnostic leaves from one plant. Sampled leaves were rinsed with tap water and deionized water, dried at 65 °C and then ground to a fine powder.

Branches were sampled during the pruning of the plants (described in detail in Sect. 2.2.1), after their leaves had been removed, in two different years (8 December 2020 and 20 August 2021). Similar to the diagnostic-leaf sampling, at each sampling event, eight replicates (one replicate = one plant in one pot) were sampled from each treatment. Sampled branches were rinsed with tap water and deionized water, dried at 65 °C and then ground to a fine powder.

N, P and K were extracted by wet digestion with concentrated sulfuric acid (98%) and hydrogen peroxide (Snell and Snell 1948). The N and P concentrations were determined using an auto-analyzer (Automated Photometric Discrete Analyser, Gallery Plus, Thermo Fisher Scientific, 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 (Atomic Absorption Spectroscopy, Analyst400, PerkinElmer, Waltham, MA, USA) after the samples had been digested with nitric acid and perchlorate.

2.3 Leachate Parameters

Leachate was collected five times over the study period (14 April 2020 to 8 September 2021). At each time point, eight replicates (one replicate = one plant in one pot) were sampled from each treatment. The pH of the leachate was measured using a pH meter (FC 200b, Hanna Instruments, Woonsocket, RI, USA). The N-NH₄, N-NO₃ and P-PO₄ concentrations were determined using an auto-analyzer (Automated Photometric Discrete Analyser, Gallery Plus, Thermo Fisher Scientific, Finland) and the K concentration was determined by flame photometry (Sherwood M410, Sherwood Scientific Ltd., Cambridge, UK). The concentrations of Ca, Mg, Fe, Mn and Zn were determined using atomic absorption spectroscopy (Analyst400, PerkinElmer, Waltham, MA, USA).

2.4 Statistical Analysis

The statistical analysis was performed with JMP® Pro statistical software, version 16.0 (SAS Institute Inc., Cary, NC, USA). Differences in measured parameters between treatments were evaluated by three- to five-way {N- rate, NI application, CaCO₃ application and days after transplanting (pH and chlorophyll concentrations) or year (nutrient element concentrations in the leachate, biomass production,

nutrient element concentrations in the brunch) or season (nutrient element concentrations in the leaf)} analysis of variance (ANOVA). When significant differences were found, Tukey's honestly significant difference (HSD) test at a 5% probability level was used to compare mean values of the three N levels. Since there is only one degree of freedom in CaCO₃ and NI (with or without), Student's t-test at a 5% probability level was used to compare mean values for these variables. A Pearson correlation analysis were done in order to present the relation between nutrient elements in the branches and leaves using JMP® Pro statistical software, version 15.1.0 (SAS Institute Inc., Cary, NC, USA).

3 Results

3.1 Effects of the Treatments on the Composition of the Leachate

The initial pH of the sand substrate is ~7.0 and addition of CaCO₃ increased it to ~7.3. Overall, in all sampling dates, the pH of the leachate was lower as the N level was higher (Table 1). As expected, the pH levels in the leachate in all sampling dates, were higher in the treatment with CaCO₃ than the treatment without CaCO₃, and in the treatment with NI than in the treatment without NI. There was a general trend of lower pH in the second year than the first year.

The effect of the year (the average of measurements for a given variable for a particular year of cultivation) on the composition of the leachate was significant (<0.0001, with the exception of N-NH₄) and there were significant interactions between the year and the other independent variables (N level, CaCO₃ and NI). Therefore, we present the effects of the treatments on each measured parameter separately for each year (Table 2 and 3).

As expected, NH₄ and NO₃ concentrations increased as the N level increased in both years of measurements. The ratio of NH₄/Total N and the concentrations of Ca also increased with the elevation of N level, probably as a result of the acidification effect of the applied NH₄. P, K, Mg, Fe, Mn and Zn concentrations were not affected or were affected inconsistently by changes in the N level over the two years of measurements.

The concentrations of N-NO₃ and Ca in the leachate were higher in the treatment with CaCO₃, as compared to the treatment without CaCO₃, in both years. Conversely, the concentrations of N-NH₄, P, Mg, Fe and Mn and the N-NH₄/Total N ratio were higher in the treatment without CaCO₃ than in the treatment with CaCO₃, in both years. The concentration of K was not affected by the CaCO₃ treatments in either years.

The NI had stronger impact on the leachate composition in the first than the second year, therefore we describe here

Table 1 Effects of the N, CaCO₃ and nitrification inhibitor (NI) treatments on the pH in the leachate from southern highbush blueberry (*Vaccinium corymbosum*, cv. Springhigh) plants over the course of the experiment (14 April 2020 to 20 August 2021)

		pH				
		Days after transplanting				
Variable		37	99	402	464	492
N concentration (mg L ⁻¹)						
20		7.24(0.06)a	7.23(0.06)a	6.91(0.11)a	6.73(0.10)a	6.62(0.10)a
40		7.15(0.06)ab	7.13(0.07)ab	6.68(0.14)b	6.50(0.13)b	6.44(0.12)a
80		7.12(0.06)b	7.06(0.08) b	6.36(0.11)c	6.21(0.09)c	6.15(0.09)b
CaCO ₃ application						
No		7.02(0.05)b	6.89(0.05)b	6.07(0.07)b	5.95(0.06)b	5.90(0.05)b
Yes		7.32(0.04)a	7.39(0.04)a	7.23(0.06)a	7.01(0.06)a	6.97(0.06)a
NI application						
No		6.93(0.04)b	7.09(0.06)a	6.55(0.12)b	6.43(0.10)a	6.34(0.10)a
Yes		7.41(0.03)a	7.19(0.05)a	6.75(0.09)a	6.54(0.08)a	6.46(0.08)a
Variable	df	Probability of <i>F</i> < 0.05				
N concentration (mg L ⁻¹)	2	0.020	0.049	< 0.0001	< 0.0001	< 0.0001
CaCO ₃ application	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
NI application	1	< 0.0001	n.s	0.007	n.s	n.s
Block	7	n.s	n.s	n.s	n.s	n.s
N × CaCO ₃	2	0.002	n.s	0.001	0.0006	0.003
N × NI	2	n.s	n.s	n.s	n.s	n.s
NI × CaCO ₃	1	0.002	0.006	n.s	0.047	n.s
N × CaCO ₃ × NI	2	0.006	n.s	n.s	n.s	n.s

