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Article *in* Journal of Soil Science and Plant Nutrition · December 2019

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ORIGINAL PAPER

Vegetative and Physiological Responses of "Emerald" Blueberry to Ammoniacal Sources with a Nitrification Inhibitor

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Received: 22 July 2019 / Accepted: 19 November 2019

Abstract
The nitrogen nutrition in blueberry has been studied by some authors; however, the effect of N-nitrate or N-ammoniacal fertilizers with nitrification inhibitors on plant growth and physiology has not been known. The aim of this investigation was to study the effectiveness and physiological implications of ammoniacal fertilizers, with or without nitrification inhibitors in blueberry. An experiment was conducted on 1-year "Emerald" blueberries grown in 20-L plastic pots. Our data indicate that ammonium-containing fertilizers promote vegetative growth and increase the leaf nitrogen concentration and gas exchange in plants, possibly due to higher nitrogen root absorption compared with nitrate. On the other hand, fertilization with ammonium with a nitrification inhibitor increases the leaf chlorophyll concentration compared with the addition of ammonium without a nitrification inhibitor and nitrate. However, the ammonium supply decreases the concentration of calcium and potassium in leaves. Our data suggest, for the first time, that fertilization with ammonium accompanied by a nitrification inhibitor is an effective strategy to improve the nitrogen status and promote plant development in "Emerald" blueberry.

Keywords Nitrate · Ammonium · DMPP · Photosynthesis · Chlorophyll · *Vaccinium* spp.

1 Introduction

In recent years, the cultivation of blueberry (*Vaccinium* spp.) has increased significantly due to the high consumer demand for this berry worldwide. Among other properties, blueberry is characterized by a high antioxidant compound concentration in its skin, which significantly contributes to the prevention of several human diseases (Michel et al. 2019). Blueberries grow in soils with a high organic matter concentration and acidic pH (< 5.5) (Alt et al. 2017; Vargas and Bryla 2015) and are frequently cultivated on raised beds mulched with sawdust (Ehret et al. 2014). In addition, blueberry is a small shrub and produces low-weight fruits compared with other fruit crops, which make it a crop characterized by lower nutritional requirements (Bryla et al. 2012; Vargas and Bryla 2015). However, of all the essential mineral nutrients for plants, nitrogen (N) is the one most absorbed by this species. In fact, the N extracted from a blueberry orchard is about 1 to 2 kg per ton of fruit removed, which justifies the need to employ fertilization management in several farming systems (Retamales and Hancock 2012).

Nitrogen significantly influences plant development like no other mineral nutrient, since it plays a crucial role as a structural component of several fundamental molecules like amino acids, proteins, enzymes, plant energy systems (e.g., ATP), amides, peptides, hormones as well as secondary metabolites (Barker and Bryson 2007; Leghari et al. 2016). In berry crops, N status strongly affects orchard longevity and productivity, root and shoot growth rate, and berry quality.

In most agricultural soils, nitrate (NO_3^-) and ammonium (NH_4^+) are the most common N sources available for plants. Despite the first mineral N source in the soil derived from organic matter and atmosphere being NH_4^+, NO_3^- is frequently the primary N source present in the soil (Heil et al. 2016). Indeed, in Mediterranean environments, the average NH_4^+ concentration is frequently 10–1000 times lower than NO_3^-, rarely exceeding 50 μM. This occurs because NH_4^+, once in the soil, is rapidly oxidized to nitrite (NO_2^-) by two groups of microorganisms: ammonia and nitrite-oxidizing bacteria. The first group (e.g. *Nitrosomonas* spp. and *Nitrosococcus* spp.) initiates the nitrification process by oxidizing ammonia (NH_3) to hydroxylamine (NH_2OH), which is catalyzed by the NH_3

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Published online: 06 December 2019

monooxygenase enzyme, and then oxidizes NH_2OH to nitrite (NO_2^-) via the hydroxylamine oxidoreductase enzyme (Coskun et al. 2017). The second group (e.g. *Nitrobacter* spp.) completes the process by producing NO_3^- via the NO_2^- oxidoreductase enzyme (Coskun et al. 2017; Hayatsu et al. 2008). More recently, however, it has been observed that other microorganisms like the "commamox" bacteria of the genus *Nitrospira* are also able to perform both oxidative steps (Coskun et al. 2017). In addition, some factors, as the pH of the soil, strongly determine the availability of NO_3^- and NH_4^+ for plants. On one hand, NO_3^- is highly available in both acidic and alkaline soil conditions (Crisóstomo et al. 2014; Darnell et al. 2015). Otherwise, NH_4^+ is mostly available at acid soil pH, because as the pH increases, it becomes more prone to volatilization and nitrification processes, which are closely determined by the activity of bacteria sensitive to temperature, O_2 availability, and soil acidity (Miller and Hawkins 2007).

