Statistical Model for Describing Macronutrient Impacts on Container Substrate pH Over Time

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Abstract. Although many factors that influence substrate pH have been quantified, the effect from fertilizers continues to be elusive. A multifactorial experiment was conducted to test macronutrient effects using a rarely used statistical method known as the central composite design. Five nutrient factors, including nitrogen (N) carrier ratio (NH_4^+ vs. NO_3^-) and concentrations of phosphorus (P) (as H_2PO_4^-), potassium (K), combined calcium (Ca) and magnesium (Mg), and sulfur (S), were varied at five levels each encompassing the proportionate range of these nutrients in commercial greenhouse fertilizers. Although a typical factorial experiment would have resulted in $5^5 = 3125$ treatments, the central composite design reduced the number to 30 fertilizer treatments. An experiment was conducted twice in which ‘Evolution White’ mealy-cup sage (*Salvia farinacea* Benth.) was grown in 14-cm-diameter pots (1.29 L) in a 3 peat:1 perlite (v/v) substrate amended with non-residual powdered calcium carbonate to raise the substrate pH to ≈ 5.6 to 5.8. Harvests occurred after 3 and 6 weeks of growth. A statistical model described substrate pH over time with significant effects including four main effects of N carrier ratio, P, K, and combined Ca and Mg; three squared terms of N carrier ratio, P, and K; and seven interaction effects. The resulting model was used to calculate substrate pH levels between 25 and 45 days after planting, and it showed that N carrier had the greatest impact on substrate pH.

Substrate pH, the activity of free protons (H^+) in aqueous solution, has been of concern to horticulturists for years because it has a pronounced effect on nutrient availability (Sparks, 2003), and it gives insight into plant rhizosphere processes. The effects of pH range from field scale across large acreages to measuring pH variations at the millimeter scale in rhizospheres during growth and nutrient uptake. Extensive efforts have been made to manipulate and control substrate pH at these various scales and in specific

research areas, including container plant production.

The pH of natural or synthetic container substrates originates from an amalgamation of factors that are either inherent in the mix or a consequence of production procedures and chemical processes that occur during crop growth. These factors include buffering capacity of substrate components (Factor 1), reactivity rate of limestone (Factor 2), water buffering capacity (Factor 3), chemical fertilizer effect (Factor 4), physiological fertilizer effect (Factor 5), plant system effects (Factor 6), and microbial transformations (Factor 7). Other factors like nutrient deficiencies and fungicide drenches (Peters and Kackley, 2006; Taylor, 2007) can influence substrate pH; however, their occurrence is variable in comparison with the others and not majorly significant for those wishing to model substrate pH change for a crop.

The majority of growers raise propagules in soilless, usually sphagnum–peatmoss-based substrate. Peatmoss (Factor 1) is organic soil with an inherent pH range of 3.0 to 4.0 (Dole and Wilkins, 2005). To raise the pH to the recommended range of crop

production pH (5.4 to 6.6), base is added, typically in the form of limestone (Factor 2; Argo and Fisher, 2002; Dole and Wilkins, 2005). Limestone can be classified based on its reactivity with smaller particles of higher specific surface dissolving rapidly and larger particles dissolving more slowly to provide longer-term pH buffering (Fisher et al., 2006; Rippy, 2005). During growth, a continuous supply of water is needed to maintain plant turgidity. The presence of alkalinity (bicarbonates and carbonates) in the water supply, other ions like hydroxides (OH^-), ammonia, borates, organic bases, phosphates, and silicates (Bailey, 1996), and abnormally high or low water pH (Petersen, 1996) can influence substrate pH (Factor 3). Extensive research has been done to derive quantitative measurements of these first three factors—the inherent substrate pH (Rippy, 2005), limestone reactive and residual fractions (Fisher et al., 2006), and alkalinity from irrigation water (Bailey and Bilderback, 1998). The areas that are still elusive and deserve focus are the chemical and physiological effects of fertilizers and the effects from the plant system and microbial transformations.

Once the propagules are planted, adequate nutrition is needed for growth. Substrate nutrients must be frequently replenished as a result of plant uptake, leaching of the substrate during open-system irrigation of containers, and limited substrate volume to capture and resupply nutrients (Nelson, 2012; Smith and Lopes, 2008). Fertilizers have an impact on pH that is chemical and physiological in effect.

Active acidity/basicity (Factor 4) refers to how the fertilizers chemically react in water as well as cation exchange reactions that occur in the substrate. Most fertilizers are reported to not have a pronounced chemical effect relative to acids injected into irrigation water for alkalinity control, but those that contain urea phosphate or citric acid can significantly influence water pH (Peters and Kackley, 2006).