Means within columns for each parameter that are followed by the same letter are not significantly different, according to Tukey's honestly significant difference (HSD) test at 5% probability level for the N treatments and Student's *t*-test at 5% probability level for the CaCO₃ and the NI treatments. $n = 8$. The numbers in parentheses represent SE. The ANOVA statistical analysis and the HSD and Student's *t*-tests were performed with JMP® Pro statistical software, version 15.1.0 (SAS Institute Inc., Cary, NC, USA)

the effects during the first year. The concentrations of N-NH₄ and P, and the N-NH₄/Total N ratio were higher in the treatment with NI than in the treatment without NI. The concentrations of NO₃, Ca, Mg and Zn were higher in the treatment without NI than in the treatment with NI.

Interaction effects between the independent variables were observed for most of the measured nutrient elements, especially in the second year of cultivation (Tables 2 and 3). However, these interaction effects are mainly due to the strength of the effect and do not show opposing trends and are therefore not shown.

3.2 Chlorophyll Concentrations, Foliage Coverage and Biomass Production

3.2.1 Biomass Production

The biomass production of leaves, branches and the whole-plant foliage (sum of the leaves + branches) were affected by the treatments and their interactions (Table 4). In the first year of cultivation, an increase in N level was associated with a decrease in biomass production of branches, leaves and the whole-plant foliage. In the second year, a similar

effect of the N level was observed only for branch biomass. Amendment of the substrate with CaCO₃ and NI application through the irrigation were associated with lower biomass production, as compared to the treatments without CaCO₃ or without NI, in the first and second years. Significant interactive effects of the treatments on the biomass of the branches, leaves and total foliage were observed in the second year (Table 4). Therefore, the effects of NI on the cumulative biomass of the branches, leaves and total foliage over two years (Year 1 + Year 2), at each N level, are presented separately for the with- and without-CaCO₃ treatments (Fig. 1).

The biomass production of the leaves, branches and total foliage in the absence of CaCO₃ (Fig. 1) decreased as the N level increased, independently of the NI treatment. In contrast, in the presence of CaCO₃ and the absence of NI, the biomass production of each of the plant organs and the total foliage biomass increased as the N level increased (Fig. 1), associated with the acidification by high NH₄ level. An opposite effect of N level was obtained with NI and with CaCO₃ (Fig. 1).

The relative biomass production decreased as the pH of the leachate rose above 6 (Fig. 2). According to the regression line equation, an increase of 1 pH unit was associated

Table 2 Effects of the N, CaCO₃ and nitrification inhibitor (NI) treatments on the major nutrient element concentrations (N, P, and K) and on the ratio of N-NH₄/Total N in the leachate from pots planted with southern highbush blueberry (*Vaccinium corymbosum*, cv. Springhigh) plants over the course of the experiment (14 April 2020 to 20 August 2021)

Variable	N-NH ₄		N-NO ₃		N-NH ₄ /Total N		P-PO ₄		K	
	mg L ⁻¹				%		mg L ⁻¹			
	First	Second	First	Second	First	Second	First	Second	First	Second
N concentration (mg L ⁻¹)										
20	7.74(1.00)c	2.15(0.35)c	12.9(1.06)c	14.6(1.08)c	38.1(4.56)c	13.6(2.13)c	22.5(1.03)a	46.7(2.77)ab	143(2.2)a	179(4.9)b
40	19.9(2.13)b	13.0(1.49)b	20.5(1.94)b	34.4(2.41)b	48.8(4.52)b	28.9(3.36)b	22.2(1.22)a	49.0(3.48)a	145(3.2)a	238(5.9)a
80	49.5(3.73)a	51.0(5.27)a	30.6(4.08)a	64.7(6.17)a	63.3(4.04)a	45.9(4.58)a	19.7(1.06)a	42.7(1.64)b	144(2.4)a	172(4.1)c
CaCO ₃ application										
No	29.9(2.77)a	39.7(4.00)a	14.4(1.11)b	17.3(1.62)b	58.7(3.24)a	52.7(3.00)a	26.2(0.74)a	63.7(1.79)a	145(2.3)a	213(5.2)a
Yes	21.5(2.64)b	4.48(1.76)b	28.3(2.87)a	58.5(3.46)a	41.5(4.04)b	6.34(1.95)b	16.7(0.80)b	28.5(1.80)b	144(2.0)a	179(3.9)a
NI application										
No	14.8(2.21)b	23.7(3.29)a	32.0(2.50)a	35.8(3.38)a	26.4(2.75)b	31.1(2.86)a	19.8(1.04)b	49.1(2.06)a	145(2.3)a	206(4.9)a
Yes	36.6(2.78)a	20.4(2.97)b	10.6(1.39)b	40.0(3.79)a	73.7(3.05)a	27.9(3.17)b	23.1(0.71)a	43.1(2.13)b	143(1.9)a	187(4.2)b
Variable	df									
N concentration (mg L ⁻¹)	2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	n.s	0.004	n.s	<0.0001
CaCO ₃ application	1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	n.s	n.s
NI application	1	<0.0001	0.0008	n.s	<0.0001	0.037	0.001	0.0001	n.s	<0.0001
Block	7	n.s	<0.0001	<0.0001	n.s	<0.0001	0.001	0.011	<0.0001	<0.0001
N×CaCO ₃	2	0.006	<0.0001	n.s	n.s	<0.0001	n.s	<0.0001	n.s	n.s
N×NI	2	<0.0001	<0.0001	n.s	n.s	n.s	n.s	<0.0001	n.s	<0.0001
NI×CaCO ₃	1	0.0003	<0.0001	n.s	<0.0001	<0.0001	0.003	n.s	n.s	n.s
N×CaCO ₃ ×NI	2	0.024	<0.0001	n.s	n.s	n.s	n.s	<0.0001	n.s	n.s