As for blueberry, some patterns have been observed related to N absorption by roots. In this context, some *Vaccinium* spp. commonly used for commercial purposes, such as highbush blueberries (*Vaccinium corymbosum* L.), tend to prefer NH_4^+ rather than NO_3^-. This has been attributed to the higher NH_4^+ availability in soils with an acidic pH, which are widespread in those areas where the origin of this group of genotypes has been described (Alt et al. 2017; Leitzke et al. 2015; Machado et al. 2014). Some authors have tried to explain the effect of different N sources on vegetative and physiological variables in blueberry; however, there is still no clear agreement between the conclusions resulting from each experiment. In this sense, some investigations indicate that blueberry reacts better to N-NH_4^+ as the main N source in the soil or in hydroponic solutions (Claussen and Lenz 1999). By contrast, other authors have reported that bud and root growth as well as photosynthetic activity are positively influenced by the application of NO_3^- compared with NH_4^+ (Crisóstomo et al. 2014; Merhaut and Darnell 1996). The edaphoclimatic condition of the genotype origin area may influence its response to the N source. For example, *Vaccinium arboreum*, a species which evolves in a soil pH close to 6.5, has a higher dry matter accumulation when fertilized with NO_3^- source compared with NH_4^+. Moreover, *Vaccinium corymbosum* L., predominantly adapted to more acid soil pH, showed a better behavior under the presence of NH_4^+ in the soil (Darnell and Cruz-Huerta 2011; Poonnachit and Darnell 2004). However, scientific information related to the effect of different N sources on physiological variables of blueberry such as photosynthesis, the mineral status of plants, or in the leaf chlorophyll concentration is very scarce in the available literature.

Although a significant fraction of plants properly absorb and metabolize NO_3^-, it is possible to highlight several NH_4^+ advantages as a N source for plant nutrition: (1) due to the NH_4^+ chemical properties (a positively polyatomic ion), it is more stable and less susceptible to leaching into the soil compared with NO_3^-. In fact, in a permanent-charge paddy soil, ^{15}N-labeled NH_4^+ sulfate was prone to leach nine times less than NO_3^-, being 8.2% and 78% of added ^{15}N, respectively (Zheng-Qin et al. 2010); (2) NH_4^+ absorption facilitates the uptake of other minerals by roots (Fe, Zn, Cu), since NH_4^+ uptake induces an acidification in the rhizosphere due to the proton excretion via the H^+-ATPase, favoring the reduction of Fe^{+3}, Zn^{+3}, and Mn^{+4} to more soluble forms (Marschner 1995); (3) NH_4^+ requires less energy to be metabolized at cellular level, since the energy cost to reduce NO_3^- to NH_4^+ inside the plant cell consumes about 12–26% of photosynthetically generated reductants (Loulakakis et al. 2009). Some studies conducted under field and hydroponic conditions in fruit species like avocado (Granja and Covarrubias 2018) and grapevine (Molina and Covarrubias 2019), respectively, have demonstrated some benefits of N-NH_4^+ nutrition compared with N-NO_3^- in vegetative and physiological variables such as shoot growth, leaf chlorophyll concentration, nutritional status, and leaf gas exchange. However, in order to improve the effectiveness of the positive effects induced by NH_4^+ in some plants, it may be important to employ strategies focused on maintaining the NH_4^+ concentration in the soil at medium-to-low levels by slowing down the oxidation of NH_4^+. In such a context, the use of nitrification inhibitors applied to the soil has recently become the focus of intensive research initiatives (Coskun et al. 2017). Some of these inhibitors, including nitrapyrin, dicyandiamide, 2-amino-4-chloro-6-methylpyrimidine, and the highly specific 3,4-dimethylpyrazole phosphate (DMPP), have been used to suppress NH_4^+ nitrification. In an experiment conducted on strawberry, NH_4^+ with DMPP applications increased fruit size, ascorbic acid concentration, and leaf chlorophyll content compared with plants treated with NH_4NO_3 (Martínez et al. 2015). In citrus trees, Martínez-Alcántara et al. (2013) reported that the addition of N-NH_4^+ with 1% DMPP increased the fertilizer-N uptake, plant biomass, and fruit yield associated with a reduced N loss by leaching as a result of the reduced NH_4^+ nitrification rate. Another experiment conducted on grapevines cultivated in a calcareous soil showed that the application of N-NH_4^+ + DMPP increased leaf chlorophyll content and leaf stomata length compared with plants treated with N-NO_3^-, suggesting that this treatment is effective in preventing Fe chlorosis in grapevines located in calcareous soils (Covarrubias et al. 2014).

The present study is focused on determining the effect of different N sources, with and without a nitrification inhibitor, on physiological and vegetative variables of "Emerald" blueberry, an interspecific origin based largely on *Vaccinium corymbosum* L. with some genes from *Vaccinium darrowi* Camp.

2 Materials and Methods

2.1 Plant Material, Experimental Conditions, and Treatments

The experiment was conducted from May 2017 to April 2018 (2017–2018 season) at the Experimental Station of the Facultad de Ciencias Agronómicas, Universidad de Chile (Santiago, Chile). In autumn 2017, 1-year micropropagated highbush blueberries cv. Emerald were acclimated under shady nets for 3 weeks and transferred to 20-L plastic pots (one plant per pot) filled with a substrate composed of soil collected from the experimental station (33% v/v), sand (33% v/v), and vermiculite (33% v/v). The pots were covered with light reflecting aluminum foil to keep the soil temperature below 30 °C, and were placed under a structure covered with a black shade net (50% of shading) at a distance of 0.75 × 1 m. The trial was provided with a drip irrigation system with 2 L h^{-1} on-line microdrip emitters (one per plant). In spring 2017, the blueberries were pruned to two 10–20 cm long shoots per plant. During the experiment, flowers and fruits were removed from plants in order to reduce variability in the experiment.

