

Article

A Base Layer of Ferrous Sulfate-Amended Pine Bark Reduces Phosphorus Leaching from Nursery Containers

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Abstract: Phosphorus (P) fertilizers applied to container-grown nursery crops readily leach through pine bark-based substrates and can subsequently runoff and contribute to surface water contamination. The objectives of this research were to determine the effect of adding a layer of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ -amended pine bark (FSB) to the bottoms of nursery containers on P leaching characteristics. Phosphorus and iron (Fe) leaching in response to FSB layer height (4 or 7.5 cm), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ rate (0.3, 0.6, or 1.2 $\text{kg} \cdot \text{m}^{-3}$ Fe), and form (i.e., granular versus liquid) used to formulate the FSB layer, and the inclusion of dolomite in the FSB layer were also investigated. Greenhouse studies lasting 15 and 19 weeks were conducted, in which 2.5 L nursery containers containing the FSB layer treatments below non-amended pine bark substrate were fertilized with 199 or 117 mg P from a soluble or controlled-release fertilizer, respectively. Leachate resulting from daily irrigation was collected and analyzed for P and Fe weekly. All FSB treatments leached less P than the control (non-amended pine bark only), with P reductions ranging from 22% (4 cm FSB with 0.3 $\text{kg} \cdot \text{m}^{-3}$ Fe) to 73% (7.5 cm FSB with 1.2 $\text{kg} \cdot \text{m}^{-3}$ Fe). Phosphorus leaching decreased linearly with an increase in Fe rate or layer height. The amount of Fe that leached from containers with FSB was <5% of that applied, regardless of the Fe rate. Granular- and liquid-applied $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ with or without dolomite were equally effective at reducing P leaching. Adding 0.6 $\text{kg} \cdot \text{m}^{-3}$ Fe to the bottom 500 cm^3 of pine bark increased P adsorption by 0.053 $\text{mg} \cdot \text{cm}^{-3}$ P, which equates to 17.9 mg P adsorbed per gram of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ added. Results from this research suggest that including an FSB layer in the bottom of nursery containers is an effective strategy for reducing P runoff from container-based nursery production sites.

Keywords: phosphorus; ferrous sulfate; iron; pine bark; substrate; container; nursery; leaching; dolomite



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1. Introduction

Nursery stock is the highest-grossing horticultural specialty crop sector in the U.S., with over \$4.5 billion in annual sales, representing about one-third of all specialty crop sales [1]. Approximately 80% of U.S. nursery operations produce crops in containers [1]. Substrates commonly used for container-based production, predominantly pine bark in eastern U.S., are inherently low in plant-essential nutrients and have poor nutrient-holding capacities [2]. Frequent replenishment of the substrate with nutrients is, therefore, essential to ensure a salable crop is produced. However, the constant presence of a soluble or solubilizing fertilizer in a substrate that poorly retains nutrients, paired with frequent (often daily) irrigation, results in excess nutrients leaching from containers. Phosphate is particularly prone to leaching from pine bark-based substrates [3–7]. For example, Yeager and Barrett [5] showed that when 3 $\text{kg} \cdot \text{m}^{-3}$ of superphosphate was mixed into a substrate composed of a 2:1:1 ratio (by volume) of pine bark:peatmoss:sand, 76% of the applied P leached from the substrate in 3 weeks with once-daily irrigation.

Nutrients that drain from nursery containers can subsequently runoff to surface waters. Phosphorus and nitrogen (N) runoff to surface waters has been linked to eutrophication and harmful algal blooms that are responsible for annual “dead zones” that plague the Gulf of Mexico, Chesapeake Bay, Lake Erie, Florida Everglades, Lake Okeechobee, and other economically and ecologically important water bodies [8]. The impact of agricultural P runoff on surface water quality has resulted in increased environmental regulation, a trend that will likely continue in an effort to remediate and preserve impaired waterways. For example, Maryland’s Water Quality Improvement Act of 1998 requires all agricultural operations (including ornamental plant nurseries) grossing \geq \$2500 to submit N and P management plans and file annual reports on N and P applications [9]. More recently, Florida enacted the Senate Bill 712 (the “Clean Waterways Act”) in 2020 which requires all agricultural landowners and growers to submit N and P application records to the Florida Department of Agriculture and Consumer Services; individuals who fail to do so may be reported to the Florida Department of Environmental Protection for “regulatory action”.

Fertilizing with a controlled-release fertilizer (CRF) instead of soluble forms is among the most widely implemented best management practices (BMPs) for fertilizer management in container-based nursery production according to survey studies in Virginia and Alabama [10,11]. However, P leaching from CRF-fertilized containerized crops can be substantial. Million and Yeager [12,13], Million et al. [14], and Tyler et al. [15,16] reported that as much as 27% to 35% of P from CRF was recovered in the leachate of containerized crops grown in a pine bark-based substrate.

Several P-sorbing substrate components have been evaluated for their effectiveness in improving P retention while maintaining a labile P supply for plant uptake. Williams and Nelson [17] increased P adsorption in a Sphagnum peat-based substrate without negatively affecting the growth of *Dendranthema × grandiflorum* (Ramat.) Kitamura ‘Sunny Mandalay’ by incorporating 0.32 kg m^{-3} Al from $\text{Al}_2(\text{SO}_4)_3$ into the substrate. However, amending a substrate with $\text{Al}_2(\text{SO}_4)_3$ imposes the risk of Al toxicity in plants, as was observed by Williams and Nelson [17], when Al incorporation rates exceeded 0.32 kg m^{-3} . Calcined clay products added to a pine bark- or peat-based substrate at 8% to 20% (by volume) has been shown to reduce P leaching by 34% to 73% compared to the non-amended control while successfully producing containerized woody and herbaceous plant species [18,19]. In contrast, Owen et al. [20] reported that effluent from pine bark amended with 11% clay contained as much as 90% more dissolved reactive P compared to that from pine bark amended with sand, suggesting some calcined clay products increase P leaching. Variability in the quality attributes of calcined clay products and the relatively high cost of shipping to continually use the recommended high incorporation rates may collectively explain the poor adoption of calcined clay products by the U.S. nursery industry [21]. Other materials that have more recently been shown to reduce P leaching when added to container substrates include dolomite [22], sulfate-based micronutrient fertilizer [22], activated alumina [23], and flue gas desulfurization gypsum [24,25].

Modifying the charge properties of conventional substrate components (e.g., pine bark) through cationization is a novel but possibly simpler approach to improving P retention during containerized crop production. Cationization can be accomplished by amending organic materials with metal salts; the metal cations adsorb to functional groups in the organic material resulting in an increase in PO_4^{3-} binding sites. Metal-loaded agricultural by-products (e.g., sugarcane bagasse, coir pith, wood particles, okara) have been studied extensively for their capacity to sequester phosphate (PO_4^{3-}) from wastewater [26,27]. Relative to other metal compounds that have been used to cationize organic materials [e.g., ZnCl_2 , ZrO_2Cl , $\text{La}(\text{NO}_3)_3$], iron salts are less expensive, non-toxic, and more readily available for purchase [27]. Ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; FS) is one such soluble iron compound that is routinely used in containerized nursery production as a pre-plant Fe fertilizer, often as a component of a complete micronutrient fertilizer [28]. When mixed into a pine bark substrate along with superphosphate, FS has been shown to reduce the amount of water-extractable P from the substrate [29]. However, the effects of

amending a substrate with relatively high rates of FS (i.e., 1.5 to 3 kg·m⁻³) on P leaching from soluble and controlled-release fertilizers has not yet been investigated.

To avoid possible reductions in P availability to plants, while still mitigating P leaching from the container, we propose adding a layer of FeSO₄-amended pine bark (FSB) to the bottoms of nursery containers with the remainder of the container filled with a standard pine bark substrate. Accordingly, P that is released from the fertilizer and leaches past the root zone may be adsorbed to the FSB before draining from the container. Growing containerized nursery and greenhouse crops in strategic layers of substrate, sometimes referred to as substrate stratification, has recently been proposed to decrease fertilizer requirements [30], reduce weed growth and weed seed germination [31,32], mitigate crop water stress during drought [33], and reduce peat use [34]. However, stratifying a substrate to include a P-sorbing base layer has not yet been explored. The objectives of this research were to investigate P and Fe leaching characteristics in response to FSB layer height, FS rate and form (i.e., granular versus liquid) used to formulate the FSB layer, and the presence of dolomite in the FSB layer. Specific hypotheses being tested include, (1) an FSB layer in the bottom of nursery containers reduces P leaching and (2) the degree to which P leaching is reduced is affected by the FSB layer height and the FS rate, FS form, and the presence of dolomite in the FSB layer.