The physiological effect (Factor 5) or potential acidity/basicity (PAB) stems from the balance of cation vs. anion uptake by plants and use of charged ions to maintain plant electroneutrality. Charged ions like H^+ , OH^- , and bicarbonates offer the plant cell a fluid currency to maintain the electrical gradient during nutrient ion uptake (Dakora and Phillips, 2002). A plant cell typically has an electrical potential of -100 to -200 mV, which is established in part as a result of a diffusion potential (Assmann, 2010) and H^+ pumping by H^+ -ATPases (Reid and Hayes, 2003). Once this charge is established, cations can passively enter the cell resulting from the potential across the membrane. They are usually transported through antiports inward in response to expulsion of a H^+ to the substrate. To overcome the strong H^+ gradient, anions must be actively transported into the cell. Anions either enter the cell as bicarbonate or OH^- are expelled, or they can be transported inward through a symport with a H^+ . Both cases result in the net loss of a H^+

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from the substrate. If the charge of cations and anions taken up is equal and no other factors are significant, there should be no net change in substrate pH attributable to the fertilizer physiological effect. However, whenever the cation and anion uptake charge ratio shifts dominantly toward either charge, pH begins to decrease (greater cation uptake) or increase (increased anion uptake). Which ion form is taken up is determined by factors like nutrient species, plant species, ion availability, and diurnal effects (Reid and Hayes, 2003). The physiological effect is dominated by the uptake of the form of the macronutrient N. Uptake of NH_4^+ in excess of nitrate NO_3^- results in a net excretion of H^+ , whereas net H^+ consumption results from uptake of NO_3^- in excess of NH_4^+ (Marschner, 1995).

There are also plant system effects on substrate pH (Factor 6). Whereas PAB (Factor 5) can vary based on which fertilizers are used during production, plant system effects (Factor 6) depend more on the physiology of the plant. Plant root respiration, organic acid exudation, and root exfoliation can influence substrate pH. Of these factors, respiratory production of CO_2 and consequently formation of carbonic acid is likely to be the biggest driver (Marschner, 1995). An additional physiological effect is the influence of microbial flora (Factor 7) in the rhizosphere on substrate pH resulting from metabolic processes like nitrification (Lang and Elliott, 1991; Van Miegroet and Cole, 1984).

Efforts have been made to quantify the overall effect from fertilizers, but pieces of the puzzle have been missing from each attempt. Pierre (1933) developed a method for titrating flamed fertilizer material to determine cation and anion ratios and fertilizer PAB to allow manufacturers to incorporate lime into mixed fertilizers to help neutralize the acidic-forming effect of fertilizers [see Hoffmeister (1985) and Johnson et al. (2010) for a review of the procedure]. Pierre assumed the acid-base balance was the primary driver for the fertilizer effect and classified the elements accordingly. Acidic elements (half of N, one-third of P, S, and chlorine) would increase soil acidity, and basic elements [Ca, Mg, K, and sodium (Na)] would increase soil basicity. Additionally, Pierre (1933) believed that all of the acidity from NH_4^+ came from nitrification in field soil and that plants took up a negligible amount of NH_4^+ ; thus, his method omitted acidification from plant uptake of NH_4^+ , which does occur (Tisdale et al., 1985). Additionally, his method was developed for field soil, which has limited application to containerized soil-less substrates. Even he commented on the difficulties in developing a quantitative method based on field measurements (Pierre et al., 1970). Pierre's method is the dominant one used by the entire horticulture/agricultural industry and is applied to containerized production (Tisdale et al., 1985). Although many people within the industry feel this system is more qualitative than quantitative, it continues to be the most popular system for assessing fertilizer effects on substrate pH.

Johnson et al. (2010) revisited inaccuracies of determining PAB in their article, again asserting that Pierre's method is inaccurate for determining PAB values for fertilizers, and they stated that a new method to predict fertilizer acidity is needed by the horticulture industry. Johnson et al. (2013) followed this work with a new approach to quantify the effects from fertilizers on substrate pH. They grew *Impatiens*, *Pelargonium*, and *Petunia* and observed that as the N carrier increased in NH_4^+ , a drop in pH was observed. Their method used parameter estimates based on the N carriers in the fertilizer to calculate how much base would be needed to counteract the acidity from acid fertilizers and how much base was produced from basic fertilizers. The impact on substrate pH was not constant across species, providing evidence that a species effect does occur. Although they saw a close correlation with N carrier and substrate pH, they stated that their model was not able to account for the effects of other macronutrients on substrate pH.

To develop a model that can describe and predict pH shifts resulting from fertilizer application over time, one would need quantitative data from all macronutrients while considering interactions between nutrients. As stated, N may be the dominant nutrient affecting pH, but all nutrient-ion uptake causes exchange of acid-base ions to maintain electroneutrality. One approach to evaluating multiple fertilizer-ion effects of N carrier ratio and P, K, Ca, Mg, and S concentrations is to conduct a large multifactorial experiment. Here we applied a more condensed statistical design called the central composite design (CCD, see "Materials and Methods"). The CCD allows one to test multiple variables at once at multiple levels. Therefore, the objectives of our experiment

were to identify macronutrients that have a significant effect on substrate pH and to create a model for illustrating how substrate pH changes over time, particularly for the model crop mealy-cup sage (*Salvia farinacea*).