The treatments tested were (i) plants fertilized with $Ca(NO_3)_2$ (control); (ii) plants fertilized with $(NH_4)_2SO_4$; (iii) plants fertilized with NH_4NO_3; (iv) plants fertilized with $(NH_4)_2SO_4$ + the nitrification inhibitor DMPP; and (v) plants fertilized with NH_4NO_3 + the nitrification inhibitor DMPP. The fertilizers were applied to the soil. Nitrate nitrogen-fertilized plants (control) received 1000 mL of $Ca(NO_3)_2$ solution (0.25 g N L^{-1}) every week from the onset of shoot expansion, once all the plants had at least 3 fully expanded leaves, until the end of the season (autumn colors). During the season, plants had received 6 g N. The same amount of N supplied in the plants treated with $Ca(NO_3)_2$ was also applied to plants treated with $(NH_4)_2SO_4$ or NH_4NO_3 through the application of 1000 mL solutions (0.25 g N L^{-1}) every week. In treatments iv and v, nitrification inhibition was maintained during the experiment with DMPP at doses of 1% of the N amount supplied.

Each pot for all treatments was irrigated daily with one 2 L h^{-1} out-line microdrip emitter maintaining a constant level of soil moisture close to field capacity (40% saturation). The irrigation frequency and the amount of water to replace to each pot were determined by mass balance, using the variation in the pot weight as a reference. Weeds were manually removed, and pest and disease protection was regularly carried out. Moreover, to provide the demand of blueberries for other essential minerals, an additional supply of the following solutions (1000 mL) was occasionally added to the pots: 2.5 mM K_2SO_4; 1 mM $MgSO_4 \times 7H_2O$; 1 mM KH_2PO_4; 4.60 μM $MnCl_2 \times 4H_2O$; 23.2 μM H_3BO_3; 0.06 μM Na_2MoO_4; 0.40 μM $ZnSO_4 \times 7H_2O$; 0.19 μM $CuSO_4$, and 50 μM de Fe-EDDHA.

A Latin square design (5 × 5) was used to take into account the sun exposure and the drip line as possible independent sources of variance, so each treatment was replicated five times. The experimental plot for each treatment was composed of one plant.

2.2 Leaf Chlorophyll Concentration and Plant Growth

Leaf chlorophyll concentration was measured every 14 days during the season on four points of the first completely expanded leaf of 2 shoots per plant using a SPAD meter (SPAD Minolta 502, Konica Minolta, Osaka, Japan). The SPAD value was previously calibrated with leaf chlorophyll concentration in 15 leaves with different degrees of chlorosis according to Wellburn (1994).

During the season, the length of the same shoots selected for the chlorophyll determinations was determined every 14 days. During the spring flush growth, the length of shoots in each experimental unit was determined, whereas during the summer flush growth, the length of all the lateral shoot branches from the spring shoots was measured. At the end of the experiment (April 2018), plants were divided into roots, shoots, and leaves for dry mass determinations.

2.3 Leaf Gas Exchange

Leaf gas exchange was measured on one plant per replicate using an infrared gas analyzer (IRGA, LCi-ADC, London, United Kingdom). During the experiment, measurements were performed on the first completely expanded mature leaf inserted in the middle third of two shoots per plant. Net photosynthesis (μmol $CO_2 m^{-2}$ s^{-1}) was measured after 40–60 s, when foliar CO_2 uptake was steady in the leaf chamber. The measurements were made by taking air from 1.5 m above the canopy. The area of the leaf chamber was 6.25 cm^2. Gas exchange measurements including CO_2 assimilation rate and stomatal conductance (g_s) were taken at 9:00–10:30 a.m., 11:30 a.m. − 12:30 p.m., 1:30–2:30 p.m., and 3:30–4:30 p.m. during the spring (at 66 days after full bloom; DAFB) and during the summer (at 153 DAFB).

2.4 Leaf Mineral Concentrations

In January 2018, at 95 days of treatment, samples of 8 healthy and fully expanded leaves were collected from each experimental unit. The leaves were collected from the middle third of shoots developed during the season. The samples were oven-dried at 75 °C, ground to pass a 40-mesh screen, and 200 mg of each sample were subjected to acid digestion with nitric acid 8 mL and hydrogen peroxide 2 mL at 180 °C for 20 min in a microwave reaction system (Multiwave PRO, Anton Paar 3200, Austria). The concentrations of P, K, Ca, Mg, Fe, Mn, Zn, and Cu were determined by using microwave

plasma atomic emission spectroscopy (MP-AES 4200, Agilent Technologies, USA). Total leaf N concentrations were determined by using the Kjeldahl method.

2.5 Statistics

The analysis of variance (ANOVA) was performed under the framework of mixed linear models (MLM). Data related to chlorophyll concentration, shoot growth, and leaf gas exchange were analyzed, considering temporal correlations, using the "lme" function in the "nlme" package in R. In case of significant differences between treatments, the multiple comparisons DGC test ($\alpha = 0.05$) was used. The statistical software used was InfoStat v. 2013.