Means within columns for each parameter that are followed by the same letter are not significantly different, according to Tukey's honestly significant difference (HSD) test at 5% probability level for the N treatments and Student's *t*-test at 5% probability level for the CaCO₃ and the NI treatments, *n* = 8. The numbers in parentheses represent SE. The ANOVA statistical analysis and the HSD and Student's *t*-tests were performed with JMP® Pro statistical software, version 15.1.0 (SAS Institute Inc., Cary, NC, USA)

Table 3 Effects of the N, CaCO₃ and nitrification inhibitor (NI) treatments on the Ca and Mg concentrations and on the minor nutrient element concentrations (Fe, Mn and Zn) in the leachate from pots planted with southern highbush blueberry (*Vaccinium corymbosum*, cv. Springhigh) plants over the course of the experiment (14 April 2020 to 20 August 2021)

Variable	Ca		Mg		Fe		Mn		Zn	
	mg L ⁻¹				µg L ⁻¹					
	Second	First	Second	First	Second	First	Second	First	Second	First
N concentration (mg L ⁻¹)										
20	59.0(4.62)c	94.8(6.54)b	32.3(1.04)b	46.8(2.63)b	1225(129)a	2454(203)b	400(40.1)a	801(79.5)c	280(19.1)a	437(33.0)b
40	80.4(6.95)b	154(10.5)a	36.1(1.60)ab	58.7(2.57)a	1255(129)a	3048(270)a	480(50.8)a	1189(132)a	251(15.9)a	603(45.3)a
80	102(8.6)a	171(15.0)a	37.7(1.79)a	38.9(2.14)c	1435(152)a	2066(133)c	500(51.9)a	974(73.2)b	315(25.9)a	618(59.3)a
CaCO ₃ application										
No	55.2(2.80)b	72.4(3.47)b	37.9(1.43)a	53.4(2.37)a	1524(117)a	3710(183)a	587(40.2)a	1616(75)a	293(17.3)a	789(36.4)a
Yes	106(6.9)a	208(9.0)a	32.9(0.98)b	42.9(1.77)b	1086(101)b	1336(102)b	333(32.9)b	359(69.7)b	271(16.8)a	316(17.5)b
NI application										
No	96.8(6.53)a	143(9.2)a	37.1(1.39)a	47.3(1.99)a	1329(108)a	3082(170)a	522(37.3)a	1017(68)a	301(15.6)a	513(27.7)b
Yes	64.1(4.66)b	137(9.3)a	33.7(1.07)b	49.0(2.22)a	1282(116)a	1963(128)b	397(40.7)b	959(88.4)a	263(18.4)a	592(38.5)a
Variable	df									
N concentration (mg L ⁻¹)	2	<0.0001	<0.0001	0.024	<0.0001	n.s	<0.0001	n.s	<0.0001	<0.0001
CaCO ₃ application	1	<0.0001	<0.0001	0.003	<0.0001	0.006	<0.0001	<0.0001	n.s	<0.0001
NI application	1	<0.0001	n.s	0.036	n.s	n.s	<0.0001	0.015	n.s	0.017
Block	7	n.s	0.002	0.004	<0.0001	n.s	<0.0001	<0.0001	<0.0001	<0.0001
N×CaCO ₃	2	n.s	<0.0001	n.s	0.018	n.s	<0.0001	<0.0001	n.s	<0.0001
N×NI	2	0.022	<0.0001	0.005	<0.0001	n.s	<0.0001	<0.0001	n.s	0.003
NI×CaCO ₃	1	0.029	n.s	n.s	0.017	n.s	<0.0001	n.s	n.s	n.s
N×CaCO ₃ ×NI	2	n.s	0.042	n.s	n.s	0.0003	n.s	0.015	n.s	<0.0001

Means within columns for each parameter that are followed by the same letter are not significantly different, according to Tukey's honestly significant difference (HSD) test at 5% probability level for the N treatments and Student's *t*-test at 5% probability level for the CaCO₃ and the NI treatments, *n* = 8. The numbers in parentheses represent SE. The ANOVA statistical analysis and the HSD and Student's *t*-tests were performed with JMP® Pro statistical software, version 15.1.0 (SAS Institute Inc., Cary, NC, USA)

Table 4 Effects of N, CaCO₃ and nitrification inhibitor (NI) treatments on the dry biomass of branches, leaves and total foliage of southern high-bush blueberry (*Vaccinium corymbosum*, cv. Springhigh) plants over 17 months of growth (14 April 2020 to 20 August 2021)

Variable		Year 1 (7 Dec 2020)			Year 2 (20 Aug 2021)		
		Branches	Leaves	Foliage	Branches	Leaves	Foliage
g dry weight plant ⁻¹							
N concentration (mg L ⁻¹)							
20		39.6(4.48)a	42.2(4.11)a	81.8(8.49)a	264(20.4)a	244(16.6)a	507(36.7)a
40		33.9(4.46)ab	39.9(5.46)ab	73.8(9.85)ab	249(12.8)ab	269(10.3)a	517(21.9)a
80		25.3(4.12)b	29.5(4.31)b	54.8(8.33)b	214(14.4)b	235(14.2)a	448(28.4)a
CaCO ₃ application							
Without		45.7(3.76)a	51.6(3.80)a	97.2(7.44)a	287(10.8)a	289(6.9)a	576(17.0)a
With		20.2(2.31)b	22.8(2.62)b	43.0(4.85)b	197(13.0)b	209(12.8)b	406(25.3)b
NI application							
Without		40.3(3.78)a	45.3(3.69)a	85.5(7.39)a	252(12.6)a	270(9.60)a	522(21.4)a
With		25.6(3.28)b	29.1(3.84)b	54.8(7.03)b	232(14.2)a	228(12.6)b	460(26.6)b
Variable	df	Probability of $F < 0.05$					
N concentration (mg L ⁻¹)	2	0.005	0.012	0.006	0.020	n.s	n.s
CaCO ₃ application	1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NI application	1	<0.0001	<0.0001	<0.0001	n.s	0.001	0.020
Block	7	<0.0001	<0.0001	<0.0001	n.s	n.s	n.s
N × CaCO ₃	2	0.040	n.s	n.s	<0.0001	<0.0001	<0.0001
N × NI	2	n.s	0.018	n.s	<0.0001	0.002	0.0001
NI × CaCO ₃	1	n.s	n.s	n.s	n.s	n.s	n.s
N × CaCO ₃ × NI	2	n.s	n.s	n.s	0.039	0.021	0.025

Means within columns for each parameter that are followed by the same letter are not significantly different, according to Tukey's honestly significant difference HSD test at 5% probability level for the nitrogen level and Student's *t*-test at 5% probability level for the CaCO₃ and NI treatments. $n = 8$. The numbers in parentheses represent SE. The ANOVA statistical analysis and the HSD and Student's *t*-tests were performed with JMP® Pro statistical software, version 15.1.0 (SAS Institute Inc., Cary, NC, USA)

with 26% decrease in biomass production. A similar relationship was also observed between the pH of the leachate and the foliage coverage (Figure S1).

