Ammonium Uptake Is the Main Driver of Rhizosphere pH in Southern Highbush Blueberry

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Abstract. Unlike most horticultural crops, blueberry (*Vaccinium* spp. section cyanococcus) prefers low-pH (4.2–5.5) soils. Other plants can acidify their rhizosphere to create a hospitable microenvironment. Southern highbush blueberry (SHB; *Vaccinium corymbosum* interspecific hybrids) plants do not acidify their rhizosphere in response to Fe deficiency, but other factors that affect rhizosphere pH have not been elucidated. We report results from two hydroponic experiments exploring N uptake effects on the rhizosphere pH of 'Emerald' SHB. Ammonium (NH_4^+) uptake led to rhizosphere acidification, whereas nitrate (NO_3^-) uptake led to rhizosphere alkalization. When grown in a split-root hydroponic system, roots that took up NH_4^+ acidified the rhizosphere to a greater extent that roots not exposed to NH_4^+. Rhizosphere acidification was observed even in a nontreated control. These results suggest that NH_4^+ uptake is the main driver of rhizosphere pH in SHB. N form effects suggest that fertilization with NO_3^- might lead to undesirable rhizosphere alkalization.

Soil pH is a major factor mediating plant agricultural performance. Plants in family Ericaceae (i.e., acid-loving plants) thrive in acidic soils (pH 4.2–5.5) where NH_4^+ is the most abundant form of N (Marrs and Bannister, 1978). When acid-loving plants are grown in neutral or high-pH soils, they exhibit stress, nutritional deficiencies, stunted growth, and low survival (Finn et al., 1993; Marrs and Bannister, 1978; Paya-Milans et al., 2017; Tsuda et al., 2014). Acidic soils are rare in agricultural and landscape settings. Thus, growers must find naturally acidic soils or rely on costly soil amendments (Almutairi et al., 2017; Williamson et al., 2015) or soilless substrates (Kingston et al., 2017) for the production of ericaceous crops, such as blueberries (*Vaccinium* spp. section cyanococcus). Development of cultivars and practices that reduce dependence on these inputs requires a better understanding of the processes that affect rhizosphere pH.

Some herbaceous and woody plants can acidify their rhizosphere (Gogorcena et al., 2001; Hawkins et al., 2008; Lei et al., 2015; Santi and Schmidt, 2009; Suzko et al., 2018; Valentinuzzi et al., 2015). Previous research suggests that plants acidify their rhizosphere to create more hospitable microenvironments for nutrient uptake and growth (Lei et al., 2015; Suzko et al., 2018). Plants acidify their rhizosphere by using plasma membrane-bound H+-ATPases (Santi and Schmidt, 2009) and/or by exuding organic acids (Marschner et al., 1987). Cells have a basal level of H+-ATPase activity, but several stimuli—such as cation uptake—can enhance this activity (Haynes, 1990). P and Fe deficiencies also increase H+-ATPase activity in some plants, as roots acidify the rhizosphere to solubilize these nutrients (Gogorcena et al., 2001; Jimenez et al., 2007; Lei et al., 2015).

N uptake is a major factor affecting rhizosphere pH because this element can be taken up as a cation and/or an anion. NH_4^+ uptake leads to rhizosphere acidification as cells extrude H+ to maintain their electrical charge balance (Marschner et al., 1991). On the other hand, NO_3^- uptake leads to rhizosphere alkalization as the symport mechanism responsible for NO_3^- movement across the plasma membrane removes H+ from the rhizosphere (Meharg and Blatt, 1995). Thus, the N form taken up by the plant can enhance or counteract H+-ATPase-mediated rhizosphere acidification.

SHB (*Vaccinium corymbosum* interspecific hybrids) is an economically important acid-loving plant (Perez and Ferreira, 2017). SHB plants take up N preferentially in the NH_4^+ form (Merhaut and Darnell, 1995). Although SHB plants do not acidify their rhizosphere in response to Fe deficiency (Nunez et al., 2015), other factors that affect SHB rhizosphere pH are unknown. This research aims to quantify the effect of NH_4^+ uptake on SHB rhizosphere pH change. We hypothesized that NH_4^+ uptake is the main driver of rhizosphere acidification by SHB. We tested this hypothesis in two separate hydroponic experiments.