2. Materials and Methods

2.1. Experiment 1: Effect of FeSO₄ Rate and FSB Depth

2.1.1. Substrate Characteristics

Hammermilled pine (*Pinus palustris*) bark substrate that had been aged outdoors for at least 6 months was obtained from a commercial nursery-substrate manufacturer (T.H. Blue Inc., Eagle Springs, NC, USA). The substrate particle size distribution was determined by passing approximately 35 g oven-dried (60 °C) substrate through 12.5, 6.3, 4, 2.8, 2, 1.4, 1, 0.71, 0.5, 0.35, 0.25, 0.18, and 0.106 mm soil sieves using a RX-29/30 Ro-Tap test sieve shaker (W.S. Tyler, Mentor, OH, USA) for 5 min per each of three samples (278 oscillations/min, 150 taps/min). Percentage (by mass) of the substrate comprising coarse (<12.5 and ≥2.8 mm), medium (<2.8 and ≥0.71 mm), and fine (<0.71 mm) particles is reported in Table 1.

Table 1. Physical properties of the pine bark substrate (*n* = 3).

Substrate Property	Mean	SD
Particle size distribution (% by mass)		
Coarse ^z	55	5.1
Medium	30	3.4
Fine	15	1.7
Air space ^y (% by vol.)	30	4.0
Container capacity (% by vol.)	45	2.0
Bulk density (g·cm ⁻³)	0.17	0.002

^z Coarse particles were <12.5 and ≥2.8 mm, medium particles were <2.8 and ≥0.71 mm, and fine particles were <0.71 mm. ^y Air space, container capacity, and bulk density were determined according to the North Carolina State University Porometer Method [30].

Substrate physical properties (air space, container capacity, and total porosity) were determined according to the method described by Fonteno and Harden [35]. The substrate was packed in 347-cm³ aluminum cores (7.6 cm tall, 7.6 cm i.d.) and then attached to North Carolina State University Porometers (Horticultural Substrates Laboratory, North Carolina State University, Raleigh, NC, USA) for determination of air space. There were three replications for each substrate. Cores were weighed, oven-dried for 4 d at 60 °C, and weighed again to determine container capacity (i.e., the percent volume of a substrate that is filled with water after the substrate is saturated and allowed to drain). Total porosity was calculated as the sum of air space and container capacity. All physical properties (total porosity, air space, container capacity) were calculated as the algebraic mean of the core.

Bulk density was determined using an oven-dried (60 °C) substrate in 347-cm³ cores. The substrate air space, container capacity, and bulk density are reported in Table 1.

Pine bark initial electrical conductivity (EC) and pH were (\pm sd) $117 \pm 17 \mu\text{S}\cdot\text{cm}^{-1}$ and 4.3 ± 0.04 , respectively, and water-soluble and insoluble total nutrient contents are provided in Table 2. Electrical conductivity, pH, and water-soluble nutrient concentrations in the pine bark were determined in saturated media extracts (SMEs; [36]) from 200 cm³ pine bark samples ($n = 4$). The substrate initial moisture content and volume of DI water added for SMEs was recorded and later used to equate nutrient concentrations in aqueous extracts to mg extractable nutrients per cm³ substrate. Electrical conductivity and pH were measured using a conductivity meter (Fisher 06-662-61; ThermoFisher Scientific, Weltham, MA, USA) and pH/ion analyzer (MA 235; Metler Toledo, Columbus, OH, USA). Water-soluble nutrient concentrations were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES; Agilent 5110, Agilent Technologies, Santa Clara, CA, USA) after vacuum-filtering the extract through a cellulose fiber filter (Whatman #1; Cytiva, Marlborough, MA, USA) and then passing a 10 mL aliquot of filtrate through a 0.45 μm polyethersulfone (PES) syringe filter (CH4525-PES; ThermoFisher Scientific). To measure total Fe and P concentrations in pine bark, $\approx 100 \text{ cm}^3$ oven-dried (105 °C) pine bark was ground to <0.5 mm using a Wiley Laboratory Mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA), digested with concentrated HNO₃ and HCl (USEPA Method 3051 [37]), and analyzed via ICP-OES.

Table 2. Water soluble and insoluble elemental concentrations in non-amended pine bark determined via saturated media extracts [36] and aqua regia digestion (USEPA Method 3051 [37]), respectively ($n = 5$).

Element	Soluble		Insoluble	
	Mean	SD	Mean	SD
			$\mu\text{g}\cdot\text{cm}^{-3}$	
P	0.11	0.01	24.58	2.94
K	18.72	0.69	158.19	12.02
Ca	4.40	0.24	428.82	33.15
Mg	1.77	0.08	108.55	14.32
S	0.44	0.02	48.94	4.37
Cu	0.01	0.00	3.57	0.94
Fe	0.46	0.02	508.52	144.40
Mn	0.17	0.01	19.63	1.50
Mo	0.00	0.00	0.17	0.09
Zn	0.95	0.04	4.48	0.16
Na	8.08	0.39	1.35	0.21
Al	1.47	0.08	490.48	14.97

2.1.2. Substrate Treatments

In total, 5 single-container replicates each of the 6 substrate treatments were created by filling 2.5 L black nursery containers (PF310, #1; Nursery Supplies, Inc., Chambersburg, PA, USA) with two layers of pine bark stacked vertically. The bottom layer was FSB, which consisted of 500 cm³ (V1; 4 cm deep) or 1000 cm³ (V2; 7.5 cm deep) pine bark amended with 5.93 kg·m⁻³ pulverized dolomite (ECOPHRST; National Lime and Stone Co., Findlay, OH, USA) and one of three Fe rates: 0.3 (R1), 0.6 (R2), or 1.2 (R3) kg·m⁻³ Fe from granular FeSO₄·7H₂O (QC Corporation, Baltimore, MD, USA). The control, as well as the upper 1500 cm³ (V1; 9.5 cm deep) or 1000 cm³ (V2; 6 cm deep) layer of the 6 treatments, consisted of non-amended pine bark. The purpose of the dolomite, which had a calcium carbonate equivalent of 105% and contained 21% Ca and 12% Mg (by weight), was to buffer pH from the acidifying effects of FS hydrolysis. The initial pH of the FSB, regardless of FS rate, was between 5.9 and 6.3. Dolomite and granular FS were incorporated into the pine bark by evenly distributing the amendments over the 500- or 1000-cm³ pine bark aliquots

and then hand-mixing each for 30 s in a plastic, 36 × 23 × 14 cm (l × w × h) rectangular tub. Amended pine bark incubated for 7 d in sealed, 2 L, high-density polyethylene buckets to allow the Fe to react with the pine bark before the substrate was layered into the nursery containers.

2.1.3. Leachate Collection

Substrate containers were each nested in a 2 L, white, high-density polyethylene leachate bucket, such that the bottom of the substrate container wedged snugly against the inner wall and 6.5 cm above the bottom of the leachate bucket. This design prevented debris and non-target irrigation water from falling directly into the leachate buckets and prevented leachate evaporation between samplings. The leachate buckets were sheathed with sections of black plastic nursery pots to eliminate light penetration and subsequent algae growth in the leachate. The 35 substrate containers nested in 2 L leachate buckets and 5 empty buckets to collect applied fertilizer solution were completely randomized in a 4 × 10 grid with 25 cm spacing between containers on a bench in a glass greenhouse at the Ohio Agricultural Research and Development Center (40.7818° N, 81.9305° W) (Figure 1). The average high and low daily temperatures in the greenhouse over the course of the experiment were (\pm sd) 20.9 ± 1.7 °C and 16.7 ± 1.6 °C, respectively.



Figure 1. Photograph of Experiment 1 substrate containers, spray stakes, and leachate buckets.