3.2.2 Chlorophyll Levels in the Leaves

The chlorophyll concentrations in the leaves were affected by the treatments throughout the growing seasons (Table 5). At most measurement times, the chlorophyll concentrations in the leaves at the intermediate and high N levels were higher than at the low N level, opposite to the effect of the N level on biomass production (Table 4). In addition, at most measurement times, the chlorophyll concentrations in the leaves of plants grown in the presence of CaCO₃ were lower than those of plants grown without CaCO₃. The chlorophyll concentration in the NI treatment was higher than that observed in the treatment without NI, in contrast to the effect of the NI on biomass production (Table 4). No interactive effects of the three independent variables on the chlorophyll concentrations in the leaves were observed for most of the sampling dates.

3.3 Nutrient Elements Concentrations in the Leaves and Branches

3.3.1 Nutrient Elements Concentrations in the Leaves

Concentrations of nutrient elements in the leaves were affected by the sampling season (Table 6). N and Zn concentrations increased from spring to autumn, whereas, the highest concentrations of P, K, Ca, Mg, and Fe were observed in the summer. While the lowest concentrations of P, K, Ca and Mn were obtained in the spring, similar concentrations of Mg and Fe were obtained in the autumn and spring samplings. Mn concentrations in the autumn were similar to those observed in the summer. Since the sampling season affected the concentrations of the nutrient elements, the effects of the main treatments and their interactions are presented separately for each season (Table 6).

In the summer, N and P concentrations increased as the N level increased (Table 6) whereas other nutrient elements were not affected. Also in the summer, P, Mg, Mn and Zn concentrations were higher in the treatment without CaCO₃ than in the treatment with CaCO₃. The NI treatment only

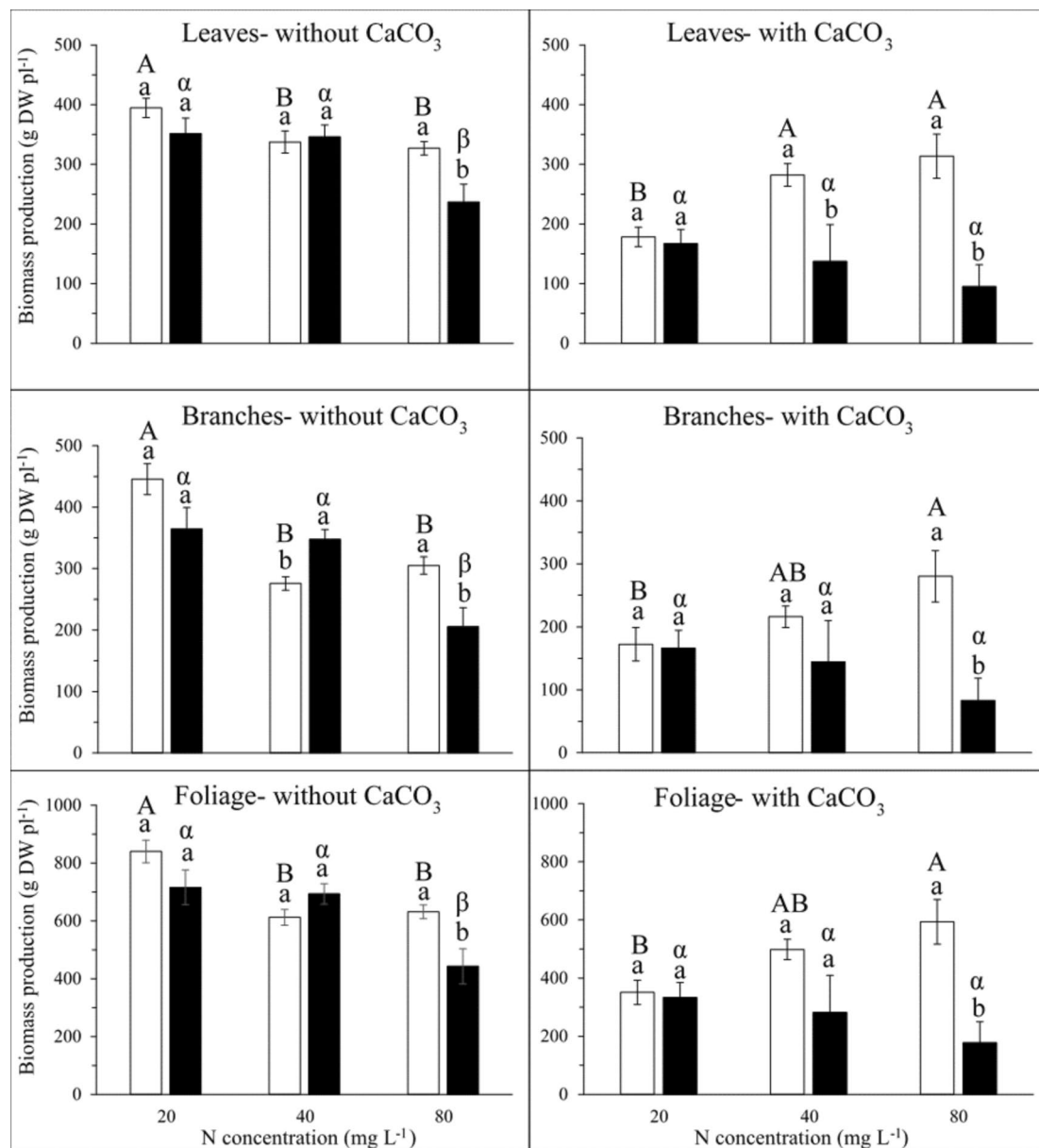


Fig. 1 Effects of the N level with nitrification inhibitor (NI, filled column) or without NI (empty column) on the cumulative dry biomass of leaves, branches and total foliage of southern highbush blueberry (*Vaccinium corymbosum*, cv. Springhigh) plants over two years (Year 1 + Year 2) of cultivation in sandy substrate without and with CaCO_3 . Means of each parameter in each subgraph followed by the same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at 5% probability level for the N treatments. Means of each parameter at each N level in each subgraph