Materials and Methods

Experimental design. We conducted two experiments to evaluate the combined chemical and physiological fertilizer effects of macronutrients in potted substrate on substrate acidification while controlling the other factors discussed. We evaluated effects of five different nutrient factors at five input levels each. Whereas a full multifactorial experimental design would have resulted in $5^5 = 3125$ treatments, we used a CCD that reduced the number to 30 fertilizer treatments plus two fallow-pot controls (deionized water additions without fertilizers) for a total of 32 treatments. The CCD is a statistical design for factorial experimentation that reduces the number of treatment levels needed to generate a quadratic response surface [see Chapter 8 in Cochran and Cox (1957) and Chapter 15 in Box et al. (1978) for more information]. It relies on central concentration points of each parameter (nutrient factor) trialed, axial points that push the extremes of the concentration levels, and median points between the central and axial points (Fig. 1). Treatments (planted pots) were arranged in a randomized complete block design. For Expt. 1, we performed two destructive harvests of five plants per treatment at 26 and 47 d after planting. Expt. 2 was the same except three plants were destructively harvested in each treatment after 24 d and 42 d.

Substrate formulation and liming. A 3 sphagnum peatmoss:1 horticultural-grade perlite (v:v) substrate (Sun Gro Horticulture,

Fig. 1. Diagram of the central composite design. Visualizing the central composite design with 30 treatments from five nutrient factors at five levels each can be difficult. Therefore, we present a simplified schematic illustrating 15 treatments from three nutrients at five levels. Nitrogen levels increase from the front to the back of the diagram (right sides of smaller cubes), phosphorus concentrations increase from the left to the right (tops of the smaller cubes), and potassium concentrations increase from the bottom to the top (fronts of the smaller cubes). The values 1, 2, 3, 4, and 5 then correspond with very low, low, middle, high, and very high nutrient concentrations that are representative of the range of fertilizers used in the industry. This design allowed us to model substrate pH based on the ranges of the five nutrient factors used.

Bellevue, WA) was formulated based on the densities determined using methodology described by Barnes et al. (2013). Aquagro 2000G™ (Aquatrols®, Paulsboro, NJ) was used at the industry formulation rate of 0.6 g·L⁻¹ of sphagnum peatmoss at shipping moisture level. To determine how much base was needed to achieve a target pH of 5.6 to 5.8, substrate samples were incubated with varying amounts of calcium carbonate powder (CaCO₃ precipitated powder; Fisher, Fair Lawn, NJ) and a moisture content five times that of the container capacity. Samples were stirred for 10 s daily for 2 d to ensure sufficient reaction, and the final pH was recorded with an Accumet Excel XL15 pH meter (Fisher Scientific, Pittsburgh, PA). Using data from the incubations, powdered CaCO₃ was then applied to substrates used for the CCD experiments. For Expts. 1 and 2, the substrate pH averages were 5.72 (range, 5.59 to 5.81) and 5.79 (5.73 to 5.86), respectively.

Pretreatment plant production. ‘Evolution White’ mealy-cup sage seeds were sown on 5 Mar. 2012 into 288-cell plug trays (2.1 cm × 2.1 cm × 3.2 cm deep) containing Sungro Metro Mix substrate (Sun Gro Horticulture, Bellevue, WA). They were germinated inside a white polyethylene tent on a heating mat set at 24 °C that was situated inside a glass greenhouse at 35°N latitude in Raleigh, NC. On germination, the trays were moved out of the tent and off the heating mat and were fertilized at each irrigation with 13N–0.9P–10.8K Cal-Mag (SQM North America, Atlanta, GA) at a concentration of 150 mg·L⁻¹ N. Supplemental light from high-pressure sodium lamps was provided for 12 h·d⁻¹. Greenhouse day and night temperature set points were 20 and 18 °C, respectively. On 6 Apr. 2012 seedlings were transplanted into 1206-cell packs (3.9 cm × 3.9 cm × 5.7 cm deep) using the formulated 3 peat-moss:1 perlite mix described earlier. Supplemental lighting was discontinued, but the fertilization and temperature regimes were maintained. On 25 Apr. 2012, plants were transplanted into 14-cm-diameter plastic pots (1.29 L), and fertilizer treatments were initiated. For Expt. 2, the same procedures were followed except for the following. Seeds were sown on 8 June 2012. Supplemental high-pressure sodium light was not used. Plugs were transplanted into 1206-plug cell packs on 4 July 2012, fertigated with 17N–2.2P–14.1K (Greencare Fertilizers, Inc., Kankakee, IL) at 100 mg·L⁻¹ N, and grown at 21 to 24 °C day/17 to 20 °C night. Seedlings

were then transplanted into pots for the beginning of treatment on 20 July 2012.

To determine the amount of mix to put into each pot, 10 pots were completely filled with substrate, leveled off, and dropped from a height of ≈10 cm to set density. Filled pots were weighed, the average weight was determined, and pots were filled with substrate by weight rounding down to the nearest tens unit. In Expts. 1 and 2, pots were filled with 260 and 270 g of substrate (54.01 and 55.36 g dry peat equivalent/L substrate), respectively.