Materials and Methods

Plant material and acclimation period. One-year-old rooted cuttings of 'Emerald' SHB were washed clean of substrate using tap water. Cuttings were transplanted to a previously described hydroponic system (Poonnachit and Darnell, 2004) in which each plant was exposed to 2 L of constantly aerated nutrient solution. Plants were acclimated in a nutrient solution containing 0.5 mM KNO_3, 0.5 mM K_2PO_4, 1.0 mM $MgSO_4$, 0.5 mM $CaCl_2$, 0.045 mM H_3BO_3, 0.01 mM $MnSO_4$, 0.01 mM $ZnSO_4$ with 0.3 μM $CuSO_4$, 0.2 μM Na_2MoO_4, and 45 μM Fe provided as Sequestrene 330 (10% iron(III)-diethylenetriamine pentaacetic acid) (Becker Underwood, Inc., Ames, IA). Nutrient solution was buffered to pH 5.5 using 5.0 mM 2-(4-morpholino)-ethane sulfonic acid (MES). Nutrient solution pH was adjusted back to pH 5.5 using 1 M HCl or 1 M KOH as needed, three times each week. Fresh nutrient solution was provided every 7 d to maintain nutrient concentrations. Debris, algae, and detached roots were removed from the reservoir to minimize impact on solution pH. Acclimation lasted 21 d in both experiments. Plant fresh weight (FW) was measured nondestructively at the end of acclimation.

All experiments were carried out in a temperature-controlled greenhouse set to maintain air temperatures between 15.6 and 26.7 °C. Natural light was supplemented by metal halide lamps with a photosynthetic photon flux density output of 367 nmol·m^{-2}·s^{-1} for 14 h every day.

Expt. 1: Nitrogen form effect. After acclimation, one half of the plants were transferred to a nutrient solution in which 5 mM NO_3^- (delivered as KNO_3) was the only form of N. The other half of the plants received a nutrient solution in which 5 mM NH_4^+ [delivered as $(NH_4)_2SO_4$] was the only form of N. Additional salts were not added to compensate for K+ and SO_4^- concentrations. Considering that the magnitude of N uptake is much greater than K+ and SO_4^- uptake (Havlin et al., 2013), it is reasonable to assume that most changes in rhizosphere pH were the result of N form as opposed to these counterions. In addition, KNO_3 and $(NH_4)_2SO_4$ have been used as blueberry fertilizers in previous scientific studies with SHB (Nunez et al., 2015). Nutrient solutions were adjusted to pH 5.5 during nutrient solution preparation, but they did not contain any pH buffer. All other nutrient concentrations were the same as during the acclimation period. Nutrient solution in reservoirs with no plants was used as the control for each treatment. Nutrient solution pH, N uptake, and leaf chlorophyll content were measured periodically. The treatment period lasted 35 d.

This experiment followed a completely randomized design with one variable (N form) and two treatments (NH_4^+ and NO_3^-). Individual plants were the experimental unit. There were four single-plant replications per treatment. All data except for rhizosphere pH

were analyzed using one-way analysis of variance (ANOVA) in R version 3.5.1 (R Core Team, 2017). Rhizosphere pH data were analyzed using a linear mixed model (see section Rhizosphere pH measurement).

Expt. 2: Ammonium uptake effect. After acclimation, plants were transplanted to a split-root hydroponic system. Plants were held upright using garden stakes and Velcro straps (Supplemental Fig. 1). Root systems were separated into two parts of similar volume. Each half of the root system was placed in a different 1.5-L reservoir (termed reservoir A and reservoir B). In reservoir A, all plants were exposed to a complete, buffered nutrient solution that contained 5 mM NH_4^+. NH_4^+ concentration treatments were delivered in reservoir B. Seven plants were exposed to a nutrient solution containing 5 mM NH_4^+ in reservoir B, whereas seven different plants received a nutrient solution without N in reservoir B. NH_4^+ was delivered as $(NH_4)_2SO_4$ in all solutions. Nutrient solutions were adjusted to pH 5.5 during nutrient solution preparation. Solutions in reservoir A were buffered with 5.0 mM MES. Solutions in reservoir B were not buffered to track pH change. All other nutrient concentrations were the same as during the acclimation period. In summary, treatments were (reservoir A/reservoir B) NH_4^+/NH_4^+ and $NH_4^+/$ 0N. Reservoirs with no plants were used as controls for each treatment. Nutrient solution pH, N uptake, and leaf chlorophyll content were measured periodically. Root vitality was measured 30 d after transplant to the split-root system. The treatment period lasted 35 d. At the end of this period, plants were harvested destructively to measure plant and organ dry weight and tissue N content.