Substrate containers were liquid-fertilized daily with a commercially available water-soluble fertilizer (Jack's Professional 20N–4.4P–16.6K Peat-Lite, JR Peters Inc., Allentown, PA, USA) with target N, P, and K concentrations of 100, 21.8, and 83 mg·L⁻¹, respectively. Concentrated liquid fertilizer stock was mixed into reverse-osmosis (RO) water at a 1:100 ratio using an injector (model D14MZ2, Dosatron International, Clearwater, FL, USA) and applied using 6.9 mL·s⁻¹ (6.6 GPH) double-spray-pattern, pressure-compensating spray stakes (Netafim USA, Fresno, CA, USA) that had been trimmed to 8 cm in length and inserted into the substrate in the center of each container. An 8-gauge steel wire was threaded through the container lips on opposite sides of the container and around the spray stake to ensure spray stakes remained vertical and at the same height over the course of the experiment. The first liquid fertilization consisted of four 10 s cycles with ≈3 min rest periods to bring the substrate to container capacity; thereafter, containers were

automatically liquid-fertilized daily for 15 weeks (105 d) at 0730 hr in three 4 s cycles with 5 min rest periods. Spray stakes were twisted 90° every 7 d to improve substrate wetting uniformity and ensure all substrate was in contact with the fertilizer solution. Buckets containing leachate or applied fertilizer solution were weighed every 7 d to approximate leachate and applied fertilizer volume (i.e., 1 g \approx 1 mL). Fifty-milliliter samples were subsequently collected from each bucket and analyzed for P and Fe concentrations and pH. Remaining leachate was discarded, and leachate buckets were replaced with clean ones.

Leachate pH was measured immediately after collection using a pH/ion analyzer (MA 235; Metler Toledo, Columbus, OH, USA). A portion of each leachate sample was stored at -20°C until the end of the study (91 d) before being thawed, digested, and analyzed for P and Fe concentrations using ICP-OES. Leachate samples were digested with HNO_3 and HCl facilitated with a microwave digestion system (MARS 5.0, CEM Corporation, Matthews, NC, USA) according to USEPA Method 3015A [38]. An additional 10 mL subsample from each replicate of the applied fertilizer solution was syringe-filtered through a 0.45 μm PES membrane and analyzed for $\text{PO}_4\text{-P}$ concentrations via an ion chromatography system (Dionex ICS-6000; Thermo Fisher Scientific, Waltham, MA, USA) each week to confirm target nutrient concentrations were being applied.

2.1.4. P and Fe Remaining in Pine Bark

At 15 weeks after experiment initiation (WAI), the upper and lower substrate layers were individually removed from each container. The substrate within control containers was divided like V1 treatments (i.e., the bottom 500 cm^3 was collected separately from the upper 1500 cm^3). Saturated media extracts were then performed on 200 cm^3 substrate samples collected from each layer to measure pH and water-soluble Fe and P concentrations, as described in Section 2.1.1. The substrate initial moisture content and volume of DI water added for SMEs was recorded and later used to calculate the mass of water-extractable P and Fe in each layer. Total Fe and P concentration and content in each layer were determined as described in Section 2.1.1.

2.2. Experiment 2: Effect of Dolomite and FS Form

2.2.1. Substrate Treatments

Pine bark, FS, dolomite, and nursery containers used in Experiment 2 were the same as those used in Experiment 1. Experiment 2 included five single-container replicates each of a control (i.e., no amendments) and four FSB treatments consisting of a 500 cm^3 base layer (4 cm deep, 25% of total substrate volume) of pine bark amended with 0.6 $\text{kg}\cdot\text{m}^3$ Fe from either granular (GFS) or liquid (LFS) FS and with (+DL) or without (−DL) 5.93 $\text{kg}\cdot\text{m}^3$ pulverized dolomite. Ferrous sulfate heptahydrate solutions for the LFS treatment were made by dissolving 1.48 g granular FS in 86 mL of 0.004 M H_2SO_4 to acidify the solution to $\text{pH} < 2.5$, minimizing Fe^{2+} oxidation and subsequent precipitation of Fe^{3+} hydroxides prior to mixing into the substrate [39]. The dolomite and granular or liquid FS were incorporated into the pine bark by evenly distributing the amendments over 500 cm^3 pine bark aliquots and then hand-mixing each for 30 s. To mimic industry conditions, no additional steps were taken to prevent the oxidation of Fe^{2+} once the FS was mixed into the substrate. To ensure all treatments had the same initial moisture content (i.e., 75% by weight), treatments that did not receive liquid FS were moistened with 86 mL deionized water prior to adding amendments. Amended pine bark incubated for 7 d in sealed high-density polyethylene buckets to allow Fe to react with pine bark before substrate was layered into nursery pots and irrigated.

The upper portion of the substrate profile in all treatments consisted of a 1500 cm^3 layer (9.5 cm deep, 75% of total substrate volume) of pine bark amended with 4.45 g polymer-coated CRF (117 mg P; 19N–2.6P–10.8K, 3- to 4-month release, Harrell's, Lakeland, FL, USA) which is equivalent to the manufacturer-recommended medium incorporation rate of 2.97 $\text{g}\cdot\text{L}^{-1}$. Dolomite, FS, and CRF were weighed and thoroughly hand-mixed into pine bark separately for each container to ensure independence of replicates.

The 25 substrate containers, as well as 5 additional empty, 2 L buckets to collect applied irrigation water, were completely randomized in a 5 × 6 grid with 25 cm spacing between containers on a bench in a glass greenhouse at the Ohio Agricultural Research and Development Center (Figure 2). Average maximum and minimum daily temperatures in the greenhouse over the course of the experiment were (\pm sd) 36 ± 4.5 °C and 21 ± 2.5 °C, respectively. Substrate containers were each overhead irrigated with municipal water applied via a full-cone sprayer tip (TG-1, TeeJet Technologies, Springfield, IL, USA) with a diaphragm-type anti-drip valve mounted ≈ 25 cm above the substrate surface of each substrate container. Water pressure was regulated at 137.9 kPa, resulting in a nozzle flow rate of $7.2 \text{ mL}\cdot\text{s}^{-1}$. The first irrigation consisted of three 6 s cycles with 5 min rest periods which brought the substrate to container capacity with minimal leaching. Every 24 h thereafter for 19 weeks, substrate containers were irrigated in two 6 s cycles with 5 min rest periods resulting in 0.45 cm water applied daily.



Figure 2. Photograph of Experiment 2 substrate containers, irrigation system, and leachate buckets. Photograph was taken prior to sheathing the white leachate buckets with black plastic.

2.2.2. Leachate Collection

Substrate containers were nested inside leachate collection buckets as previously described in Section 2.1.2. Irrigation collection buckets were covered with lids that had a 1 cm hole in the center of each, and the buckets were elevated such that the sprayer tip housing plugged the hole allowing all sprayed irrigation water to be captured inside the bucket for subsequent measurements while minimizing evaporation. Every 7 d, the leachate volume from each substrate container was recorded, and a 50 mL sample was collected from each to analyze for P and Fe concentrations and pH. The remaining leachate was discarded and leachate buckets were replaced with clean ones.

Methods and equipment used to analyze leachate P and Fe concentrations and pH were the same as in Experiment 1, with the following exceptions. Leachate P and Fe concentrations were determined within 4 h of collection in both filtered ($0.45 \mu\text{m}$ polyvinylidene difluoride membrane; Millipore Sigma, Burlington, MA, USA) and non-filtered, digested (USEPA Method 3015A [38]) samples via ICP-OES. The purpose of analyzing both filtered and non-filtered digested leachate was to determine whether particulate P forms (e.g., $\text{Fe}\cdot\text{PO}_4$ precipitates) were leaching from nursery pots due to added Fe.

2.2.3. P remaining in Pine Bark

At 19 WAI, the lower and upper substrate layers were separately removed from containers, and CRF-free substrate samples were analyzed for pH and water-soluble Fe and P content in SMEs as described in Experiment 1. All CRF was removed from the substrate prior to performing SMEs. The initial substrate moisture content and volume of DI water added for SMEs was recorded, allowing for the calculation of mg extractable nutrients per unit substrate volume. A modified diethylenetriaminepenta-acidic acid (DTPA) SME [36] was also performed on a substrate sample from the FSB and analyzed for Fe and P concentrations.