Fertilizer formulation and application. For the experimental design, 30 nutrient solution treatment formulations were derived from the CCD (Table 1, see experimental design above) using ranges of macronutrients commonly found on fertilizer labels in proportion to N (see below). Nutrient solutions were formulated (Table 2) at a concentration of 7 mM N (98 mg·L⁻¹ N). Nitrogen was

supplied in the following NH₄⁺-N/NO₃⁻-N ratios (0:100, 15:85, 40:60, 65:35, and 80:20); H₂PO₄ quantified as P₂O₅ at 10%, 25%, 50%, 85%, and 100% (w/w) of N; K quantified as K₂O at 35%, 55%, 100%, 150%, and 170% (w/w) of N; Ca + Mg in a 3:1 ratio at 25%, 35%, 50%, 65%, and 75% (w/w) of N; and S at 10%, 17%, 30%, 43%, and 50% (w/w) of N. Single-strength nutrient solutions were formulated in deionized water from the following salts: NH₄NO₃, KNO₃, (NH₄)₂SO₄, NH₄Cl, NaNO₃, KH₂PO₄, NaH₂PO₄·H₂O, Ca(NO₃)₂·4H₂O, Mg(NO₃)₂·6H₂O, NaSO₄, K₂SO₄, CaCl₂·2H₂O, MgCl₂·6H₂O, and KCl. Micronutrients were supplied to all nutrient treatment solutions from the following sources and concentrations: FeDTPA, 72 μM; H₃BO₃, 22.5 μM; MnCl₂·4H₂O, 4.5 μM; ZnCl₂, 0.75 μM; CuCl₂·2H₂O, 0.75 μM; and Na₂MoO₄·2H₂O, 0.05 μM. For both experiments, pH for the 30 nutrient solutions

Table 1. Treatment numbers, corresponding levels (Table 2) of each nutrient used for the 30 treatments in the central composite design, and observed treatment substrate pH values for the two experiments in which the starting substrate pH values were 5.72 and 5.79 for Expts. 1 and 2, respectively.

Treatment	Nutrient parameter level ^a					Treatment pH			
	NH ₄ ⁺ :NO ₃ ⁻	P	K	3 Ca:1 Mg	S	Expt. 1 (n = 5)		Expt. 2 (n = 3)	
						Day 26	Day 47	Day 24	Day 42
1	2	2	2	2	4	5.95	5.82	5.79	5.81
2	2	2	2	4	2	5.80	5.78	5.78	5.38
3	2	2	4	2	2	5.82	5.69	5.84	5.64
4	2	2	4	4	4	5.73	5.45	5.59	5.45
5	2	4	2	2	2	5.89	5.95	6.04	6.01
6	2	4	2	4	4	5.79	5.59	5.75	5.65
7	2	4	4	2	4	5.78	5.64	5.75	5.75
8	2	4	4	4	2	5.66	5.48	5.72	5.54
9	4	2	2	2	2	5.35	4.97	5.25	5.09
10	4	2	2	4	4	5.31	5.12	5.26	4.92
11	4	2	4	2	4	5.30	4.80	5.14	4.80
12	4	2	4	4	2	5.16	4.94	5.03	4.85
13	4	4	2	2	4	5.35	5.11	5.16	5.06
14	4	4	2	4	2	5.30	5.06	5.12	4.94
15	4	4	4	2	2	5.29	5.09	5.16	5.09
16	4	4	4	4	4	5.30	4.99	5.14	4.86
17	3	3	3	3	3	5.60	5.28	5.56	5.30
18	3	3	3	3	3	5.49	5.22	5.43	5.24
19	3	3	3	3	3	5.48	5.25	5.44	5.32
20	3	3	3	3	3	5.54	5.20	5.39	5.26
21	1	3	3	3	3	6.00	6.01	6.08	6.22
22	5	3	3	3	3	5.25	5.16	5.21	4.86
23	3	1	3	3	3	5.39	4.95	5.29	4.96
24	3	5	3	3	3	5.58	5.23	5.32	5.31
25	3	3	1	3	3	5.62	5.44	5.56	5.46
26	3	3	5	3	3	5.49	5.13	5.40	5.10
27	3	3	3	1	3	5.55	5.13	5.50	5.22
28	3	3	3	5	3	5.49	5.18	5.43	5.21
29	3	3	3	3	1	5.50	5.28	5.47	5.33
30	3	3	3	3	5	5.52	5.17	5.38	5.25

^aP = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; NH₄⁺ = ammonium; NO₃⁻ = nitrate.

Table 2. Millimolar concentrations of each nutrient at each of five treatment levels.

Level	Nutrient ion concentrations (mM)				
	NH ₄ ⁺ :NO ₃ ⁻	P	K	3 Ca:1 Mg ratio	S
1	0:7 (0% NH ₄ ⁺) ^a	0.138 (10% N) ^a	0.728 (10% N)	0.458:0.252 (25% N)	0.306 (10% N)
2	1.05:5.95 (15% NH ₄ ⁺)	0.345 (25% N)	1.145 (25% N)	0.642:0.353 (35% N)	0.520 (17% N)
3	2.8:4.2 (40% NH ₄ ⁺)	0.690 (50% N)	2.081 (50% N)	0.917:0.504 (50% N)	0.917 (30% N)
4	4.55:2.45 (65% NH ₄ ⁺)	1.173 (85% N)	3.122 (85% N)	1.192:0.655 (65% N)	1.314 (43% N)
5	5.6:1.4 (80% NH ₄ ⁺)	1.380 (100% N)	3.538 (100% N)	1.375:0.756 (75% N)	1.528 (50% N)

^aPercentages in parentheses are proportions (by weight) relative to total N inputs of 7 mM.