This experiment followed a completely randomized design with one variable (NH_4^+ concentration in reservoir B) and two treatments (0 mM NH_4^+ and 5 mM NH_4^+). Individual plants were the experimental unit. There were seven single-plant replications per treatment. All data except for rhizosphere pH were analyzed using one-way ANOVA in R version 3.5.1 (R Core Team, 2017). When appropriate, reservoir A and reservoir B were compared via paired one-tail *t* tests (α = 0.05). Rhizosphere pH data were analyzed using a linear mixed model (see section Rhizosphere pH measurement).

Rhizosphere pH measurement. Nutrient solution pH was measured three times per week during the treatment period in both experiments using a standardized portable pH meter (Accumet AP110; Fisher Scientific, Hampton, NH). Solution cross-contamination and disturbance of fine roots was avoided during pH measurement. Rhizosphere pH for a single treatment on a single day was compared with controls using one-sample single-tail *t* tests (α = 0.05) performed in R version 3.5.1 (R Core Team, 2017). Treatments were compared through linear mixed-effect analysis in R version 3.5.1 using package lme4 (Bates et al., 2015). Midweek pH data were used. Treatment, treatment duration, and their interaction were

designated as fixed effects; repeated measures per subject and week were designated as random sources of error. Statistical significance was determined by likelihood ratio tests comparing the full model against a model without the effect being investigated. Linear regression slopes are provided only as a reference.

Nitrogen uptake. Nutrient solution volume was measured at the end of each week. About 20 mL solution was collected in scintillation vials from each reservoir. Samples were filtered through a fine-wire mesh to remove debris, acidified with 1 drop 1 N HCl, and stored frozen at –20 °C. Samples were submitted for analysis at an institutional laboratory (University of Florida IFAS Analytical Research Laboratory, Gainesville, FL), where NO_3^- and NH_4^+ concentrations were measured by automated colorimetry according to Environmental Protection Agency protocols 353.2 and 351.2, respectively (U.S. Environmental Protection Agency, 1993a, 1993b). Both NO_3^- and NH_4^+ concentrations were measured in all nutrient solution samples. Nutrient solution volumes and concentrations were used to determine N content after 7 d of plant exposure. N removal from the nutrient solution was computed by subtracting N content after 7 d from the N content of fresh nutrient solution. N uptake was normalized according to whole-plant FW. Nitrification rates were estimated from the NO_3^- concentration at day 7 in control reservoirs that received NH_4^+-containing nutrient solution.

Chlorophyll status and N content. Plants were harvested destructively after 5 weeks of treatment. Canes, leaves, and roots from each reservoir were dried to a constant weight and ground until they passed through a #20 mesh screen. N content in these samples was measured through combustion–thermal conductivity at a commercial laboratory (Waters Agricultural Laboratories, Camila, GA) according to Association of Analytical Communities protocol 2.4.02 (Association of Analytical Communities, 2012). Chlorophyll status was measured nondestructively using a leaf greenness SPAD 502 meter (Konika Minolta, Osaka, Japan). Unitless measurements were made on three positions on the youngest fully expanded leaf of each plant at the end of the acclimation period and during week 3 of treatment.

Root viability. Relative electrolyte leakage (REL) was used to assess root viability per McKay and White (1997). Root tissue samples ($\approx$250 mg) were collected from each reservoir. Root samples contained a third-order root and all the attached lower order roots. This sampling method has been used previously to study SHB roots (Nunez et al., 2015). Roots were blotted dry, submerged in 16 mL deionized H_2O, and incubated for 24 h under continuous shaking at $\approx$60 rpm. Electrical conductivity was measured immediately after incubation and after autoclaving for 30 min at 121 °C using a standardized conductivity meter (Accumet XL30, Fisher

Scientific). Deionized water incubated with no roots was used as an assay control.