2.3. Statistical Analysis

Experiment 1 leachate P concentration data were subjected to a two-way repeated measures analysis of variance (ANOVA) with one between-subjects factor, substrate treatment (control, V1R1, V1R2, V1R3, V2R1, V2R2, and V2R3) and a repeated measures factor, time (weeks 1–15). The repeated measures analysis was accomplished via covariance structure modeling [40], in which the most appropriate covariance structure was selected by fitting data to various homogeneous and heterogeneous covariance structures available in the software package (JMP Pro 17, SAS Institute Inc., Cary, NC, USA) and subsequently comparing corrected Akaike information criterion (AIC_c) values. The antedependent structure provided the lowest AIC_c values and was, therefore, used to determine significance of the time \times substrate treatment interaction and the component main effects. Slight differences in flow rate among individual fertigation emitters, which occurred randomly across treatments but were consistent over time, resulted in a higher cumulative volume leached from some containers than others by week 15. Analysis of covariance (ANCOVA) was used to analyze substrate treatment effects on cumulative P leached while controlling for the covariate, leachate volume. Cumulative leached Fe mass data were log-transformed to correct for non-constant variance prior to ANOVA and post hoc means separation, but means from the original non-transformed values were reported. Phosphorus mass that remained in the substrate in soluble or insoluble form were analyzed using one-way ANOVA, and post hoc means separation was determined using Tukey's honestly significant difference test. The relationship between the P and Fe concentration in the substrate at week 15 was assessed using linear regression.

In Experiment 2, the substrate treatment effect on the linear relationship between total P (x) and dissolved P (y) was assessed by determining the significance of the treatment \times total P interaction. A one-way ANOVA was performed followed by single degree-of-freedom contrasts to partition sums of squares for the main effects of FS form and DL presence and their interaction on cumulative P leached per container and water- or DTPA-extractable P remaining in the substrate [41]. Single degree-of-freedom contrasts were also used to compare the control to specific groups of treatments (e.g., control vs. treatments with DL, control vs. treatments without DL). A *t*-test was used to assess the effect of extractant (water vs. DTPA) on P content extracted from the bottom substrate layer. Leachate P concentrations were subjected to a two-way repeated measures ANOVA with one between-subjects factor, treatment (control, GFS + DL, LFS + DL, GFS – DL, and LFS – DL), and a repeated measures factor, time (weeks 1–19). The repeated measures analysis was accomplished via covariance modeling as described previously. When the treatment \times time interaction was significant, single degree-of-freedom contrast statements were performed to assess the FS form and DL presence main effects and the FS form \times DL presence interaction for each of the 19 sampling times. Contrast statements were also used to compare the control to all other substrate treatments or specific groups of treatments at each sampling time. All statistical analyses were carried out using statistical software (JMP Pro 17, SAS Institute Inc.).

3. Results

3.1. Experiment 1

3.1.1. Leachate Phosphorus

The repeated measures ANOVA indicated leachate P concentration was significantly affected by treatment, time, and the treatment \times time interaction ($p < 0.001$). Accordingly, treatment effects on leachate P concentration were analyzed and reported at each level of time (Table 3). The applied P concentration measured in the fertilizer collection buckets each week ranged from 21.9 to 23.4 mg·L⁻¹ and averaged 22.6 ± 0.5 sd mg·L⁻¹ P over the course of the experiment. The leachate P concentration from the control was 10.7 mg·L⁻¹ lower than the fertilizer concentration at week 1; however, it increased over time and was within 3 mg·L⁻¹ of the fertilizer-P concentrations from week 6 to 15. The V1R1 treatment had the lowest effect on leachate P, with 20% to 42% lower leachate P concentrations than the control until 6 WAI, then similar concentrations to the control from 7 to 15 WAI. Treatments V1R2 and V2R1 reduced P leaching relative to the control for the first nine weeks, after which leachate P concentrations were similar to those from the control with a few exceptions (i.e., weeks 12 and 15 for V1R2). Leachate P concentrations from V1R3, V2R2, and V2R3 were lower than those from the control at all 15 sampling periods, and V2R3 generally had lower leachate P concentrations than all treatments from 7 to 15 WAI.

Table 3. Weekly phosphorus (P) concentrations applied (20N–4.4P–16.6K–0.1Fe water-soluble fertilizer) or leached from 2.5 L nursery pots containing 2000 cm³ pine bark substrate with the bottom 500 (V1) or 1000 (V2) cm³ amended with 5.9 kg·m⁻³ dolomite and 0.3 (R1), 0.6 (R2), or 1.2 (R3) kg·m⁻³ ferrous iron (Fe²⁺) from FeSO₄·7H₂O. The control and upper 1500 (V1) or 1000 (V2) cm³ of substrate comprised non-amended pine bark.

Treatment	Week														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fertilizer	21.9	22.5	22.6	22.2	22.3	22.4	23.1	23.4	23.0	22.4	22.4	22.9	22.9	22.2	23.0
Control	11.2 a ^z	12.9 a	18.1 a	17.7 a	18.8 a	21.1 a	20.6 a	20.7 a	21.2 a	21.6 a	21.7 a	21.8 a	22.7 a	22.5 a	25 a
V1R1	6.5 bc	5.6 b	9.8 b	10.7 b	13.4 b	16.8 b	17.7 ab	18.9 a	19.6 a	20.7 a	21.1 a	21 ab	22.5 a	22.2 a	24.3 ab
V1R2	4.7 cd	5.7 b	9.5 b	10.4 bc	12.5 b	13.0 c	14.9 abc	15.8 b	16.5 b	17.9 ab	18.4 a	18.4 bc	19.9 a	20.0 a	21.5 bc
V1R3	3.9 de	3.2 bc	5.9 bc	6.8 bcd	5.9 cd	9.0 de	11.2 c	9.7 c	11.2 c	11.9 cd	12.8 b	13.6 d	15.5 b	16.2 b	17.2 d
V2R1	7.4 b	3.5 bc	6.4 bc	6.7 cd	8.8 c	11.4 cd	14 bc	14.7 b	16.6 b	17.3 ab	19.6 a	19.8 ab	20.5 a	20.1 a	22.7 ab
V2R2	4.4 cde	2.4 bc	3.7 cd	4.4 de	4.8 d	7 ef	9.7 cd	10.2 c	11.7 c	14.2 bc	14.3 b	15.6 cd	16.1 b	16.8 b	18.5 cd
V2R3	2.3 e	1.0 c	0.9 d	1.4 e	3.7 d	4.8 f	4.1 d	4.7 d	6.2 d	7.7 d	7.8 c	8.9 e	9.8 c	9.7 c	11.2 e
<i>p</i> -value Fert. vs. control ^y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	<0.0001	<0.0001	<0.0001	0.0005	<0.0001	0.0024	0.0370	<0.0001	0.0001	0.0213	0.2274	0.0120	0.3913	0.5459	0.0064

^z Means, excluding fertilizer, followed by the same letter within a column are not significantly different (Tukey's Honest Significant Difference test; $\alpha = 0.05$). ^y *p*-values from *t*-tests comparing applied fertilizer-P concentration means ($n = 5$) to mean P concentrations leached from the control ($n = 5$) within each week.

Cumulative P leached per container over time is illustrated in Figure 3. In all FSB treatments, P accumulated in the leachate more slowly at the beginning of the study than at the end. After 15 weeks of daily fertilization, each container received a total of ≈ 199 mg P, 65% of which leached from the control containers. All treatments containing FSB leached less P than the control, ranging from a 22% reduction in V1R1 to a 73% reduction in V2R3 by 15 WAI (Figure 3). Among FSB treatments, increasing the Fe rate within an FSB volume or increasing the FSB volume within an Fe rate decreased P leaching. However, treatments containing the same mass of Fe as a result of inversely proportional Fe rates and FSB volumes (e.g., V1R2 versus V2R1 and V1R3 versus V2R2) leached similar amounts of P.