followed by the same letter are not significantly different according to Student's *t*-test at 5% probability level for the NI treatments. In each panel, a and b refer to the effect of the NI, A and B refer to the effect of the N level in the treatment without NI and α and β refer to the effect of the N level in the presence of the NI. $n=8$. The error bars represent SE. The statistical analysis was performed with JMP® Pro statistical software, version 15.1.0 (SAS Institute Inc., Cary, NC, USA).

affected the P and Mg concentrations, which were higher in the treatment with NI than in the treatment without NI.

In the autumn, higher N levels were associated with higher concentrations of N, Mg and Fe (Table 6). The addition of CaCO_3 was associated with higher concentrations of K, Ca and Zn. However, the addition of CaCO_3 was associated with

lower concentrations of Mg and Mn. The addition of NI was associated with higher concentrations of P and K, as compared to the treatments without NI. In all sampling seasons, Mn concentrations were consistently affected by the interaction between the three treatments. However, these interaction are mainly due to the strength of the effect and do not show

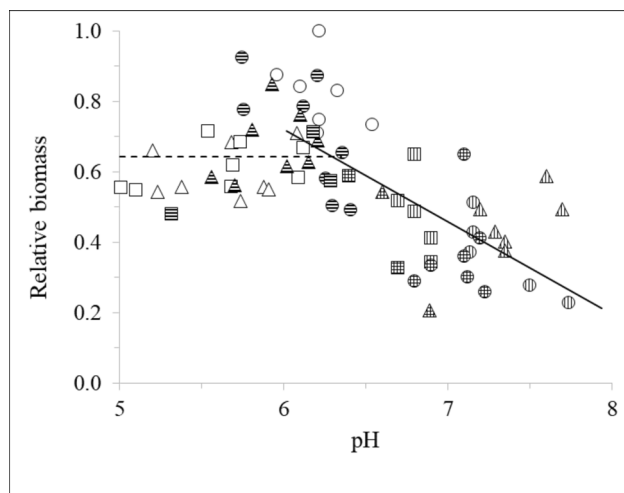


Fig. 2 Relationship between the pH of the leachate in August 2021 (end of the experiment) and the total foliage biomass of southern highbush blueberry (*Vaccinium corymbosum*, cv. Springhigh) plants over 2 years of growth in response to N-NH₄ levels: 20 (circle), 40 (triangle) and 80 (square) mg L⁻¹ applied via a fertigation system with CaCO₃ (vertical line), with NI (horizontal line), with CaCO₃ and NI (grid lines), and without CaCO₃ or NI (empty). Each symbol represents the relative plant biomass accumulated in two years for a given pot as a function of the pH of the leachate measured at the end of the growing period (August 2021) from that pot. pH > 6.0: $y = -0.26x + 2.29$, $R^2 = 0.49$, $p < 0.0001$; pH < 6.0: $y = 0.64$. Best-fit linear regression were fit using JMP software, version 15.1.0 (SAS Institute Inc., Cary, NC, USA)

opposing trends, therefore just the overall effects of the treatments on leaf Mn are shown and described above.

The concentrations of most of the nutrient elements were not affected by the treatments in the spring, with the exception of the increase in leaf N concentration with the increase of the treatment N dose. In addition, the P and Mg concentrations were lower in the CaCO₃ treatment than in the treatment without CaCO₃ and the concentration of K was higher in the NI treatment than in the treatment without NI (Table S2).

3.3.2 Nutrient Elements Concentrations in the Branches

At the end of the first year of cultivation, the concentrations of K and Ca in the branches were higher in the CaCO₃ treatment than in the treatment without CaCO₃ (Table S3). In contrast, the concentrations of Mg and Mn were higher in the treatment without CaCO₃ than in the treatment with CaCO₃. Also, the application of NI was associated with lower concentrations of Mg, as compared to the treatment without NI. At the end of the second year, the concentrations of N and Mg increased with the increase in the N level. In addition, the concentrations of Ca were higher in the treatment with CaCO₃ than in the treatment without CaCO₃. In contrast, the concentrations of Mg and Mn were lower in the CaCO₃ treatment than in the treatment without CaCO₃. In addition,

the concentration of Mn was higher in the treatment without NI than in the treatment with NI. Overall, there were no interactive effects of the three independent variables on the concentrations of the nutrient elements in the branches that were consistent across the two sampling seasons.

3.3.3 Relationships Between Nutrients in Leaves and Branches

Analysis of the relationships between the concentrations of the nutrient elements in the branches, showed significant and relatively high correlation coefficient ($r > 0.6$) between N and P, between N and K and between P and K concentrations (Table S4). In addition, a low but significant correlation coefficient was shown between Mg and Fe concentrations. A significant correlation coefficient was also shown between the concentration of Ca and Fe, but the level of correlation coefficient was very different between years. Among all the other elements no interactions were presented for the same year or between the first and the second year.

In the analysis of the relationship between the concentration of nutrient elements in the branches and in the leaves, a significant relationship was shown between the concentration of P and Mg in the branches and their concentration in the leaves in two years of sampling (Table S4). In addition, a significant but low correlation coefficient was shown between the concentration of P in the branches and Fe in the leaves. The relationships between all the other elements in the different organs were either not significant at all or were only significant in one of the years.