Results and Discussion

Nitrogen form effect on rhizosphere pH. In our experiment, cumulative NH_4^+ uptake by 'Emerald' SHB was significantly greater than NO_3^- uptake (5.66 mg N/g FW vs. 4.11 mg N/g FW, P = 0.03). Nitrification in the nutrient solution was negligible (average, 0.50 µmol NO_3^-/reservoir/d). Although it is possible that the low replication number in this experiment affected the analyses, these trends are consistent with previous studies in which SHB and other acid-loving plants exhibited a preference for NH_4^+ uptake over NO_3^- uptake (Merhaut and Darnell, 1995; Poonnachit and Darnell, 2004; Townsend, 1966). Low NO_3^- uptake is also consistent with the significantly lower chlorophyll status observed in NO_3^--grown plants compared with NH_4^+-grown ones during week 3 of treatment (36.28 SPAD vs. 59.78 SPAD, P = 0.02). FW and dry weight (DW) data were lost as a result of human error, but observations indicated that biomass accumulation mirrored N uptake and chlorophyll status trends.

NH_4^+-grown 'Emerald' SHB plants acidified their rhizosphere consistently and significantly within 3 d of each solution change (Fig. 1, $P \leq 0.0001$ in all cases). NO_3^--grown plants alkalized their nutrient solution starting on day 5 of the experiment ($P \leq 0.01$ in all cases). N form-dependent rhizosphere acidification and alkalization is consistent with previous reports in blueberry (Merhaut and Darnell, 1995; Nunez et al., 2015), and other woody (Gogorcena et al., 2001; Hawkins et al., 2008; Marschner et al., 1991; Suzko et al., 2018; Valentinuzzi et al., 2015) and herbaceous (Lei et al., 2015; Santi and Schmidt, 2009) plants. When analyzed through a linear mixed-effect model, the interaction between N source and treatment duration affected rhizosphere pH significantly (χ^2 = 10.18, df = 1, $P \leq 0.01$). Midweek rhizosphere pH of NH_4^+-grown plants was nearly constant (linear regression slope = – 0.01), whereas midweek rhizosphere pH of NO_3^--grown plants increased over time (linear regression slope = 0.15). N source also affected rhizosphere pH (χ^2 = 48.64, df = 1, $P \leq 0.001$). NO_3^- uptake raised rhizosphere pH by an average of 3.52 units compared with NH_4^+ uptake (Supplemental Fig. 2). Treatment duration did not affect rhizosphere pH significantly (χ^2 = 3.59, df = 1, P = 0.06).

Although NH_4^+-containing fertilizers are the basis for blueberry nutrition, NO_3^- is not absent from the blueberry rhizosphere. Fertilizers used for conventional (Kennedy, 2011; Liu et al., 2012; Smith and Harris, 2017) and organic (Larco et al., 2013) blueberry production can contain mixes of NH_4^+ and NO_3^-. Also, nitric acid—which dissociates into NO_3^-—can be used to acidify blueberry fertigation (Gallegos-Cedillo et al., 2018). In addition, blueberry soils can
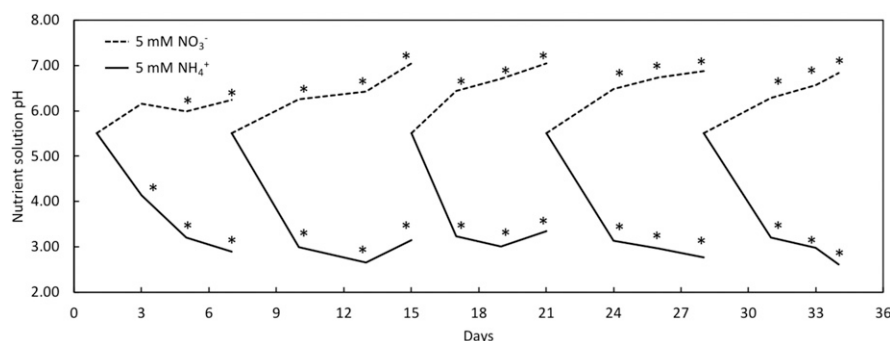
Fig. 1. Rhizosphere pH of 'Emerald' southern highbush blueberry plants grown in a complete nutrient solution containing 5 mM NO_3^- (dashed lines) or 5 mM NH_4^+ (solid lines). Fresh nutrient solution at pH 5.5 was delivered every week. Asterisks indicate significant differences from nutrient solution pH in reservoirs with no plants at $P \le 0.05$.
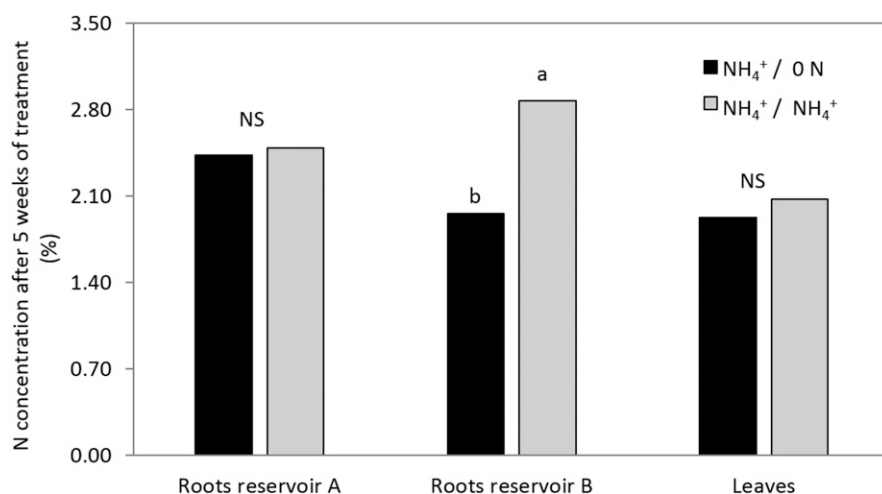