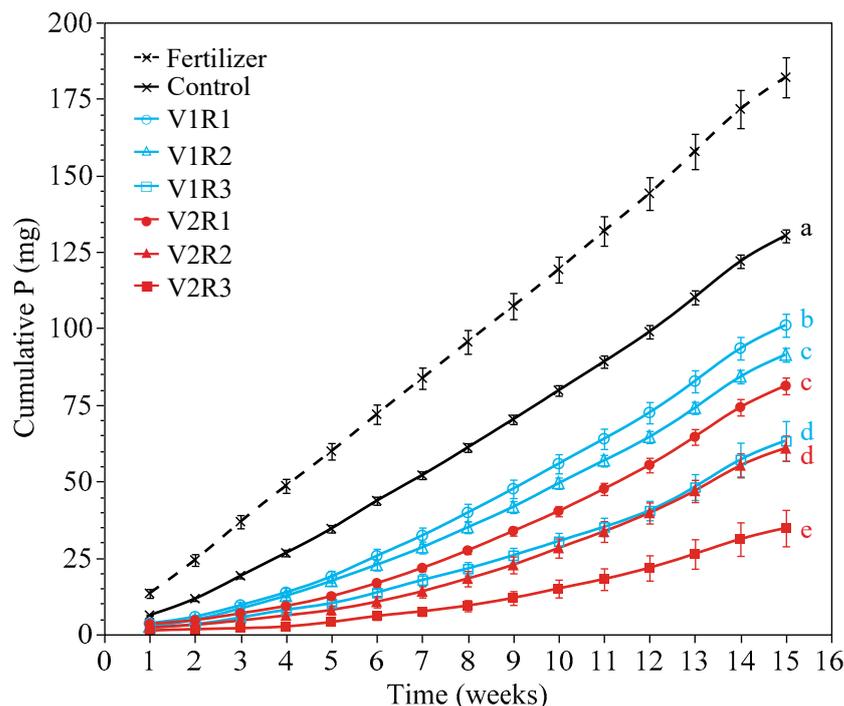


Figure 3. Mean (\pm SE) weekly cumulative phosphorus (P) mass applied to (dotted line; 20N–4.4P–16.6K–0.1Fe water-soluble fertilizer) or leached from (solid lines) 2.5 L nursery pots containing 2000 cm³ pine bark substrate with the bottom 500 (V1) or 1000 (V2) cm³ amended with 5.9 kg·m⁻³ dolomite and 0.3 (R1), 0.6 (R2), or 1.2 (R3) kg·m⁻³ ferrous iron (Fe²⁺) from FeSO₄·7H₂O. The control and upper 1500 or 1000 cm³ of substrate comprised non-amended pine bark. Cumulative P mass leached at 15 WAI was not significantly different among treatments with the same corresponding letter(s) according to Tukey’s Honestly Significant Difference test ($p \leq 0.05$).

3.1.2. Substrate Phosphorus

Soluble P concentrations in the top substrate layer measured after the final liquid fertilizer application were affected by FSB volume and Fe rate, but the FSB volume \times Fe rate interaction was not significant (Table 4). Soluble P concentrations in the top substrate layer of V2 treatments were 40% higher than those in the top layer of V1 treatments, and P concentrations in the top layer decreased linearly with increasing Fe rate in the bottom layer. Soluble P concentrations in the top layer of the control did not differ significantly from those in the top layer of the FSB containers.

Similar to the top substrate layer, soluble P concentrations measured in the FSB layer after the final liquid fertilizer application were affected by FSB volume and Fe rate, but the FSB volume \times Fe rate interaction was not significant (Table 4). Soluble P concentrations were 33% lower in the V1 compared to the V2 FSB layer, and increasing the Fe rate from 0.3 (R1) to 1.2 kg·m⁻³ (R3) resulted in a linear decrease in soluble P. The soluble P concentration in the bottom 500 cm³ of the control substrate was higher than all other pooled means and treatment means except for R1 (pooled across FSB volume) and V1R1, in which soluble P concentrations were similar. The 130 mg P that leached from control containers (Figure 3) in addition to 29.8 mg soluble P remaining in the substrate [(16 $\mu\text{g}\cdot\text{cm}^{-3} \times 1500 \text{ cm}^3$) + (11.5 $\mu\text{g}\cdot\text{cm}^{-3} \times 500 \text{ cm}^3$)/1000; calculated from Table 4] at the end of the study equates to 159.8 mg P (80% of applied P) that was not sorbed by the substrate. The insoluble P concentration in the FSB layer, pooled across treatments, had a positive linear relationship with insoluble Fe concentration in the FSB layer (Figure 4).

Table 4. Effect of a 500 cm³ (V1) or 1000 cm³ (V2) layer of FeSO₄·7H₂O-amended pine bark (FSB) with 0.3 (R1), 0.6 (R2), or 1.2 (R3) kg·m⁻³ ferrous iron (Fe²⁺) and 5.9 kg·m⁻³ dolomite in the bottom of 2.5 L nursery containers on water-soluble P concentrations in the bottom or top substrate layer after being irrigated with 20N–4.4P–16.6K–0.1Fe water-soluble fertilizer daily for 15 weeks. The control and upper 1500 or 1000 cm³ of substrate comprised non-amended pine bark.

Treatment	Substrate P Conc.	
	Top	Bottom
	μg·cm ⁻³	
Control	16.0	11.5
FSB vol. pooled means		
V1	14.0	7.9 ^{*,z}
V2	19.8	6.3 ^{**}
Fe rate pooled means		
R1	18.7	9.8
R2	17.4	7.7 ^{***}
R3	14.7	3.5 ^{***}
FSB vol. × Fe rate		
V1R1	15.5	11.4
V1R2	14.2	8.5 ^{**}
V1R3	12.3	3.9 ^{***}
V2R1	21.9	8.3 ^{***}
V2R2	20.5	6.9 ^{***}
V2R3	17.1	3.1 ^{***}
Significance ^y		
FSB volume	***	***
Fe rate	L *	L ***
Interaction	NS	NS

^z Asterisks adjacent to means indicate values are significantly different from the control within the same column according to Dunnett's test at $\alpha = 0.05$ (*), 0.01 (**), or 0.001 (***). ^y Significance of single degree-of-freedom contrast statements to test main effects of and interaction between FSB volume and Fe rate while excluding the control. Contrasts were also used to determine the significance of the linear (L) and/or quadratic (Q) relationship between Fe rate and soluble or insoluble P. Asterisks indicate significance at $\alpha = 0.05$ (*), 0.01 (**), or 0.001 (***). NS indicate non-significant ($p > 0.05$) differences.

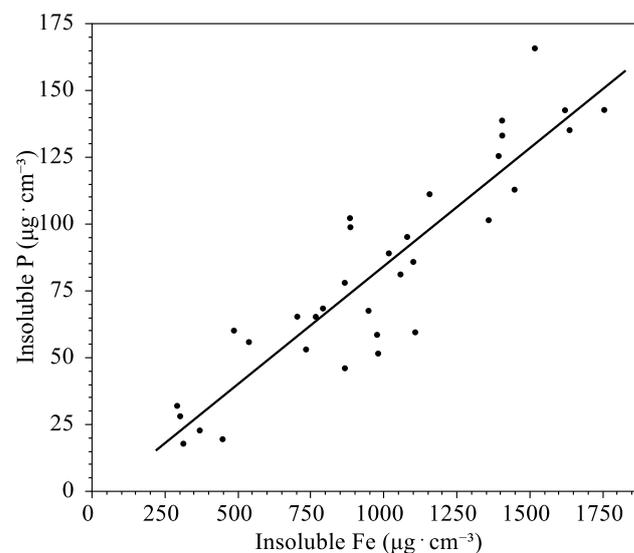


Figure 4. Insoluble phosphorus (P) concentration measured in a 500 or 1000 cm³ bottom layer of FeSO₄·7H₂O-amended pine bark (FSB) in 2.5 L nursery containers linearly regressed over insoluble iron (Fe) concentration in the FSB layer after receiving a total of ≈ 199 mg P applied over the course of 15 weeks from daily applications of 20N–4.4P–16.6K–0.1Fe water-soluble fertilizer. The 500 or 1000 cm³ FSB layer was beneath a 1500 or 1000 cm³ layer, respectively, of non-amended pine bark. Data were pooled across FSB volume. Insoluble P = $-4.052 + 0.088 \times$ Insoluble Fe; $R^2 = 0.83$.

3.1.3. Leachate Iron

Leachate Fe over time stayed within a narrow concentration range of between 0.3 and 2.6 mg·L⁻¹ in all treatments, including the control (Figure 5a). The R1 treatments, particularly V2R1, tended to have lower leachate Fe concentrations than R3 treatments; however, this relationship was not consistent over time, and substrate treatments were always within 1.5 mg·L⁻¹ of the control. The cumulative Fe mass that leached by week 15 differed among treatments, with lowest the Fe amounts leaching from V1R1, V2R1, and V2R2 (Figure 5b). Interestingly, the control, which received no FS, leached more Fe than the V2R1 treatment.