In the analysis of the relationship between the nutrient elements concentrations in the leaves, a weak but significant relationship was shown between most of the elements during spring (Table S5). However, a significant and consistent correlation coefficient was shown throughout the three sampling dates between P and Mg, between P and Fe, between Ca and Fe, and between Ca and Mn. In addition, a significant and consistent correlation coefficient was shown during the two sampling dates, between the concentrations of N and P, between P and K, between P and Mn, between K and Ca, Mg, Fe and Mn, between the Ca and Mg, between Mg and Fe, between Mn and Zn, and between Fe and Mn. However, in most cases the correlation coefficients were low ($r < 0.5$).

4 Discussion

The treatments applied in this study significantly affected the chemistry of the leachate, as expected. An elevation in the level of applied N-NH₄ was associated by a decrease in pH, due to the nitrification processes in agreement with numerous previous publications (for example, Tamir et al. 2013). However, as with elevating the level of applied N-NH₄ the ratio of

Table 5 Effects of N level, CaCO₃, and nitrification inhibitor (NI) treatments on the chlorophyll concentrations in diagnostic leaves of southern highbush blueberry (*Vaccinium corymbosum*, cv. Springhigh) plants over 17 months of growth (14 April 2020 to 20 August 2021)

Days after transplanting	140	213	409	442	458	478
Variable	$\mu\text{g cm}^{-2}$					
N concentration (mg L ⁻¹)						
20	32.2(0.90)a	41.3(0.96)a	36.7(0.69)b	37.8(0.56)b	38.0(0.64)b	39.7(0.62)b
40	34.4(1.77)a	44.0(1.21)a	39.2(0.44)a	39.9(0.41)a	40.4(0.50)a	41.1(0.55)ab
80	31.7(0.83)a	43.6(0.90)a	39.4(0.52)a	40.5(0.33)a	40.5(0.46)a	41.5(0.47)a
CaCO ₃ application						
Without	34.3(1.05)a	43.6(0.92)a	39.1(0.47)a	39.9(0.42)a	40.7(0.45)a	42.0(0.44)a
With	31.2(1.14)b	42.4(0.99)a	37.7(0.55)b	38.6(0.44)b	38.5(0.50)b	39.4(0.51)b
NI application						
Without	31.5(0.91)b	40.0(0.69)b	38.1(0.54)a	38.4(0.46)b	38.8(0.48)b	39.7(0.49)b
With	34.0(1.33)a	46.0(1.05)a	38.7(0.48)a	40.1(0.40)a	40.5(0.48)a	41.7(0.48)a
Variable	df	Probability of $F < 0.05$				
N concentration (mg L ⁻¹)	2	n.s	n.s	0.002	0.002	0.001
CaCO ₃ application	1	0.012	n.s	0.049	0.022	0.0006
NI application	1	0.034	< 0.0001	n.s	0.005	0.006
Block	7	< 0.0001	n.s	n.s	n.s	n.s
N × CaCO ₃	2	0.037	0.003	n.s	n.s	n.s
N × NI	2	n.s	0.032	n.s	n.s	n.s
NI × CaCO ₃	1	n.s	n.s	n.s	n.s	0.023
N × CaCO ₃ × NI	2	n.s	n.s	n.s	n.s	n.s

Means within columns for each parameter that are followed by the same letter are not significantly different, according to Tukey's honestly significant difference (HSD) test at 5% probability level for the N treatments and Student's *t*-test at 5% probability level for the CaCO₃ and NI treatments. $n = 8$. The numbers in parentheses represent SE. The ANOVA statistical analysis and the HSD Student's *t*-tests were performed with JMP® Pro statistical software, version 15.1.0 (SAS Institute Inc., Cary, NC, USA)

N-NH₄/Total N in the leachate increased, indicating that the nitrification process was slower as the level of applied N-NH₄ was higher. We suggest that this effect is due to a retardation of the nitrification processes under acidic pH conditions (Molina 1985; Khalil et al. 2005; Tamir et al. 2013).

The amendment of the substrate with CaCO₃ slowed down the decrease in pH compared to the substrate without CaCO₃. CaCO₃ is the soil component with the highest buffering capacity against pH changes (Bloom et al. 2005). Its dissolution as a result of the release of protons into the soil solution during nitrification processes is accompanied by the release of CO₂ and Ca (Lindsay 1979). In agreement with this mechanism, in the current experiment the concentrations of Ca in the leachate increased with the increase in the levels of applied N. This effect was higher in the treatment without NI compared to the treatment with NI, mainly in the first year.

The effect of the pH value on the intensity of the nitrification processes can also be seen in the comparisons of the treatments with and without CaCO₃. The concentrations of N-NH₄ were lower and the concentrations of N-NO₃ were higher in the treatment with CaCO₃ than without CaCO₃. The lower concentrations of P, Fe, Mn and Zn in the leachate of the treatment with CaCO₃ amendment also associate with it's higher pH compared to the treatment without CaCO₃, in

agreement with the known mechanisms of the effect of pH level on the solubility and availability of these elements in soil as function of the pH values (Lindsay 1979).

The addition of the NI slowed down the transformation processes of N-NH₄ to N-NO₃, especially in the first year. Consequently, application of NI, reduces the potential of NO₃ contamination of aquifers as shown in several studies (e.g. Qiao et al. 2015; Barth et al. 2019; Norton and Ouyang 2019; Fu et al. 2020), but slowed down or eliminated the acidification of the substrate solution as observed in the leachate.

As mentioned above, application of N-NH₄ to the substrate or soil can lead to acidification as a result of two processes: 1. N-NH₄ absorption by the plant, and 2. the nitrification process. Generally the acidification potential of the second process is higher compared to the first process (Bloom et al. 2005; Hawkesford et al. 2012). Although blueberry plants prefer to take up NH₄ rather than NO₃ (Korcak 1988; Merhaut and Darnell 1996; Alt et al. 2017), the global consumption of N by blueberries is small compared to other plants, as indicated by the low N concentrations in the leaves and other organs of blueberries (Retamales and Hancock 2012). Therefore the potential of acidification by blueberries plants due to the uptake of N-NH₄ is relatively low compared to plants that consume higher quantities of N.