Fig. 2. N concentration in roots and leaves of hydroponically grown 'Emerald' southern highbush blueberry as determined by combustion–thermal conductivity. Plants were grown in a split-root hydroponic system in which reservoir A contained a complete nutrient solution buffered to pH 5.5, and reservoir B contained a nonbuffered nutrient solution containing 5 mM NH_4^+ (treatment NH_4^+/NH_4^+) or no N (treatment $NH_4^+/0N$). Different letters indicate significant differences between treatments ($P \le 0.05$). NS = not significant.

N/g FW vs. 2.73 mg N/g FW, $P = 0.04$). N uptake from reservoir A was not significantly different between treatments (average = 2.46 mg N/g FW, $P = 0.10$). All N uptake in treatment $NH_4^+/0N$ was from reservoir A, because reservoir B did not contain N. These results are consistent with previous studies in which plants that were exposed to low nutrient concentrations in one half of their root systems took up less nutrients overall (Ma and Rengel, 2008; Shen et al., 2005).

Nutrient solution NH_4^+ concentration affected root N concentration. Roots in reservoir B in treatment $NH_4^+/0N$ exhibited significantly lower N concentrations than roots in reservoir B in treatment NH_4^+/NH_4^+ ($P < 0.001$). Roots in reservoir B in treatment NH_4^+/NH_4^+ exhibited significantly greater N concentrations than roots in reservoir A in all treatments ($P < 0.01$), suggesting there was greater NH_4^+ uptake from nutrient solutions that were not buffered. Contrasting N uptake and root N concentration in reservoir B did not affect whole-plant N status. Treatments did not affect plant DW (average = 29.38 g, $P = 0.42$), root N concentrations in reservoir A ($P = 0.76$), or leaf N concentration ($P = 0.31$, Fig. 2). All leaf N concentrations were within previously established sufficiency ranges for blueberry (Smith and Harris, 2017; Strik and Vance, 2015). In addition, leaf chlorophyll status did not change between acclimation and treatment periods (49.1 SPAD vs. 52.4 SPAD, $P = 0.21$), and it was not significantly different between NH_4^+/NH_4^+ and $NH_4^+/0N$ plants during the treatment period (55.0 SPAD vs. 49.9 SPAD, $P = 0.11$). These results suggest that overall plant N status was unaffected by the treatments in the 5-week treatment period used in this experiment. It is likely that prolonged cultivation under these conditions could lead to N-deficient plants in treatment $NH_4^+/0N$.