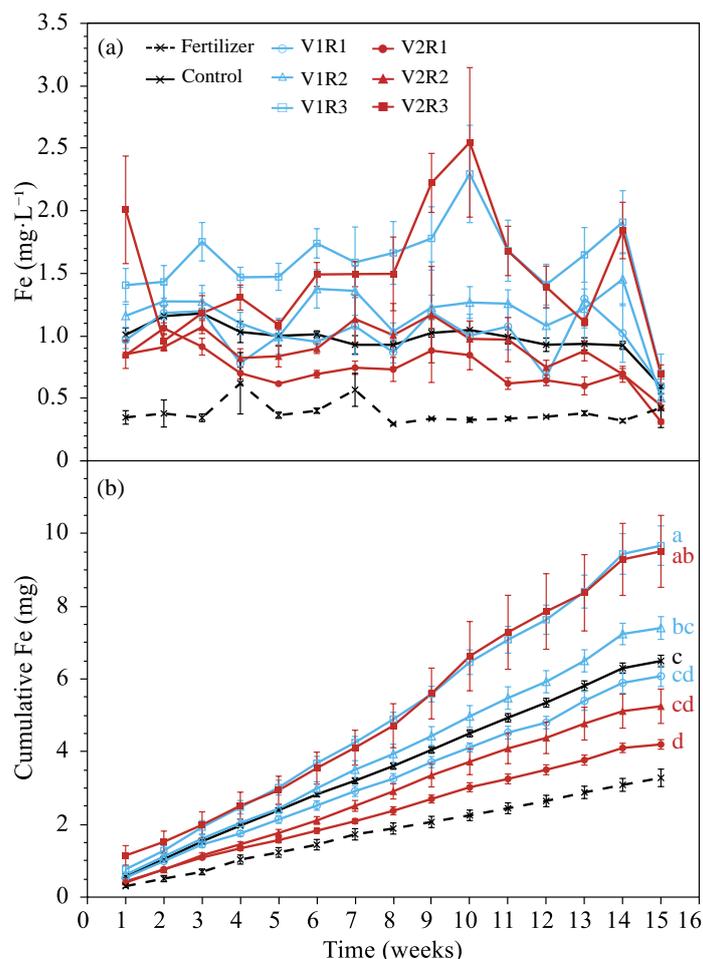


Figure 5. Mean (\pm SE) weekly total iron (Fe) concentration (a) and cumulative mass (b) applied to (dotted line; 20N–4.4P–16.6K–0.1Fe water-soluble fertilizer) or leached from (solid lines) 2.5 L nursery pots containing 2000 cm³ pine bark substrate with the bottom 500 (V1) or 1000 (V2) cm³ amended with 5.9 kg·m⁻³ dolomite and 0.3 (R1), 0.6 (R2), or 1.2 (R3) kg·m⁻³ ferrous iron (Fe²⁺) from FeSO₄·7H₂O. The control and upper 1500 or 1000 cm³ of substrate comprised non-amended pine bark. Cumulative Fe mass leached at 15 WAI was not significantly different among treatments with the same corresponding letter(s) according to Tukey’s Honest Significant Difference test ($p \leq 0.05$).

3.1.4. Leachate pH

Leachate pH from the control remained between 4.0 and 4.2 over the course of the experiment (Figure 6). Among FSB treatments, leachate pH during the first 9 WAI was generally lowest in R3 treatments and highest in V1R1; pH values of all other treatments were intermediate or similar to V1R1 depending on the week. After 9 WAI, leachate pH values were similar among FSB treatments until 15 WAI during which V1R1 leachate pH was approximately one unit lower than that from V2R1 (5.5 vs. 6.5). Leachate pH of all FSB

treatments increased for the first 4 to 6 weeks and then was generally constant with the exception of V1R1 which decreased from 6.9 at week 11 to 5.5 at week 15.

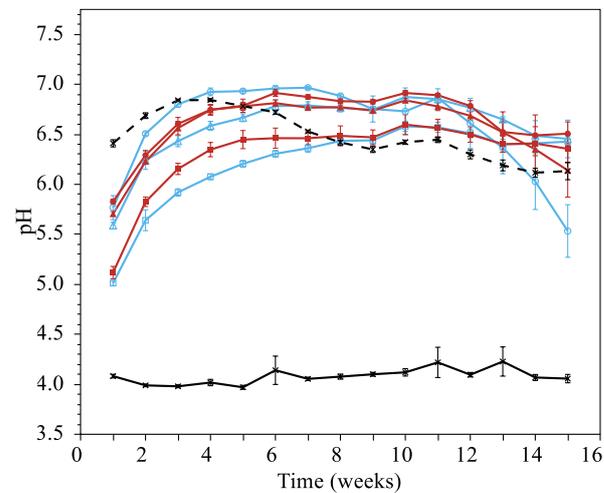


Figure 6. Mean (\pm SE) weekly pH of the applied fertilizer solution (dotted line; 20N–4.4P–16.6K–0.1Fe water-soluble fertilizer) and leachate (solid lines) from 2.5 L nursery pots containing 2000 cm³ pine bark substrate with the bottom 500 (V1) or 1000 (V2) cm³ amended with 5.9 kg·m⁻³ dolomite and 0.3 (R1), 0.6 (R2), or 1.2 (R3) kg·m⁻³ ferrous iron (Fe²⁺) from FeSO₄·7H₂O. The control and upper 1500 or 1000 cm³ of substrate comprised non-amended pine bark.

3.2. Experiment 2

3.2.1. Leachate Phosphorus

In the linear model equating dissolved (i.e., filtered to <0.45 μ m) P from total (i.e., digested) P, the treatment \times total P interaction term was not significant ($p = 0.648$); therefore, a single regression, pooled across treatments, is reported (Figure 7). Dissolved and total P concentrations in the leachate had a strong linear relationship ($R^2 = 0.994$).

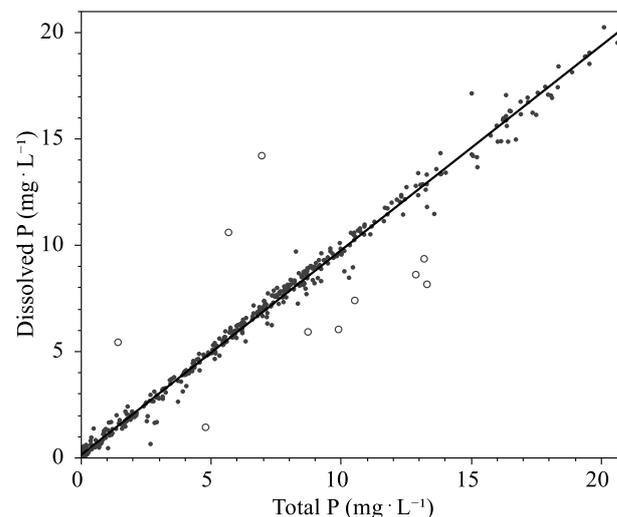


Figure 7. Dissolved P regressed over total P concentrations in leachate collected weekly for 19 weeks from nursery containers in which the bottom 500 cm³ was filled with non-amended pine bark (control) or pine bark with FeSO₄·7H₂O (FS; 0.6 kg·m⁻³ Fe²⁺) mixed into the substrate in granular or liquid form with or without 5.93 kg·m⁻³ dolomite (DL). The upper 1500 cm³ of pine bark in the control and four substrate treatments contained 4.45 g/pot (3 kg·m⁻³) of a 3- to 4-month controlled-release fertilizer (19N–2.6P–10.8K). Data were pooled across substrate treatments. Open circles had externally studentized residual values of >4 or <-4 and were removed from the analysis as outliers. Dissolved P = 0.1540987 + 0.9622062 \times Total P; $R^2 = 0.994$.

Phosphorus concentration in leachate was affected by a significant treatment × time interaction ($p < 0.05$) according to the repeated measures analysis. However, the main effects of DL presence and FS form and their interaction were not significant at any sampling date (Table 5). Accordingly, leachate P concentrations were pooled across FSB treatments and compared to those of the control (Figure 8a). The FSB treatments had lower leachate P concentrations than the control at all but the final three sampling dates (i.e., weeks 17, 18, and 19), during which there were no differences. The P leaching pattern over time differed between the control and FSB substrates. Leachate P concentrations from the control increased until week 10, peaking at 18.6 mg·L⁻¹ P, then decreased through week 19. For the FSB treatments, leachate P concentrations increased between weeks 1 and 10, then remained between 7.6 and 6.9 mg·L⁻¹ through week 19.