3 Results

3.1 Leaf Chlorophyll Concentration and Plant Growth

During the first 115 days after full bloom (DAFB), treatments did not influence the leaf chlorophyll concentration in blueberries (Fig. 1). Later, between 115 and 155 DAFB, the application of all the fertilizers containing N-NH_4 increased the leaf chlorophyll concentration compared with the control, and the most efficient was the $(NH_4)_2SO_4$ + DMPP treatment (Fig. 1). At the end of the season, the higher leaf chlorophyll concentration was registered in plants treated with NH_4^+ + DMPP and NO_3NH_4 + DMPP and the lower values were recorded in the control (Fig. 1).

The spring shoot growth flush of blueberries started in September 2017, and stopped at 99 DAFB, whereas the summer shoot growth flush lasted until 182 DAFB (Fig. 2). During the spring growth flush, significant differences in the shoot length were observed between treatments 31 and 70 DAFB, in which plants treated with NH_4^+ showed a lower shoot length compared with the other treatments (Fig. 2). Later, in the summer growth flush, data revealed that the application of all the fertilizers containing N-NH_4 increased the shoot length compared with the control, and the longest shoots were recorded in plants treated with $(NH_4)_2SO_4$ + DMPP (Fig. 2).

Data collected at the end of the experiment showed that all the fertilizers containing N-NH_4^+ induced a higher total dry mass compared with the control (Table 1). As for the leaves, the plants treated with NO_3NH_4, NH_4^+ + DMPP and NO_3NH_4 + DMPP reached higher dry mass compared with the control (Table 1). Conversely, in the case of roots, the application of NH_4^+ was more efficient at increasing the dry weight in comparison with the control, whereas the other treatments reached intermediate values (Table 1). The different N sources did not influence the shoot dry mass (Table 1).

3.2 Leaf Gas Exchange

Data related to net photosynthesis and stomatal conductance did not show any interaction between the date and time of the measurements. Therefore, the results are presented as the daily average of data collected during the spring and summer periods (Fig. 3) and also in the different hours of the day for each period (Fig. 4). At 66 and 153 DAFB, blueberries reached the highest leaf net photosynthesis (Fig. 4a, b) and stomatal conductance (Fig. 4c, d) between 9:30 and 10:30 a.m., and this variable gradually decreased until reaching the lowest values at 3:30–4:30 p.m. During the spring, data indicate that the fertilization with NH_4^+ increased the leaf net photosynthesis and stomatal conductance compared with the other treatments (Figs. 3; 4a, c). Later, during the summer, the plants treated

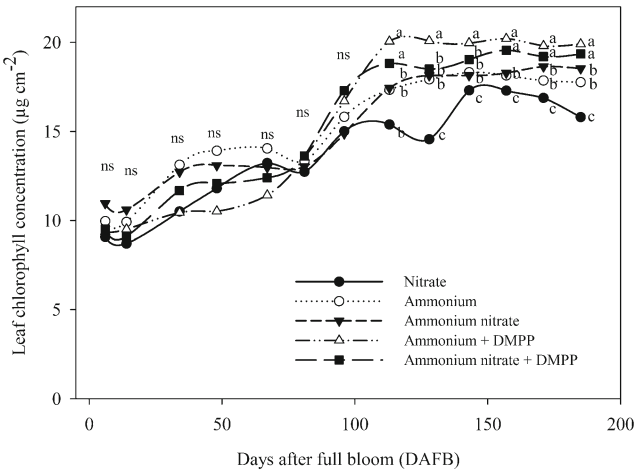

Fig. 1 Leaf chlorophyll concentration during the season of "Emerald" blueberries fertilized with different N sources. Adjusted means with different letters indicate significant differences according to the DGC test ($p < 0.05$)

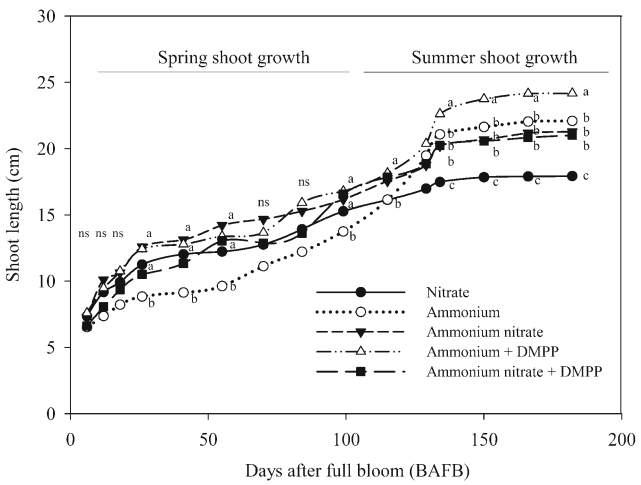

Fig. 2 Shoot length during the season of "Emerald" blueberries fertilized with different N sources. Adjusted means with different letters indicate significant differences according to the DGC test ($p < 0.05$)

Table 1 Dry mass (g) in leaves, shoots, roots, and total, determined at the end of the experiment in "Emerald" blueberries fertilized with different N sources