^bIn parentheses indicates weight of phosphorus as P₂O₅, potassium as K₂O, combined Ca and Mg, or S, all as a percent of the weight of N in the treatment. N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur; NH₄⁺ = ammonium; NO₃⁻ = nitrate.

averaged 4.2 ± 0.1 throughout the time of application.

Fertilizer applications were made to the surface of the substrate in a closed system where a 25-cm-diameter Styrofoam plate below the pot collected any leachate for reabsorption. To determine how much nutrient solution substrates could hold, two pots for each substrate batch were filled with substrate, weighed, placed in saucers, and hydrated with 700 mL H₂O through top application. Pots were rehydrated twice with the effluent at a 15-min interval between hydration events to allow the substrate to hydrate. Substrates were then weighed, and the water content held was determined by weighing the wet substrate. Initially, 400 mL of nutrient solutions was applied to the surface of planted substrate to approach container capacity. Afterward, 200 mL of nutrient solution was applied to substrate to rehydrate the substrate but not apply excess liquid that might lead to anaerobic conditions in the substrate. Substrates were fertigated at suitable frequency to prevent wilting. In Expt. 1, fertigation was initially determined by weighing four pots with plants chosen at random and determining when they reached 50% container capacity. Once sample weights began to exhibit greater discrepancy resulting from different evapotranspiration rates, substrates were fertigated based on when they were receptive to a 200-mL application. This application was determined by weight. In Expt. 2, to better determine at what point substrates needed irrigation, extra plants were grown and fertilized with 17N-2.2P-14.1K (Greencare Fertilizers, Inc., Kankakee, IL) at 100 mg·L⁻¹ N. Three plants were harvested on a weekly basis to account for accumulated plant fresh weight.

In Expt. 1, a total of 13 (one 400 mL initial and twelve 200 mL subsequent) applications were made for a total of 2.8 L of applied nutrient solution to each pot. One less 200-mL application was made in Expt. 2 for a total of 2.6 L of applied nutrient solution. Plants were grown under natural photoperiod in a glass greenhouse with air temperature set points of 21 to 24 °C day/17 to 20 °C night.

Harvest. Plants were moved into a controlled environment in the headhouse of the greenhouse facilities. Shoots were harvested for dry weight analysis. Substrates were hydrated with deionized water applied to the substrate surface until excess water leached into the saucer beneath. Samples were checked to ensure that excess water was always in the saucer. After 30 min, excess leachate was transferred to a 4-L bucket (Encore Industries, Sandusky, OH). Additional deionized water was added to the bucket to bring the total water to five times the container capacity of half of the substrate. Pots were inverted onto a cutting board. The plastic pot was removed and the contained substrate was longitudinally cut in the middle. Split subsamples were weighed and brought to within 5 g of each other. Half of the substrate was bagged and stored at 2 °C. The other half was placed in the corresponding bucket containing deionized water and leachate where it was broken apart to maximize substrate-water contact and reacted without stirring for 15 min. Then, pH was measured, and the pH meter calibration was checked with pH 4.0 and 7.0 buffer solutions after every eight samples and adjusted if necessary.

All data were analyzed using PROC MIXED, PROC GLM, and PROC RSREG in SAS (Version 9.2; SAS Institute, Cary,

NC) and SAS Enterprise Guide (Version 4.2; SAS Institute). Controls were not included in the statistical analysis of the CCD.

Results

Model for calculating pH. In Expts. 1 and 2, several parameters were significant drivers of pH change (Table 3). Because the parameters generated from both experiments were similar, we pooled the data together to generate a single model. Combining the data allowed us to strengthen our current model. With the pooled data, N carrier ratio and P, K, and Ca + Mg concentrations had significant main effects. Because S did not have a significant main effect, it was not discussed in great detail below. It was only allowed to remain in the model because two minor significant interactions were observed with S. Although N carrier ratio was the nutrient factor that was varied in these experiments, NH₄⁺ was chosen to provide a measurable ratio level for the model. Non-ionized forms for the nutrient factors were used to denote terms in the model, and brackets were added to indicate concentration. Three squared terms for NH₄, H₂PO₄, and K were significant, which signified curvature in the pH response to these parameters. Four nutrient × nutrient interactions (NH₄ × K, NH₄ × Ca + Mg, H₂PO₄ × Ca + Mg, and Ca + Mg × S) were statistically significant. Additionally, NH₄, P, K, and S had nutrient × day interactions. However, these latter four factors only contributed a small part to the model (≈2% of 87% total) and were not focused on in great detail. The following model was derived to calculate pH based on the parameters we set in this experiment:

Table 3. Statistical significance of main effects and interactions impacting substrate pH derived from Expt. 1, 2, or 1 and 2 pooled together.