Table 5. Significance of single degree-of-freedom contrasts for the effect of FeSO₄·7H₂O (FS) form, dolomite (DL) presence, and the FS form × DL interaction on leachate phosphorus (P) and iron (Fe) concentrations and pH collected weekly for 19 weeks from 2.5 L nursery pots in which the bottom 500 cm³ was filled with non-amended pine bark (control) or pine bark with FS (0.6 kg·m⁻³ Fe²⁺) mixed into the substrate in granular or liquid form with or without 5.93 kg·m⁻³ DL. The upper 1500 cm³ of pine bark in the control and 4 substrate treatments contained 4.45 g/pot (3 kg·m⁻³) of a 3- to 4-month controlled-release fertilizer (19N–2.6P–10.8K).

Analyte	Source	Week																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
P	Main effect (DL)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Main effect (FS form)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Interaction (DL × FS form)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Control vs. all others ^z	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	*	NS	NS	NS
Fe	Main effect (DL)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
	Main effect (FS form)	*	NS																	
	Interaction (DL × FS form)	**	NS																	
	Control vs. all others	***	***	***	**	**	**	***	***	***	***	***	***	***	***	***	***	***	***	***
	Control vs. –DL treatments	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
pH	Control vs. +DL treatments	***	***	**	***	***	**	***	***	NS	NS	*	NS							
	Main effect (DL)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
	Main effect (FS form)	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS								
	Interaction (DL × FS form)	NS	NS	NS	*	NS	NS	**	NS	NS	*	NS								
	Control vs. all others	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Control vs. –DL treatments	***	***	***	***	***	***	***	***	***	***	***	***	**	***	**	**	**	**	**	
Control vs. +DL treatments	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	

^z Contrast statements were used to compare the control to pooled treatments. *, **, *** $p < 0.05, 0.01, \text{ or } 0.001$. NS indicate non-significant ($p > 0.05$) differences.

By week 10, 52% and 26% of the total P mass that leached from the control and FSB treatments, respectively, was recovered in the leachate, at which point FSB treatments had reduced the P mass leached by 81% relative to the control (4.2 vs. 21.8 mg P; Figure 8b). By week 19, 42.2 and 15.7 mg P had leached from the control and FSB treatments, respectively, indicating that the FSB layer sorbed 63% of the P that would have otherwise leached from the containers. This reduction equates to 0.053 mg P sorbed per cm³ FSB or 17.9 mg P sorbed per gram of FeSO₄·7H₂O added.

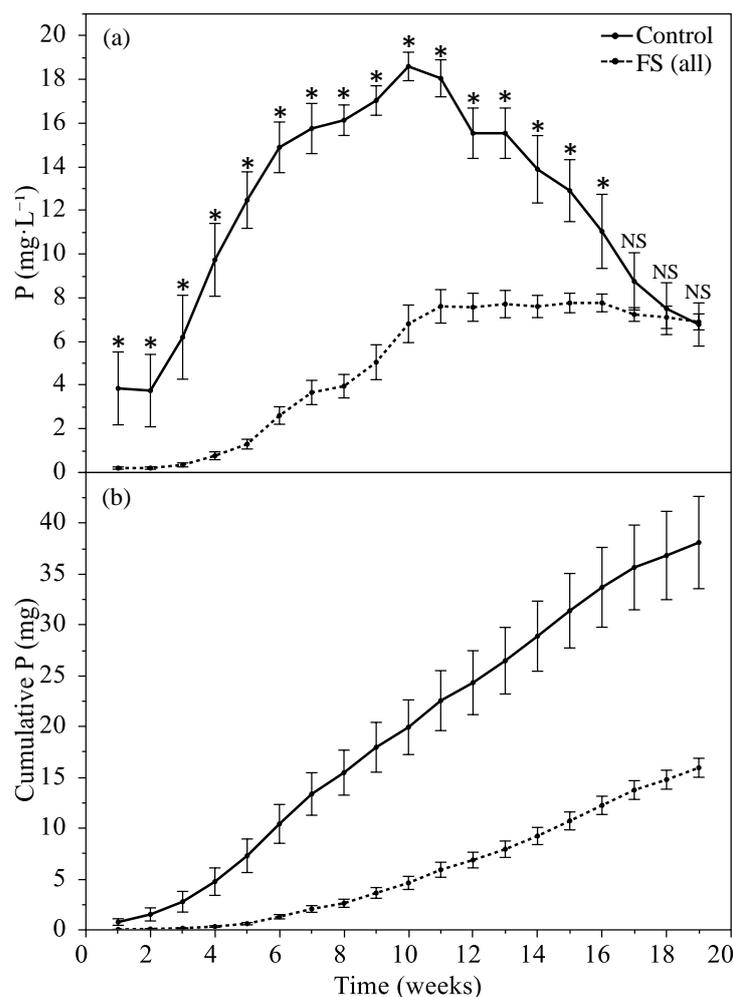


Figure 8. Mean (\pm SE) weekly phosphorus (P) concentration (a) and cumulative mass (b) in leachate per 2.5 L nursery pot in which the bottom 500 cm³ was filled with non-amended pine bark (control) or pine bark with FeSO₄·7H₂O (FS; 0.6 kg·m⁻³ Fe²⁺) mixed into the substrate in granular or liquid form with or without 5.93 kg·m⁻³ dolomite (DL). The upper 1500 cm³ of pine bark in the control and four substrate treatments contained 4.45 g/pot (3 kg·m⁻³) of a 3- to 4-month controlled-release fertilizer (19N–2.6P–10.8K). Treatments containing FS were pooled across Fe form and DL treatments (n = 20). Asterisks and NS indicate significant ($p \leq 0.05$) and non-significant ($p > 0.05$) differences, respectively, in P concentration between the control and FS treatments determined by *t*-tests.

3.2.2. Substrate Phosphorus

The FS form and FS form \times DL interaction had no significant effect on post-experiment water-extractable P in the upper substrate layer or on water- or DTPA-extractable P in the bottom layer (Table 6). Substrate P data were, therefore, pooled across FS form to show the main effects of DL and single degree-of-freedom contrasts between the pooled DL treatments and the control. Water-extractable P in the top substrate layer was similar among the control and +DL and –DL FSB treatments. In the bottom substrate layer, +DL FSB treatments had 25% less water-soluble P compared to –DL FSB treatments, but none of the FSB treatments differed from the control. DTPA-extractable P in the bottom layer was higher in the –DL FSB treatments than either the control or the +DL FSB treatments. In addition, the bottom layer had higher DTPA-extractable P than water-extractable P in the +DL and –DL FSB treatments, whereas water- and DTPA-extractable P was similar in the control.

Table 6. *p*-values from a one-way analysis of variance and contrast statements to assess significance of FeSO₄·7H₂O (FS) form (0.6 kg·m⁻³ Fe from granular- or liquid-applied FS), dolomite presence [+DL (5.9 kg·m⁻³) or -DL], and the FS form × dolomite presence interaction on cumulative phosphorus mass leached or remaining in the upper 1500 cm³ (pine bark with controlled-release fertilizer ^z only) or lower 500 cm³ layer of substrate (FS-amended pine bark) after 19 weeks of daily irrigation. Contrasts were also used to compare pooled treatments to the control ^y.

Source	Leachate	Substrate		
		Top Layer	Bottom Layer	
		Water Extract ^x	Water extract	DTPA Extract ^w
Treatment	<0.0001	0.4271	0.1043	0.0141
<i>p</i> -values				
Contrasts				
Main effect (DL)	0.6503	0.2097	0.0143	0.0145
Main effect (FS form)	0.6398	0.1961	0.9216	0.2435
Interaction (DL × FS form)	0.6401	0.8463	0.2377	0.5311
Control vs. all others	<0.0001	0.4803	0.6966	0.0132
Control vs. -DL treatments	<0.0001	0.8623	0.5222	0.0022
Control vs. +DL treatments	<0.0001	0.2614	0.1820	0.1465
Pooled means ^v				
	mg P/container		mg P/layer	
Control	42.2	21.4	5.1	4.8
-DL	15.2	22.5	5.5	8.3 **
+DL	16.3	29.2	4.1	6.3 ***

^z The CRF was polymer-coated 19N-2.6P-10.8K and was mixed into the substrate at a rate of 3 kg·m⁻³. ^y The control consisted of a 1500 cm³ top layer of CRF-amended pine bark and a bottom 500 cm³ layer of non-amended pine bark. ^x Water-soluble P content in the substrate layer determined by measuring the P concentration in 200-cm³ substrate samples via saturated media extracts (SMEs; [36]) and then converting P concentration (mg·L⁻¹) to P mass per container (mg) given the known volume of water used for SMEs and initially present in the substrate. ^w P content in the bottom layer extracted by a modified DTPA SME [36]; P concentration was converted to P mass as previously described for the water-soluble P. ^v Treatments with FS were pooled across FS form (granular- or liquid-applied) such that *n* = 5, 10, and 10 for the control, FS - DL, and FS + DL treatments, respectively. *, **, and *** in the DTPA extract column indicate a significant difference (*t*-test) between water- and DTPA-extractable P in the bottom layer at α = 0.05, 0.01, or 0.001, respectively.