Roots in reservoir B of all treatments acidified their rhizosphere consistently and significantly starting 2 d after each solution change (Fig. 3, $P \le 0.0009$ in all cases). The magnitude of rhizosphere acidification by roots exposed to NH_4^+ in their nutrient solution was comparable between Expts. 1 and 2, and it is consistent with previous reports (Marschner et al., 1991). When analyzed through a linear mixed-effect model, the interaction between NH_4^+ concentration in reservoir B and treatment duration significantly affected rhizosphere pH ($\chi^2 = 8.36$, df = 1, $P < 0.01$). Midweek nutrient solution pH remained relatively constant in treatment NH_4^+/NH_4^+ (linear regression slope = -0.17) (Supplemental Fig. 3), suggesting that rhizosphere acidification in these plants responded to a constant stimulus, such as NH_4^+ uptake. In contrast, midweek nutrient solution pH decreased over time in treatment $NH_4^+/0N$ (linear regression slope = -0.31), suggesting that rhizosphere acidification in these plants increased in response to changing conditions. It is possible that diminishing root N concentrations in reservoir B of treatment $NH_4^+/0N$ increased the rhizosphere acidification capacity in these roots (local

have high nitrification rates (Hanson et al., 2002). Our results suggest that using NO_3^- for blueberry fertilization might lead to rhizosphere alkalization. In blueberry, high rhizosphere pH can lead to nutrient deficiencies, stunted growth, and low survival (Finn et al., 1993; Tsuda et al., 2014). Thus, NO_3^- use would exacerbate the need for soil amendments and soilless substrates for blueberry cultivation. Further research is necessary to establish the extent to which $NH_4^+:NO_3^-$ combinations affect rhizosphere pH under field conditions.

The observed rhizosphere acidification in NH_4^+-grown 'Emerald' SHB plants represents a conundrum, because it is not possible to determine whether these plants acidify their rhizosphere independently from the effect of NH_4^+ uptake. Previously, rhizosphere acidification capacity has been used as a proxy to identify genotypes with broad soil adaptation (Suzko et al., 2018) or superior nutrient uptake capacity (Lei et al., 2015). Thus, determining whether SHB is

capable of acidifying its rhizosphere independently of N form might be a first step in breeding blueberries that tolerate neutral or higher pH soils. This trait has been identified as a priority for blueberry breeders in the United States and Canada (Gallardo et al., 2018).

Rhizosphere acidification with and without NH_4^+ uptake. 'Emerald' SHB plants were transplanted to a split-root hydroponic system to distinguish basal rhizosphere acidification from NH_4^+ uptake-related rhizosphere acidification. Thirty days after transplant, the REL of roots from the same reservoir was not significantly different ($P_{reservoir A} = 0.35$, $P_{reservoir B} = 0.19$). Abiotic stress increases root REL (McKay and White, 1997). REL results in this experiment suggest that roots were not stressed by manipulation and transplant to the split-root hydroponic system.

'Emerald' SHB plants in treatment NH_4^+/NH_4^+ took up significantly more N than plants in treatment $NH_4^+/0N$ (3.95 mg
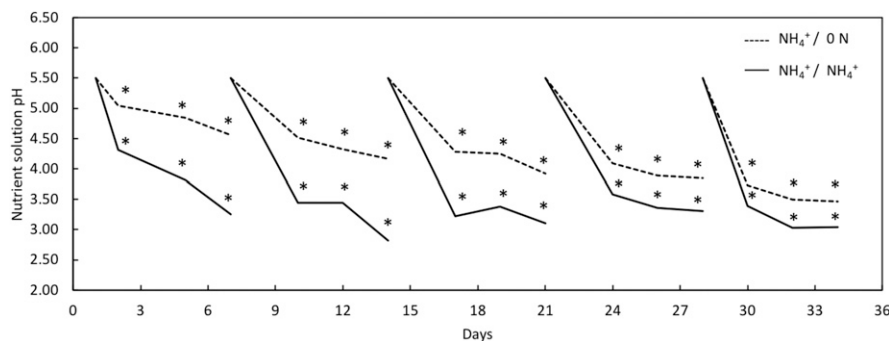
Fig. 3. Rhizosphere pH of 'Emerald' southern highbush blueberry plants grown in a split-root hydroponic system in which reservoir A contained a complete nutrient solution buffered to pH 5.5, and reservoir B contained a nonbuffered nutrient solution containing 5 mM NH_4^+ (treatment NH_4^+/NH_4^+, solid lines) or no N (treatment $NH_4^+/0N$, dashed lines). Fresh nutrient solution at pH 5.5 was delivered every week. Asterisks indicate significant differences from nutrient solution pH in bottles with no plants at $P \leq 0.05$.

response). In addition, it is possible that the entire root system in treatment $NH_4^+/0N$ responded to NH_4^+ uptake from reservoir A (systemic response). Additional research is necessary to elucidate whether local or systemic responses are involved in this apparent acclimation.