Expt. 1		Expt. 2		Experiments pooled			
Parameters	Significance ^a	Parameters	Significance	Parameters	Significance	R ² ^b	SE
Day	***	Day	***	Day	NS	11.4	0.003
NH ₄ ^x	***	NH ₄	***	NH ₄	***	63.6	0.024
H ₂ PO ₄	***	H ₂ PO ₄	***	H ₂ PO ₄	***	0.6	0.105
K	***	K	***	K	*	2.6	0.045
Ca + Mg	***	Ca + Mg	***	Ca + Mg	***	1.2	0.055
S	NS	S	*	S	NS	0.1	0.079
NH ₄ × H ₂ PO ₄	**	NH ₄ × H ₂ PO ₄	*	NH ₄ × H ₂ PO ₄	NS	—	—
NH ₄ × K	*	NH ₄ × K	NS	NH ₄ × K	*	0.1	0.004
NH ₄ × Ca + Mg	***	NH ₄ × Ca + Mg	***	NH ₄ × Ca + Mg	***	0.7	0.009
H ₂ PO ₄ × Ca + Mg	*	H ₂ PO ₄ × Ca + Mg	NS	H ₂ PO ₄ × Ca + Mg	*	0.1	0.038
Ca + Mg × S	NS	Ca + Mg × S	***	Ca + Mg × S	**	0.2	0.040
[NH ₄] ²	***	[NH ₄] ²	***	[NH ₄] ²	***	3.6	0.002
[H ₂ PO ₄] ²	***	[H ₂ PO ₄] ²	***	[H ₂ PO ₄] ²	***	0.9	0.047
[K] ²	*	[K] ²	NS	[K] ²	*	0.1	0.009
NH ₄ × day	***	NH ₄ × day	***	NH ₄ × day	***	0.6	0.000
H ₂ PO ₄ × day	NS	H ₂ PO ₄ × day	***	H ₂ PO ₄ × day	**	0.2	0.001
K × day	**	K × day	NS	K × day	**	0.2	0.001
Ca + Mg × day	NS	Ca + Mg × day	*	Ca + Mg × day	NS	—	—
S × day	NS	S × day	NS	S × day	*	0.1	0.001
				exp		0.4	0.029
				Intercept		N/A	0.134
				Total		86.8	

^a*, **, or *** indicates statistically significant differences between sample means based on F test at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively. NS indicates F test for differences between sample means had $P > 0.05$.

^bR² values indicate predictability for each term in the model, which totals 86.8%.

^cModel parameter abbreviations for nitrogen form (NH₄), phosphorus (H₂PO₄), potassium (K), combined calcium and magnesium (Ca + Mg), and sulfur (S). N/A = not applicable.

$$\begin{aligned}
\text{pH} = & 6.675 - 0.003\text{day} - 0.369[\text{NH}_4] \\
& + 0.489[\text{H}_2\text{PO}_4] - 0.109[\text{K}] \\
& - 0.291[\text{Ca} + \text{Mg}] - 0.111[\text{S}] \\
& + 0.008[\text{NH}_4 \times \text{K}] \\
& + 0.049[\text{NH}_4 \times \text{Ca} + \text{Mg}] \\
& - 0.080[\text{H}_2\text{PO}_4 \times \text{Ca} + \text{Mg}] \\
& + 0.126[\text{Ca} + \text{Mg} \times \text{S}] + 0.029[\text{NH}_4]^2 \\
& - 0.299[\text{H}_2\text{PO}_4]^2 + 0.019[\text{K}]^2 \\
& - 0.002[\text{day} \times \text{NH}_4] \\
& + 0.004[\text{day} \times \text{H}_2\text{PO}_4] \\
& - 0.002[\text{day} \times \text{K}] - 0.003[\text{day} \times \text{S}].
\end{aligned}$$

Using the terms in the model, we were able to explain 87% of the variation in the data. Of that 87%, terms for NH_4 (64%), day (11%) and NH_4^2 (4%) provided the greatest contribution to predictability (Table 3).

This model can be used to calculate the pH based on a combination of nutrient concentrations and to generate a graphic representation of the effect of each nutrient on substrate pH. To investigate the effect from each nutrient, we created a range of values in mM between the minimum and maximum for nutrient concentrations used in the experiment. The other four factors were set at the median levels used in the experiment [2.8 mM NH_4^+ , 0.690 mM H_2PO_4^- , 2.081 mM K^+ , 1.421 mM $\text{Ca} + \text{Mg}$ (0.917 mM $\text{Ca} + 0.504$ mM Mg), and 0.917 mM S], and days were set at 25 and 45 d for the average days that harvests occurred.

Nitrogen. As NH_4^+ became the more dominant N carrier, there was a sharp decrease in substrate pH (Fig. 2). For 0, 2.8, and 5.6 mM NH_4^+ , the calculated pH values were 6.14, 5.47, and 5.26 at 25 d and 6.01, 5.25, and 4.95 at 45 d, respectively.

Phosphorus. As P concentration increased, substrate pH rose but then dropped at higher concentrations used in the experiment (Fig. 3). For 0.138, 0.69, and 1.38 mM P, the calculated pH values were 5.34, 5.47, and 5.37 at 25 d and 5.07, 5.25, and 5.22 at 45 d, respectively.

Potassium. As K concentrations increased, a decrease in pH was observed (Fig. 4). This range for the decrease in pH became more significant over time. For 0.728, 2.081, and 3.538 mM K, model pH values were 5.44, 5.37, and 5.36 at 25 d and 5.42, 5.27, and 5.18 at 45 d, respectively.