Table 6 Effects of N level, CaCO₃ and nitrification inhibitor (NI) treatments on the nutrient element concentrations in diagnostic leaves of southern highbush blueberry (*Vaccinium corymbosum*, cv. Springhigh) plants during the summer and autumn

		N	P	K	Ca	Mg	Fe	Mn	Zn
Sampling season		mg g DW ⁻¹					µg g DW ⁻¹		
Spring		11.9(0.49)c	1.01(0.05)c	4.97(0.22)c	14.5(0.32)c	8.48(0.20)b	197(8.0)b	194(7.4)b	112(5.7)c
Summer		13.7(0.25)b	1.85(0.05)a	10.9(0.41)a	22.0(0.53)a	10.1(0.29)a	257(13.4)a	486(29.4)a	199(16.8)b
Autumn		15.1(0.31)a	1.55(0.05)b	9.45(0.45)b	18.8(0.44)b	8.44(0.33)b	194(8.1)b	470(19.5)a	257(20.9)a
Variable	df	Probability of $F < 0.05$							
Date of sampling	2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment		Summer							
N concentration (mg L ⁻¹)									
20		12.1(0.37)b	1.70(0.08)b	10.2(0.55)a	22.7(1.05)a	9.72(0.36)a	226(14.9)a	486(69.6)a	189(23.1)a
40		14.2(0.37)a	1.80(0.09)ab	12.1(0.90)a	21.3(0.93)a	10.0(0.41)a	262(19.0)a	439(28.2)a	183(30.9)a
80		14.9(0.40)a	2.04(0.10)a	10.5(0.59)a	22.2(0.77)a	10.7(0.66)a	279(31.3)a	534(45.7)a	226(32.3)a
CaCO ₃ application									
Without		13.9(0.29)a	1.96(0.08)a	8.59(0.30)b	22.2(0.74)a	11.7(0.37)a	273(21.0)a	637(43.1)a	228(25.9)a
With		13.5(0.40)a	1.74(0.07)b	13.3(0.58)a	21.9(0.76)a	8.62(0.31)b	238(16.1)a	336(24.1)b	172(21.1)b
NI application									
Without		13.6(0.38)a	1.74(0.07)b	11.3(0.49)a	22.7(0.83)a	9.62(0.39)b	249(21.3)a	492(46.5)a	184(23.5)a
With		13.8(0.33)a	1.96(0.08)a	10.6(0.65)a	21.4(0.65)a	10.7(0.40)a	262(16.1)a	480(36.1)a	215(24.1)a
Variable	df	Probability of $F < 0.05$							
N concentration (mg L ⁻¹)	2	<0.0001	0.007	n.s	n.s	n.s	n.s	n.s	n.s
CaCO ₃ application	1	n.s	0.013	<0.0001	n.s	<0.0001	n.s	<0.0001	0.045
NI application	1	n.s	0.015	n.s	n.s	0.011	n.s	n.s	n.s
Block	7	<0.0001	0.0003	n.s	n.s	n.s	n.s	n.s	0.013
N × CaCO ₃	2	n.s	0.020	n.s	n.s	0.0002	n.s	0.005	n.s
N × NI	2	0.028	0.047	n.s	n.s	0.003	n.s	0.0005	0.0009
NI × CaCO ₃	1	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
N × CaCO ₃ × NI	2	n.s	n.s	n.s	n.s	n.s	n.s	0.027	n.s
Treatment		Autumn							
N concentration (mg L ⁻¹)									
20		13.9(0.51)b	1.47(0.09)a	9.30(0.65)a	18.6(0.63)a	7.68(0.38)b	181(10.8)b	460(28.6)a	269(30.2)a
40		15.3(0.47)ab	1.58(0.07)a	8.97(0.64)a	17.8(0.66)a	8.22(0.53)ab	186(12.4)ab	467(33.8)a	221(39.2)a
80		16.3(0.52)a	1.62(0.08)a	10.2(0.92)a	20.0(0.89)a	9.33(0.68)a	224(16.3)a	487(35.5)a	293(35.5)a
CaCO ₃ application									
Without		14.7(0.39)a	1.54(0.06)a	8.25(0.47)b	17.9(0.51)b	9.98(0.43)a	193(10.2)a	508(28.1)a	227(30.4)b
With		15.6(0.46)a	1.57(0.08)a	10.7(0.69)a	19.7(0.68)a	6.84(0.37)b	201(12.2)a	435(23.2)b	295(26.4)a
NI application									
Without		15.1(0.45)a	1.45(0.06)b	8.45(0.56)b	18.2(0.54)a	8.09(0.51)a	192(11.1)a	473(22.6)a	270(37.7)a
With		15.2(0.37)a	1.67(0.07)a	10.5(0.69)a	19.4(0.68)a	8.73(0.44)a	201(12.1)a	473(31.1)a	252(17.1)a
Variable	df	Probability of $F < 0.05$							
N concentration (mg L ⁻¹)	2	0.002	n.s	n.s	n.s	0.028	0.027	n.s	n.s
CaCO ₃ application	1	n.s	n.s	0.001	0.039	<0.0001	n.s	0.010	0.024
NI application	1	n.s	0.016	0.007	n.s	n.s	n.s	n.s	n.s
Block	7	n.s	0.006	0.007	n.s	n.s	0.040	0.003	n.s
N × CaCO ₃	2	n.s	n.s	n.s	n.s	0.049	0.004	n.s	n.s
N × NI	2	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
NI × CaCO ₃	1	n.s	n.s	0.011	n.s	n.s	n.s	0.005	n.s
N × CaCO ₃ × NI	2	n.s	n.s	0.028	n.s	n.s	n.s	0.007	n.s

Means within the columns for each parameter that are followed by the same letter are not significantly different, according to Tukey's honestly significant difference (HSD) test at 5% probability level for the N treatments and Student's *t*-test at 5% probability level for the CaCO₃ and NI treatments. $n=8$. The numbers in parentheses represent SE. The ANOVA statistical analysis and the HSD and Student's *t*-tests were performed with JMP® Pro statistical software, version 15.1.0 (SAS Institute Inc., Cary, NC, USA)