NH_4^+ concentration in reservoir B ($\chi^2 = 7.45$, df = 1, $P < 0.006$) and treatment duration ($\chi^2 = 12.63$, df = 1, $P < 0.0004$) also affected rhizosphere pH significantly. Ammonium uptake decreased rhizosphere pH by an average of 0.75 units beyond basal rhizosphere acidification (Supplemental Fig. 3). These results are consistent with an increase in H^+-ATPase activity as a consequence of cation uptake. Molecular and genetic evidence for this phenomenon are available for model species [see Hachiya and Sakakibara (2017) for review], but not for blueberry. Root organic acid exudation increases when P and micronutrients are scarcely bioavailable (Marschner et al., 1987). Considering P and micronutrients were equally abundant in both treatments, it is unlikely that organic acid exudation was involved in rhizosphere pH change in our experiment.

Previously, lack of rhizosphere acidification in *Vaccinium* spp. was ascribed to relaxed selection in the acidic soils where these species evolved (Nunez et al., 2015). Our results contradict this notion, as 'Emerald' SHB plants are, in fact, capable of acidifying their rhizosphere. In the future, additional genotypes and related taxa should be tested to determine whether there is variation in this trait among cultivated and wild *Vaccinium* spp.

Other woody and herbaceous species are capable of acidifying their rhizosphere even when they take up NO_3^- (Gogorcena et al., 2001; Hawkins et al., 2008; Santi and Schmidt, 2009), suggesting their H^+ extrusion rates (from H^+-ATPase activity or organic acid exudation) are greater than H^+ influx rates through the H^+/NO_3^- symport. In our experiment, 'Emerald' SHB plants were capable of acidifying their rhizosphere when grown with NH_4^+ or without N, but not

when grown with NO_3^-. These results suggest that 'Emerald' SHB H^+ extrusion rates are less than H^+ influx rates caused by NO_3^- uptake. Considering that blueberry plants are exposed to NH_4^+ and NO_3^- under typical field conditions (Hanson et al., 2002), further research is necessary to determine whether they can create an acidic microenvironment around their roots in agricultural settings.

In conclusion, N uptake is the main driver of rhizosphere pH in 'Emerald' SHB. NH_4^+ uptake, in particular, appears to be essential for maintaining low rhizosphere pH. These findings advance our understanding of rhizosphere chemistry in acid-loving plants and provide a baseline for testing $NH_4^+:NO_3^-$ mixes and other *Vaccinium* spp. in the future.