Treatment	Dry mass (g)			
	Leaves	Shoots	Roots	Total
NO_3^-	17.4 ± 1.7 c	21.0 ± 1.5	26.7 ± 1.8 c	61.9 ± 1.8 b
NH_4^+	24.8 ± 1.9 b	22.0 ± 0.7	41.9 ± 3.8 a	88.8 ± 2.8 a
NO_3NH_4	30.8 ± 2.6 a	24.8 ± 3.0	32.2 ± 1.9 b	88.9 ± 4.7 a
NH_4^+ + DMPP	31.9 ± 1.5 a	20.0 ± 2.4	34.3 ± 2.2 b	87.0 ± 5.6 a
NO_3NH_4 + DMPP	31.0 ± 2.1 a	22.5 ± 2.6	31.2 ± 2.0 b	85.9 ± 6.4 a
Significance	$p = 0.001$	n.s.	$p = 0.0004$	$p < 0.0001$

In each column, the adjusted mean ± standard error is presented. Adjusted means with different letters in the same column indicate significant differences between treatments, according to the DGC test ($p < 0.05$). *n.s.*, not significant

with NH_4^+ treatment showed a higher leaf net photosynthesis and stomatal conductance compared with the plants treated with NO_3NH_4, NH_4^+ + DMPP and NO_3NH_4 + DMPP, whereas the control showed intermediate scores (Figs. 3; Fig. 4b, d).

3.3 Leaf Mineral Concentrations

The different N sources applied in the experiment influenced the leaf mineral concentration in blueberries. In this sense, the application of fertilizers with N-NH_4^+ increased the N concentration compared with the control (Table 2). In particular, the applications of NO_3NH_4, NH_4^+ + DMPP and NO_3NH_4 + DMPP were more efficient at increasing the N levels in leaves compared with NH_4^+ without DMPP (Table 2).

As for P, the leaves of blueberries treated with NO_3NH_4 + DMPP showed a higher concentration compared with NO_3^- and NH_4^+ without DMPP (Table 2). The leaf K concentration was higher in plants fertilized with NO_3^- than those fertilized with NH_4^+ + DMPP (Table 2). Data related to Ca concentration in leaves revealed that the application of NO_3^- increased its concentration in comparison with the NH_4^+, NO_3NH_4, and NH_4^++DMPP treatments (Table 2). The treatments did not influence the Mg concentration in leaves (Table 2).

Regarding microelement concentrations, the application of NO_3NH_4 + DMPP and NH_4^+ increased the Fe concentration in leaves compared with NH_4^+ + DMPP (Table 3). As for Mn, NH_4^+ without DMPP and NO_3NH_4 + DMPP treatments increased its concentration in leaves compared with NO_3^- (Table 3). The treatments did not influence the leaf concentration of Zn and Cu (Table 3).

4 Discussion

The N sources assessed in this experiment influenced the shoot growth and biomass accumulation in plants during the season. In fact, at the end of the experiment, a higher shoot length and total biomass were measured in blueberries treated with N-NH_4^+ sources compared with those treated with N-NO_3^-. These results are in line with studies reported by Spiers (1978, 1979) on blueberry Rabbiteye cv. Tifblue, and by Claussen and Lenz (1999) on the genotype 13-16A, who also observed an early shoot growth detention and a premature senescence and leaf fall in blueberries fertilized with N-NO_3^- compared with NH_4^+. In addition, Darnell et al. (2015) and Poonnachit and Darnell (2004) observed in blueberries "Sharpblue" and "Misty," respectively, a higher leaf, shoots and total dry biomass in plants treated with NH_4^+ compared to those treated with NO_3^-.

Nitrogen is the most important mineral for blueberry growth, and as for all crops, a slight or severe N deficiency may induce limitations in plant growth (Alt 2015). The differences in the vegetative expression registered in our experiment might be related to the N status of blueberries, since

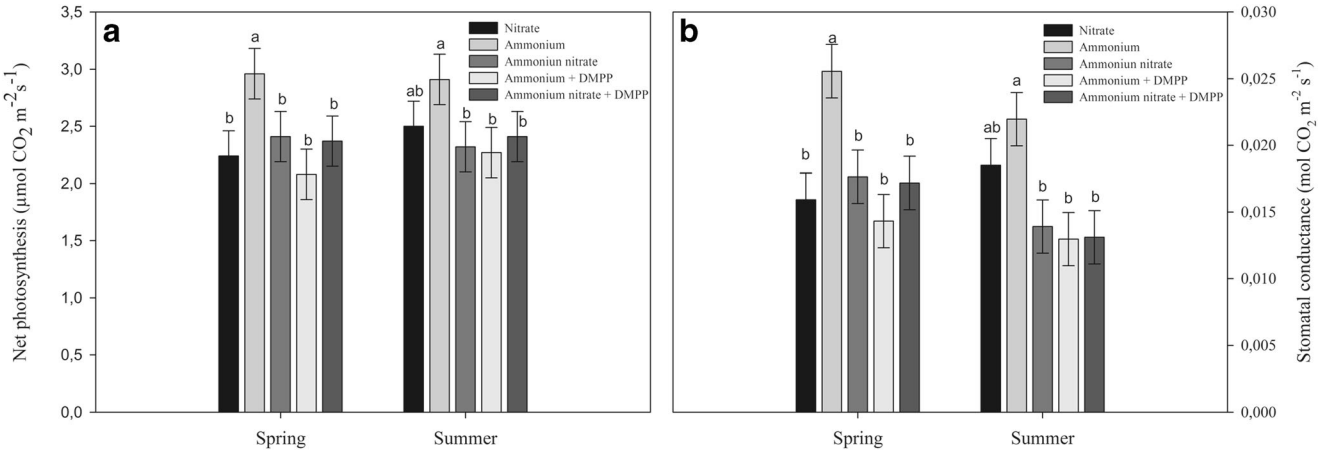

Fig. 3 Daily average of net photosynthesis (µmol CO_2 m^{-2} s^{-1}) (**a**) and stomatal conductance (mol CO_2 m^{-2} s^{-1}) (**b**) in leaves of "Emerald" blueberries fertilized with different N sources. Adjusted means with different letters indicate significant differences according to the DGC test ($p < 0.05$). Vertical bars indicate the standard error