Combined calcium and magnesium. As the concentration of $\text{Ca} + \text{Mg}$ increased, a linear decrease in substrate pH was observed (Fig. 5). For 0.71, 1.421, and 2.131 mM $\text{Ca} + \text{Mg}$, the calculated pH values were 5.53, 5.47, and 5.40 at 25 d and 5.32, 5.25, 5.18 at 45 d, respectively.

Discussion

After using the model with assigned nutrient levels, it was apparent that the N carrier ratio had the greatest impact of all the nutrient parameters on substrate pH. At 25 and 45 d, the calculated difference in substrate pH values between the high NO_3^- and high NH_4^+ levels were 0.88 (6.14 to 5.26) and

1.06 (6.01 to 4.95), respectively. In comparison with the N carrier ratio factor, the differences in pH ranges as affected by P and K at 45 d were only 0.18 and 0.24, respectively. The larger impact of N carrier most likely originated

from the sheer amount of N taken up by the plant. Nitrogen accounts for $\approx 70\%$ of the cations and anions taken up by plants (Marschner, 1995), and thus, whichever form is taken up has a strong effect on pH. From unpublished

Fig. 2. Three-dimensional representation of the effect on substrate pH from the interaction of fertilizer NH_4^+ concentration and time. Although the figure shows NH_4^+ concentration, each treatment received 7 mm nitrogen total, and the balance of nitrogen is from nitrate.

Fig. 3. Three-dimensional representation of the effect on substrate pH from the interaction of fertilizer phosphorus (P) concentration and time.

Fig. 4. Three-dimensional representation of the effect on substrate pH from the interaction of fertilizer potassium (K) concentration and time.

Fig. 5. The effect on substrate pH from the increasing fertilizer concentration of 3 Ca:1 Mg (w/w). Ca = calcium; Mg = magnesium.

data from the primary author, control tissue samples in a previous experiment with *Salvia farinacea* 'Evolution' held 3133 mmol·kg⁻¹ N, 360 mmol·kg⁻¹ P, and 1148 mmol·kg⁻¹ K, which accounted for 54%, 6%, and 20% of the total inorganic nutrients assimilated, respectively.

Rhizosphere pH is greatly influenced by N carrier (Marschner, 1995; Tisdale et al., 1985), and in this experiment when the fertilizer N carrier was 100% NO₃⁻, calculated substrate pH values were higher than the initial starting substrate pH throughout the duration of the experiment. As the N carrier became more NH₄⁺-rich, substrate pH decreased. Fertilizing with NH₄⁺ likely resulted in greater H⁺ extrusion than H⁺ consumption

or OH⁻ or bicarbonate release, and with NO₃⁻, the reverse occurred. The maximum amount of NH₄⁺ in the ratio (80%) was less than the maximum amount of NO₃⁻ (100%), yet the substrate pH value decline from 5.76 to 4.95 was greater when NH₄⁺ was the dominant N carrier than the rise of 5.76 to 6.05 when NO₃⁻ was the dominant N carrier. Raven's (1986) report that for every 3 mol of NH₄⁺ taken up, 4 mol of H⁺ was generated and for 3 mol NO₃⁻ taken, up 2 mol OH⁻ was generated helped to explain this observation.

The reason that different counterbalancing ions were exchanged with the N carrier was consistent with our current understanding of plant physiology. For NH₄⁺ uptake, it is postulated that uptake can occur through NH₃

diffusion, NH₄⁺ uniport, and H⁺-coupled symport mechanisms (Ludewig et al., 2002). Regardless of which process occurs, with NH₃ uptake, there would be a net gain of H⁺ in the substrate as a result of the dissociation of the H⁺ from the NH₄⁺ before diffusion into the cell, whereas uptake of the positively charged NH₄⁺ would result in the release of H⁺ to the substrate to maintain the membrane electrical potential. To date, only support for the uniport exists (Ludewig et al., 2002). For NO₃⁻ uptake, energy was needed for assimilation and came from the symport of the nutrient with H⁺ across the membrane (Assmann, 2010). Evidence for this method of transport came from Glass et al. (1992) where they reported that in barley, 2 H⁺:1 NO₃⁻ symport occurred. Some earlier papers discussed uptake of NO₃⁻ occurring as a result of OH⁻ efflux (Keltjens and Nijenstein, 1987) and the possible existence of an anion or OH⁻ antiporter (Haynes, 1990). However, Weissenfeld et al. (1979) indicated that OH⁻ efflux was essentially equal to H⁺ influx in aqueous solution because equilibrium conditions with respect to H₂O dissociation are rapidly achieved. To date, a OH⁻ transporter has not been found in plant root cells, and research is typically discussed as coupled with H⁺ (McClure et al., 1990). Regardless of which process was occurring, the uptake of NO₃⁻ leads to an increase in substrate pH as we observed in our study.