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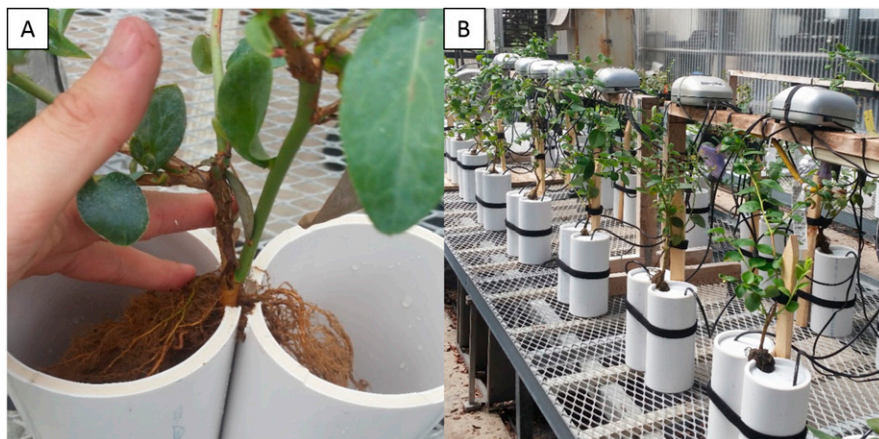
Supplemental Fig. 1. Split-root hydroponic system used to grow 'Emerald' southern highbush blueberry. (**A**) Root systems were split in two halves. Each half was placed inside a 1.5-L reservoir. (**B**) Reservoirs were held together by Velcro straps; plants were held upright using garden stakes.
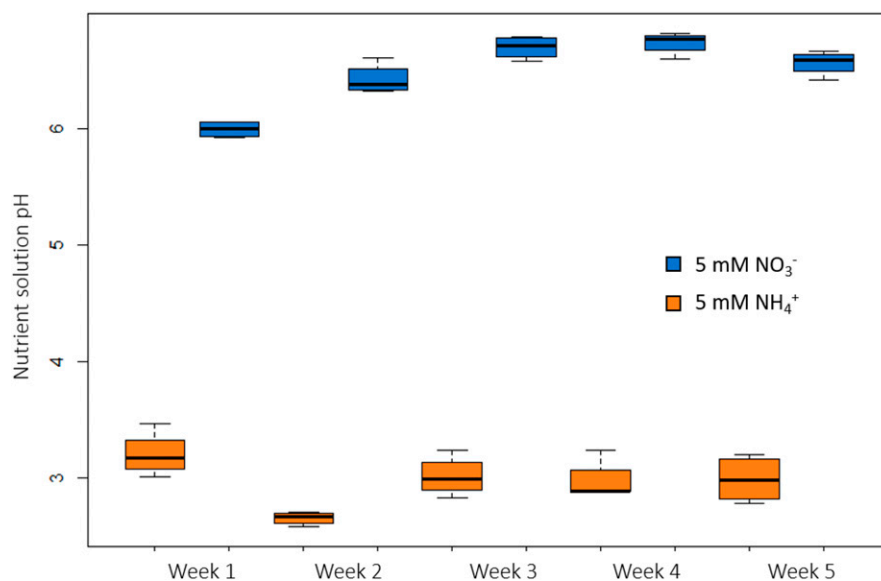
Supplemental Fig. 2. Midweek nutrient solution pH of 'Emerald' southern highbush blueberry plants grown in a complete nutrient solution containing 5 mM NO_3^- (blue) or 5 mM NH_4^+ (orange). Data shown were analyzed using a linear mixed-effect model.
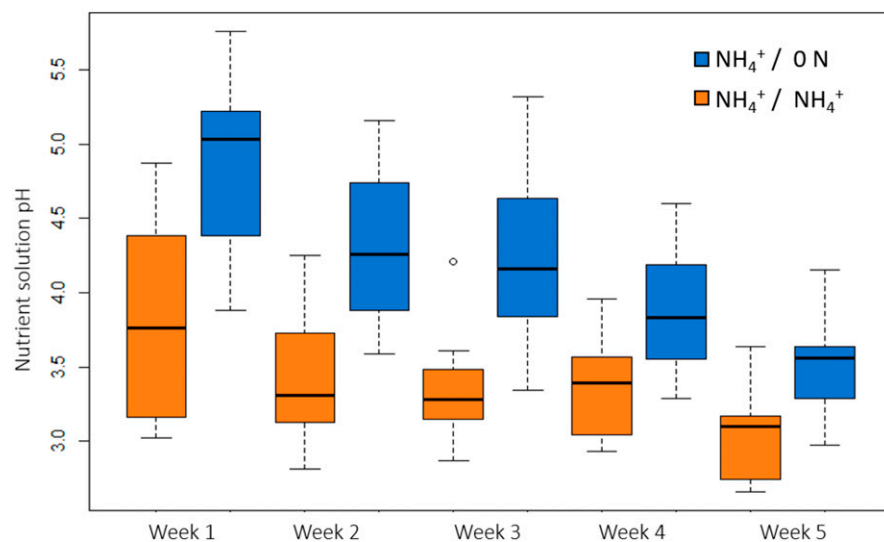
Supplemental Fig. 3. Midweek nutrient solution pH of 'Emerald' southern highbush blueberry plants grown in a split-root hydroponic system. Reservoir A contained a complete nutrient solution buffered to pH 5.5; reservoir B contained a nonbuffered nutrient solution containing 5 mM NH_4^+ (treatment NH_4^+/NH_4^+, orange) or no N (treatment $NH_4^+/0N$, blue). Data shown were analyzed using a linear mixed-effect model.