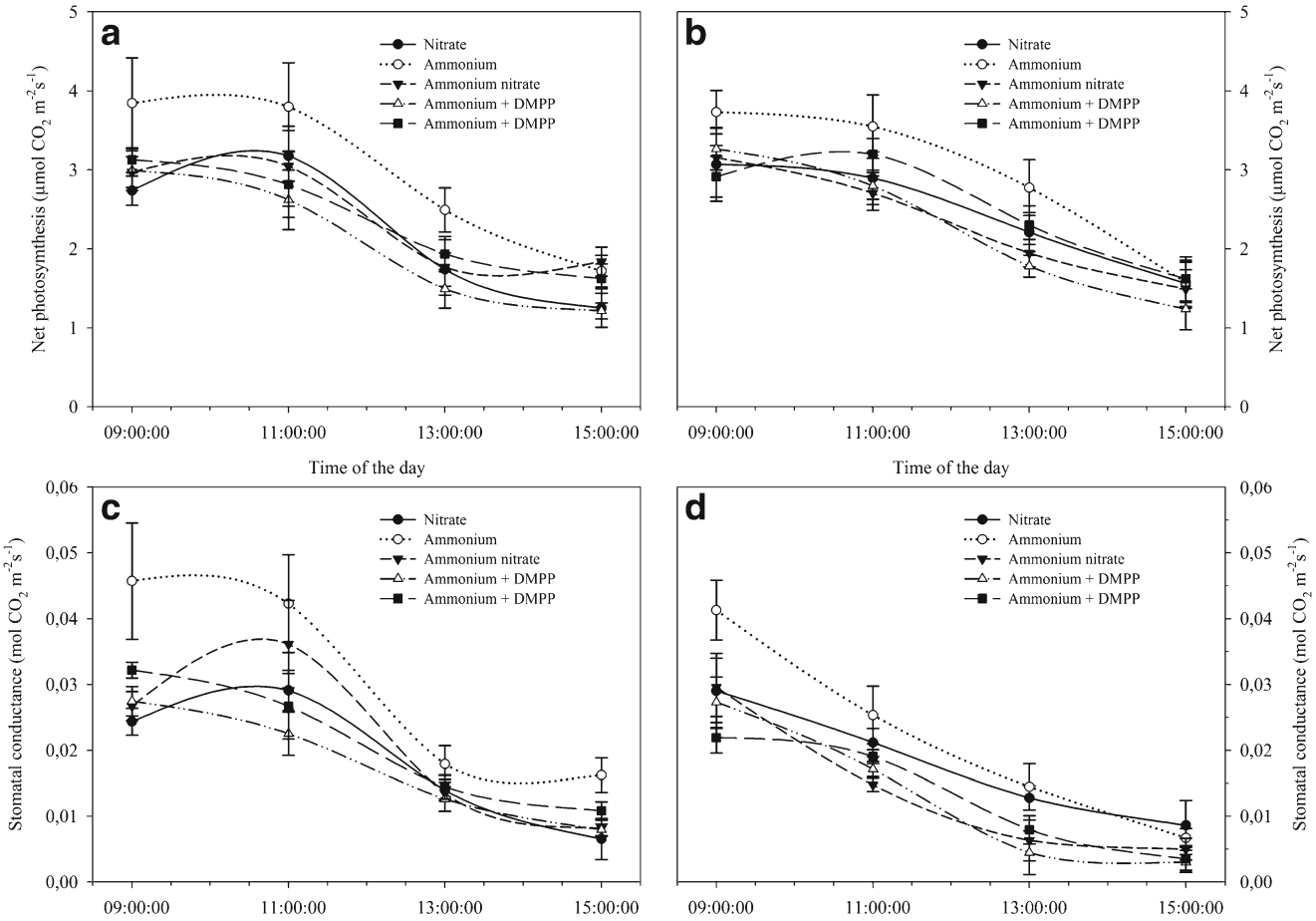

Fig. 4 Daily evolution of net photosynthesis (**a**, **b**), and stomatal conductance (**c**, **d**) in "Emerald" blueberries fertilized with different N sources, at 66 DAFB (**a**, **c**) and 153 DAFB (**b**, **d**). Adjusted means with different letters indicate significant differences according to the DGC test ($p < 0.05$). Vertical bars indicate the standard error

the plants fertilized with N-NH_4^+ showed a higher N concentration in leaves, and the plants fertilized with N-NO_3^- reached a leaf N concentration even lower than that suggested by the standard levels (Table 2). Otherwise, the higher shoot growth and biomass production induced by N-NH_4^+ may be related to the lower energy used by this N source to be metabolized within the plant cell since NH_4^+ is directly incorporated into amino acids, whereas NO_3^- must first be reduced to NH_4^+ by the activity of nitrate reductase (NR) and nitrite reductase (NiR) enzymes, whose activity requires energy (Hanson 2006; Martínez et al. 2017). In addition, Darnell et al. (2015) reported a low capacity of southern highbush blueberry to catalyze the reduction of NO_3^- to NO_2^-, and then to NH_4^+ due to a reduced activity of NR and NiR enzymes in their tissues. In