In this work we showed that there are interactions between the effects of the main treatments on biomass production of plants. An increase in the level of N with or without NI application led to inhibition of biomass production of plants grown in a substrate without CaCO_3 . In addition, an increase in the level of N with NI application led to inhibition of biomass production of plants grown in a substrate with CaCO_3 . However, an opposite effect of the levels of N on biomass production of plants was observed in the treatment without NI in a substrate with CaCO_3 . Although blueberry plants respond positively to N-NH_4 supply (Korcak 1988; Merhaut and Darnell 1996; Alt et al. 2017; Hirzel et al. 2024), this reduction is probably not due to over dose of total N (the positive effect of N level in the CaCO_3 without NI), but a results of high level of NH_4 . Many works showed that an a high ratio of N-NH_4 /Total N have negative effect on the yield, biomass production and root development in many crops: strawberry (Ganmore-Neumann and Kafkafi 1983), pepper (Bar-Tal et al. 2001), medical cannabis (Saloner and Bernstein 2022), wheat (Wang et al. 2022) and other agricultural crops (Britto and Kronzucker 2002). The negative effect of high concentration of NH_4 is probably due to the following mechanisms: 1. suppressed uptake of cationic nutrients, 2. elevated supply of carbohydrate to the root in competition with its utilization for the development of the plant and 3. Lost energy by release it out from the root (Britto and Kronzucker 2013). The lowest biomass production which was observed in the CaCO_3 + NI treatment is probably a result of the combination of high NH_4 level with relative high pH. As shown in many works, the blueberry's requirement for an acidic pH is necessary for the proper development of the plant. Similar to the buffered effect of CaCO_3 on pH decreased, the application of NI in combination with NH_4 in pH-buffered substrate reduced the acidification potential caused by NH_4 . To the best of our knowledge, this is the first time that a negative effect of a high concentration of NH_4 on blueberry development has been shown. Therefore, more comprehensive work on this subject should be carried out in the future.

The negative linear relationship between the pH of the leachate and relative biomass production above $\text{pH}=6$ is very similar to that presented by Tamir et al. (2021b) for different blueberry cultivars and species (rabbiteye and southern highbush) cultivated using different growth methods. The relationship between the biomass production and the pH of the leachate obtained in this work and by Tamir et al. (2021b) suggest that the optimal pH value for blueberry plants is lower than 5.5–6 and it is a dominant environmental factor for blueberry plant performance. However, the optimal pH value can vary in other cultivars and by environmental conditions.

The effects of NI and N level on biomass production are in contradiction to their effects on chlorophyll concentrations. The combination of higher biomass with low chlorophyll

concentration in the optimal NI and N level treatments can be a result of the "dilution effect," as has been suggested by Scagel (2003) and Turner et al. (2020) for other calcifuge plants and by Tamir et al. (2023) for blueberry plants.

The effects of the treatments on the concentrations of the nutrient elements in the leaves and in the branches are expected to be closely associated to their effects on the concentrations of the nutrient elements in the leachate. As expected, elevating the N level was accompanied by an increase in N concentrations in the leaves and branches, in agreement with previous publication on blueberry plants (Hirzel et al. 2024). On the other hand, the concentrations of Fe, Mn and Zn in the leaves were not affected consistently by the N level, in contrast to the effect of the N level on their concentrations in the leachate. The addition of NI had almost no effect on the concentrations of the nutrient elements in leaves and branches. This results are in agreement with Dreyer et al. (2020), who suggested that acidification by nitrification was more effective for Mn uptake than acidification by the roots due to NI application in calcareous soils. Although, the concentrations of all of the nutrient elements in the leaves throughout the experiment did not reveal any nutrient element deficiencies in blueberry plants, as defined by Retamales and Hancock (2012). The results presented in this study are in agreement with results presented by Messiga et al. (2021), who showed that the application of NI did not improve the yield of blueberry plants and even led to decreased yields under acidic conditions. In addition, Osorio et al. (2020) showed that the addition of NI improved the chlorophyll concentration in blueberry leaves, but did not lead to any increase in biomass or in the leaf concentrations of micronutrients or N.

In light of the fact that no deficiency values of the micronutrients and P were observed in the leaves, including under neutral and slight alkaline pH values, alongside an inhibition in the biomass production, our hypothesis is that there is another mechanism/s that causes damage to blueberry development in such pH. In our previous work (Tamir et al. 2021b, 2021a; Tamir et al. 2022) we suggested that elevating the pH above a threshold of 5.5–6.0 caused directly damage to the development of the roots, independently of the uptake of nutrient elements. This damage to the roots leads to retardation of the development and the biomass production of the entire plant.

5 Conclusions

Application of high levels of ammonium enhances the growth of blueberries in high pH-buffered substrates due to intensive nitrification processes. Application of nitrification inhibitor reduces the potential of contamination of aquifers by nitrate in such substrates, but it leads to a

decrease of biomass production, apparently as a result of slowing down the nitrification processes.

Application of high levels of ammonium inhibited the growth of blueberries in low pH-buffered substrates. In such substrates application of nitrification inhibitor is not required for protection of the environment, because the nitrification processes slows down under acidic pH conditions. The mechanism which leads negative effect of high levels of ammonium with or without nitrification inhibitor on biomass production in low pH-buffered substrates is not clear enough.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42729-025-02341-y>.

Acknowledgements We thank Raneen Shawahna for her significant contribution to the atomic absorption spectrophotometry measurements and Shiran Cohen for measuring the NH_4 and NO_3 concentrations. We thank Karen Silberman and Shai Asaf for their significant contributions to the setting up of the experiment.

Author Contributions **Guy Tamir**: Conceptualization, Methodology, Formal analysis and Roles/Writing—original draft. **Eyal Ziv**: Data curation and Formal analysis. **Asher Bar-Tal**: Conceptualization, methodology, Writing—review & editing. **Nir Dai**: Conceptualization, Methodology, Resources, Writing—review & editing.

Funding Open access funding provided by The Agricultural Research Organization of Israel. This project was funded by a grant from the Chief Scientist of the Israeli Ministry of Agriculture.

Data Availability All the raw data used in this paper is available.

Declarations

Competing Interest The authors report no declarations of interest.

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