For the other cations used in this experiment (K and Ca + Mg), the calculated substrate pH values decreased as the cation concentration increased in the fertilizer. Assuming that this effect stemmed from plant uptake, this result corresponded with what we understand from the literature about cation uptake. Uptake of K can occur with symport of H⁺ or Na (Assmann, 2010). For this high-affinity system, the efflux of two H⁺ through H⁺-ATPase activity would be needed for the influx of two positive charges with 1 K⁺:1 H⁺ symport. With the low-affinity system, K uptake occurred through specific K⁺ channels where influx was driven by a negative membrane potential generated from the efflux of H⁺ using the electronegative H⁺ pump (Maathuis and Sanders, 1994, 1997). Both systems would result in the extrusion of H⁺ into the apoplast. For Ca uptake, diffusion into the cell is mediated by channels that open and close based on the polarization of the cell membrane. When channels open allowing Ca into the cytoplasm, depolarization of the cell membrane is prevented by membrane-bound H⁺ pumps or H⁺-ATPase extruding H⁺ (Miedema et al., 2001). Although Mg²⁺ transporters and their mechanistic action of uptake of Mg from the soil have not yet been identified, the maintenance of a negative charge in the cytoplasm is important; uptake is postulated to occur through channels or transporters (Shaul, 2002).

For the anion macronutrients (P and S) used in the experiment, we expected an increase in substrate pH to occur as nutrient concentration increased. We did observe this trend for low to medium levels of P that we

used in the experiment, and at higher concentrations of P, we observed a decrease in substrate pH. We can support the effect of the rise in substrate pH based on our knowledge of anion uptake in the plant. For example, P is readily absorbed from soil through a symporter (Bloom, 2010), which means that a decrease in H^+ in the substrate soil solution occurs while a neutral charge (H^+ with $H_2PO_4^-$) transverse the cell membrane. The decrease in substrate pH at higher P levels is possibly the result of the formation of insoluble phosphate compounds. We used Visual MINTEQ (Version 3.0 beta; Dept. of Land and Water Resources Engineering, Stockholm, Sweden, <http://www2.lwr.kth.se/English/OurSoftware/vminteq/>) to determine if precipitation of P compounds could occur at the start of the experiments when the pH was at 5.76 (average starting substrate pH) and concentrations of Ca and 0.552 mM P (400 mL of the 1.38 mM applied, the highest P concentration used in the experiment) were added to the model solution. The substrate for Expts. 1 and 2 had an average of 18.8 mmol·L⁻¹ Ca from liming, and from the first addition of fertilizer, we added an additional 0.3 mM Ca for a total of 19.1 mmol·L⁻¹ Ca. If 100% of the Ca was in solution and could react with the P, then MINTEQ indicated that the solution would be nearly 40,000-fold supersaturated with respect to hydroxyapatite (saturation index of 4.6). MINTEQ indicated that hydroxyapatite precipitation, a H^+ -releasing reaction, was possible if greater than 8.5% of the Ca (1.62 mmol) in the substrate was in solution at pH 5.76. Based on the following reaction for the formation of hydroxyapatite, seven moles of H^+ are generated per mole of hydroxyapatite formed (Lindsay, 1979):

This evaluation suggests that the decrease in substrate pH at higher P concentrations could be the result of precipitation of Ca-phosphate solids. For S uptake we expected to see a similar rise in substrate pH, but the main effect of S was not significant. It was possible that uptake of S did not exhibit a significant impact on substrate pH because so little is typically taken up in comparison with the other macronutrients (Epstein and Bloom, 2005).

We recognized some of the limits to this model. The model predicts that an overall decrease in substrate pH for all the fertilizers we trialed would be observed; however, this trend contradicts some data for some fertilizer treatments like 21. Although treatment 21 had varying levels of other nutrients, it did have 0 mM NH_4^+ . We observed that the treatment 21 substrate pH levels increased over time for an average of 6.00 and 6.01 for harvests in Expt. 1 on Days 26 and 47, respectively, and 6.08 and 6.22 in Expt. 2 on Days 24 and 45, respectively. However, the

surface (Fig. 2) showed a downward trend in pH over time instead of upward for 0 mM NH_4^+ , which contradicts the rise in substrate pH that we observed for treatment 21. Our model crop for this experiment was *Salvia farinacea*, yet there are species-specific effects reported based on other crops' nutrient uptake or sensitivities to high or low pH (Jeong, 2010; Johnson et al., 2013). The model also assumes that changes in substrate pH are solely because of nutrient uptake. As we saw in our discussion with P, chemical reactions may be occurring in the substrate, which can alter the substrate pH. Cation exchange could occur between the peat surface releasing H^+ into the substrate solution. Another source of acidity could have been the potential nitrification that occurred when NH_4^+ was transformed into NO_3^- . Although we controlled for substrate, limestone, and water alkalinity in this experiment, in an industry setting, these factors could potentially play a role in influencing substrate pH.

Substrate pH has been called one of the most important variables to maintain during crop production. Extensive efforts have been made to quantify the effect on pH based on the inherent substrate acidity, neutralizing power from $CaCO_3$, and the effect of alkalinity from water sources. However, quantitative models for substrate pH shifts over time resulting from nutrient uptake are limited. From the data generated here, we were able to use a unique statistical method to model the effects of different macronutrient fertilizer compositions on substrate pH over time, and there is great power in our method and our model to be able to begin to describe and model substrate pH changes over time in a crop.

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