Table 2 Leaf concentration (%) of macroelements (N, P, K, Ca, Mg) in "Emerald" fertilized with different N sources. Sampling occurred in January 2018

Treatment	Leaf concentration (%) of macroelements				
	N	P	K	Ca	Mg
NO_3^-	1.19 ± 0.07 c	0.06 ± 0.01 b	0.85 ± 0.04 a	0.90 ± 0.11 a	0.16 ± 0.02
NH_4^+	1.72 ± 0.04 b	0.08 ± 0.01 b	0.71 ± 0.06 ab	0.66 ± 0.10 b	0.13 ± 0.02
NO_3NH_4	1.96 ± 0.07 a	0.09 ± 0.01 ab	0.70 ± 0.09 ab	0.60 ± 0.08 b	0.13 ± 0.02
NH_4^+ + DMPP	2.01 ± 0.03 a	0.10 ± 0.02 ab	0.56 ± 0.05 b	0.61 ± 0.10 b	0.13 ± 0.02
NO_3NH_4 + DMPP	1.93 ± 0.04 a	0.11 ± 0.01 a	0.67 ± 0.09 ab	0.71 ± 0.10 ab	0.13 ± 0.01
Significance	$p = 0.0009$	$p = 0.0275$	$p = 0.0054$	$p = 0.0231$	n.s.

In each column, the adjusted mean ± standard error is presented. Adjusted means with different letters in the same column indicate significant differences between treatments according to the DGC test ($p < 0.05$). *n.s.*, not significant

Table 3 Leaf concentration (mg kg^{-1}) of microelements (Zn, Cu, Fe, Mn) in "Emerald" fertilized with different N sources. Sampling occurred in January 2018

Treatment	Leaf concentration (mg kg^{-1}) of microelements			
	Zn	Cu	Fe	Mn
NO_3^-	7.9 ± 4.2	2.1 ± 0.6	64.3 ± 3.9 ab	14.8 ± 5.4 b
NH_4^+	6.1 ± 1.9	3.3 ± 1.6	73.1 ± 4.5 a	44.1 ± 9.7 a
NO_3NH_4	8.4 ± 1.4	3.1 ± 1.1	61.7 ± 4.1 ab	26.0 ± 5.4 ab
NH_4^+ + DMPP	5.6 ± 1.3	1.9 ± 0.3	56.9 ± 4.1 b	26.4 ± 7.2 ab
NO_3NH_4 + DMPP	7.7 ± 1.2	2.5 ± 0.4	71.8 ± 3.4 a	35.7 ± 5.6 a
Significance	n.s.	n.s.	$p = 0.0457$	$p = 0.0033$

In each column, the adjusted mean ± standard error is presented. Adjusted means with different letters in the same column indicate significant differences between treatments according to the DGC test ($p < 0.05$). *n.s.*, not significant

fact, in 13-16A blueberries treated with N-NO_3^-, a lower NR enzyme activity was measured in shoots and roots in comparison with plants treated with N-NH_4^+ (Claussen and Lenz 1999). Moreover, it has been reported that this effect could be a consequence of a lower induction and expression of the genes that encode such proteins (Poonnachit and Darnell 2004). It is possible that this behavior has been determined by the edaphoclimatic conditions in which each blueberry genotype originated, according to the availability of N sources, which is heavily dependent on the soil pH. Therefore, the lower growth rate registered in "Emerald" blueberries fertilized with NO_3^- may be associated with a lower N status, apparently related to a limited capacity of blueberry to uptake and metabolize NO_3^- sources.

Interestingly, the plants fertilized with NO_3NH_4 and NO_3NH_4 + DMPP exhibited a behavior similar to those treated with NH_4^+ + DMPP in the growth variables assessed, despite containing NO_3^- in their formulation. These results are consistent with those reported in blueberries "Tiflblue" by Tamada (2004), who did not observe any differences in plant growth between blueberries fertilized with NH_4^+ or NO_3NH_4. In this sense, it has been suggested that fertilizations with NO_3NH_4 may be positive in species with a poor ability to metabolize NO_3^-, since the presence of NO_3NH_4 induce signals that would promote the synthesis and expression of genes that encode enzymes involved in their assimilation pathway, allowing the plant to grow optimally in the simultaneous presence of both N sources (Yang et al. 2017). On the other hand, the N dose applied to plants (6 g N during the season), which could be considered high, possibly provided an important N concentration as NH_4^+ in the substrate of plants treated with NO_3NH_4 during the whole experiment. Thus, the N taken up by such plants may have been predominantly NH_4^+. This could contribute to explain why these plants did not differ from plants fertilized with NH_4^+ alone.

The treatments assessed influenced the leaf chlorophyll concentration from 115 DAFB (Fig. 1). From this stage, it was clear that NH_4^+, with and without DMPP, significantly increased leaf chlorophyll concentration, showing differences of up 4 μg cm^{-2} compared with the control (Fig. 1). Similar results have been reported in other fruit crops such as mandarin (Bondada and Syvertsen 2003), walnut (Liu et al. 2010), peach (Lorén 2013), and olive (Roca et al. 2018), and may be related to the lower N status registered in plants treated with NO_3^-, since the association between the N and chlorophyll concentration in leaves is well known. Moreover, the addition of NH_4^+ with DMPP proved to be the most efficient strategy to increase the leaf chlorophyll concentration in blueberries. A similar trend has also been reported for avocado and strawberry by Granja and Covarrubias (2018) and Martínez et al. (2015), respectively. Thus, DMPP probably induced a higher NH_4^+ stability in the soil, allowing it to remain unchanged for a longer period and thus to be easily absorbed by the plants.

The behavior of the leaf gas exchange during the day exhibited a similar trend, regardless of the N source applied (Fig. 4). Thus, in both spring and summer, there was a marked decrease in net photosynthesis and stomatal conductance as the time of the day progressed, possibly associated with the progressive increase in leaf temperature. Therefore, it is possible that the lower photosynthesis rate registered between 1:00 and 3:00 pm in all treatments is directly linked to a limitation in the gas exchange between the atmosphere and the leaf mesophyll due to a stomatal closure. Regarding the daily average net photosynthesis and stomatal conductance, it should be highlighted that although there were significant differences between treatments, the magnitudes of such differences were not particularly high (Fig. 3). In this sense, as evidenced by Jorquera-Fontena et al. (2016) in Brigitta blueberries, it is possible that the elimination of strong sink organs such as flowers and/or berries in our experiment induced a limitation in the maximum expression of photosynthesis. However, studies conducted by Fan et al. (2010) and Nebauer et al. (2011) on apple and citrus, respectively, reported that the roots of young plants can exert a demand for sugars similar to fruits. They also indicate that such behavior could be intensified in species like blueberry that present a continuous growth of roots during the season (Spiers 1995). Therefore, the higher photosynthetic activity recorded in plants fertilized with NH_4^+ in spring and summer (Fig. 3) could be associated with the higher root growth, which promoted a higher carbon compound transport from leaves to roots (Table 1). In addition, the higher root growth shown by plants treated with NH_4^+ (Table 1) may be interpreted as a response mechanism to a lower N status compared with the other NH_4^+ treatments (Table 2). However, multi-season experiments conducted at the field level are required to better understand the effects of the nitrogen source in leaf gas

exchange at different times of the day and moments within the season.

The fertilization with NH_4^+ sources decreased the leaf Ca and K concentrations (Table 2). This effect may be related to the ionic antagonism between these minerals and NH_4^+ in the exchange sites of the soil and the cell wall pores in roots (Crisóstomo et al. 2014; Nieves-Cordones et al. 2014; Szczerba et al. 2008). In addition, reductions in the activity of high-affinity K^+ transporters in the root plasma membrane induced by the presence of NH_4^+ have been also reported (Szczerba et al. 2008). On the other hand, it is important to note that the addition of $Ca(NO_3)_2$ probably increased the leaf Ca concentration in the control. This evidence suggests that should a fertilization program based on NH_4^+ be adopted in blueberry orchards, it would be necessary to consider supplementary fertilization with K and Ca salts. As for P, the addition of N-NH_4^+ increased its concentration in leaves (Table 2). This synergic effect between P and NH_4^+ may be associated with the acidification in the growth media promoted by NH_4^+, since the absorption of such cations releases protons (H^+) into the rhizosphere, increasing the P availability for plant uptake (Jing et al. 2010). Furthermore, our results did not evidence a clear trend related to Fe status in blueberries, and no differences were registered in Zn and Cu leaf concentration between treatments (Table 3). Such results are similar to those reported by Rosen et al. (1990) and could be related to the low concentrations at which these micronutrients are required by plants. Perhaps long-term experiments are needed to achieve effects of N sources on these minerals.

5 Conclusions

The results obtained indicate that fertilization with NH_4NO_3 or NH_4^+, with or without a nitrification inhibitor, promotes vegetative growth and increases the leaf N concentration in blueberries, possibly due to higher N root absorption compared with NO_3^-. On the other hand, the addition of these N sources accompanied by a nitrification inhibitor increases the leaf chlorophyll concentration, whereas the fertilization with NH_4^+ increases the leaf gas exchange compared with the addition of NO_3^-. However, the fertilization with NH_4^+ decreases the K and Ca concentration in leaves in comparison with the control. Our data suggest, for the first time, that the fertilization with NH_4^+ sources accompanied by a nitrification inhibitor, is an effective strategy to improve the N status and promote blueberry development.

Funding Information This study was funded by the Graduate School of the Facultad de Ciencias Agronómicas, Universidad de Chile; the Comisión Nacional de Investigación Científica y Tecnológica (CONICYT) of Chile (FONDEQUIP project "Aplicación de la analítica de minerals mediante un Sistema de espectrofotometría de emisión atómica (MP-AES) en estudios de rehabilitación ambiental y producción sostenible de alimentos funcionales" no. EQM140007); and the Regional Government of O'Higgins (project "Innovación y optimización del riego en arándanos" no. FIC 30474716-0).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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