3.2.3. Leachate Iron

The repeated measures ANOVA indicated that leachate Fe concentration was affected by treatment, time, and the treatment × time interaction (*p* < 0.05). However, within each level of time, neither the FS form nor FS form × DL interaction were significant except at week 1 (Table 5). Mean leachate Fe concentrations for the +DL and -DL treatments pooled across FS form were compared to the control at each sampling week (Figure 9a). During the first 8 weeks, the FSB treatments containing no dolomite leached Fe concentrations that were 33× to 104× and 14× to 33× the concentrations that leached from the dolomite-amended FSB treatments and control, respectively. From 9 to 19 WAI, leachate Fe concentrations in the FSB treatments without dolomite were 5× to 10× the concentrations in the dolomite-amended FSB treatments and control. Leachate Fe concentrations from -DL FSB treatments decreased sharply between 2 WAP (4.1 mg·L⁻¹) and 6 WAP (1.1 mg·L⁻¹), then remained between 1.2 and 0.6 mg·L⁻¹ for the remainder of the experiment, whereas Fe from the control and +DL FSB treatments fluctuated over the course of the experiment but were always <0.18 mg·L⁻¹.

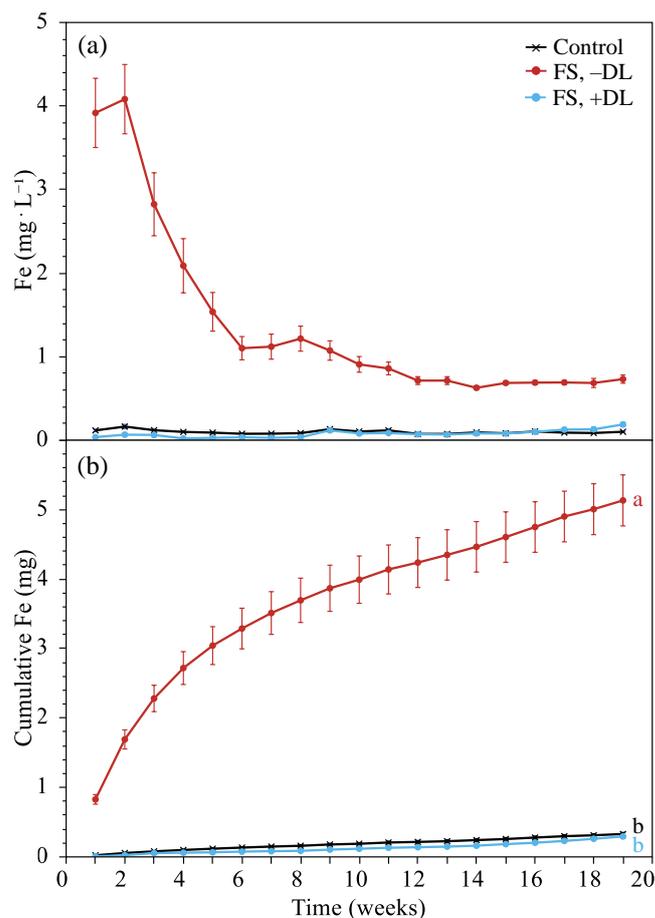


Figure 9. Mean (\pm SE) weekly total iron (Fe) concentration (a) and cumulative mass (b) leached per 2.5 L nursery pot in which the bottom 500 cm³ was filled with of non-amended pine bark (control) or pine bark with FeSO₄·7H₂O (0.6 kg·m⁻³ Fe²⁺) mixed into the substrate in granular or liquid form with or without 5.93 kg·m⁻³ dolomite (DL). The upper 1500 cm³ of pine bark in the control and four substrate treatments contained 4.45 g/pot (3 kg·m⁻³) of a 3- to 4-month controlled-release fertilizer (19N-2.6P-10.8K). Plotted FS-DL and FS + DL treatments were pooled across FS form treatments ($n = 10$). Cumulative Fe mass leached at 15 WAI was not significantly different among treatments with the same corresponding letter(s) according to Tukey's Honest Significant Difference test ($p \leq 0.05$).

Of the 298 mg Fe from FS added per container, the -DL and +DL FSB treatments leached 5.1 (2% of applied) and 0.3 (< 0.1% of applied) mg Fe per container, respectively (Figure 9b). The mass of Fe that leached from the control by week 19 was not different from the amount that leached from the +DL FS treatments.

Leachate pH

Leachate pH had a significant time \times treatment interaction ($p < 0.05$) according to the repeated measures ANOVA. At all but three sampling times, FS form and the FS form \times DL interaction were not significant, whereas DL significantly affected leachate pH at all sampling times (Table 5). Accordingly, leachate pH values pooled across FS form are reported (Figure 10). Leachate pH from the control remained between 3.8 and 4.2 over the course of the experiment. Leachate from the +DL treatments had pH values that fluctuated between a minimum of 6.3 to maximum of 7.0 with a mean of 6.7 ± 0.3 sd. In the -DL FSB treatments, leachate pH steadily increased over the course of the experiment from a minimum of 2.9 to a maximum of 3.4.

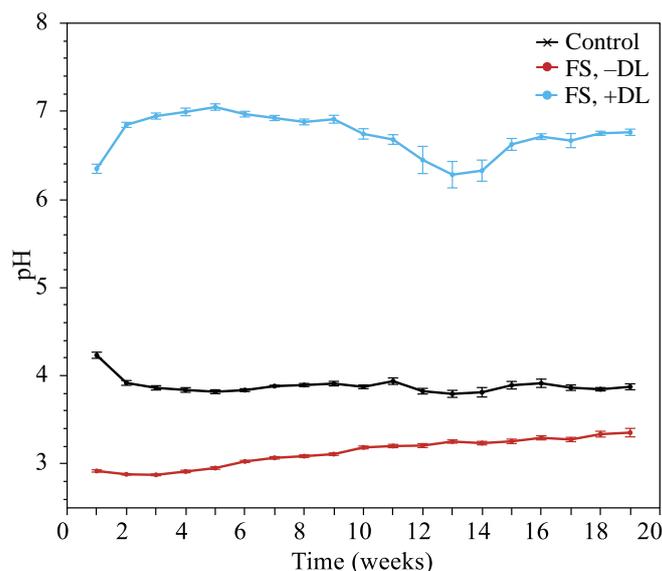


Figure 10. Mean (\pm SE) pH of leachate collected weekly from 2.5 L nursery pots in which the bottom 500 cm³ was filled with non-amended pine bark (control) or pine bark with FeSO₄·7H₂O (FS; 0.6 kg·m⁻³ Fe²⁺) mixed into the substrate in granular or liquid form with or without 5.93 kg·m⁻³ dolomite (DL). The upper 1500 cm³ of pine bark in the control and four substrate treatments contained 4.45 g/pot (3 kg·m⁻³) of a 3- to 4-month controlled-release fertilizer (19N-2.6P-10.8K). Plotted FS-DL and FS + DL treatments were pooled across FS form treatments ($n = 10$).

4. Discussion

4.1. Effect of FS on P Leaching

In Experiment 1, 81% of the 199 mg P applied to the control substrate as fertilizer either leached from the container or remained soluble in the substrate, demonstrating that non-amended pine bark has a low P adsorption capacity. The high susceptibility of P to leach from pine bark-based substrates corroborates results reported by Yeager and Wright [42] and Yeager and Barrett [5,7] who showed that pine bark-based substrates poorly retain P when applied as soluble liquid fertilizer or incorporated into the substrate as single superphosphate.

Results from both experiments indicate that mixing FS into pine bark reduces P leaching from nursery containers. Improved P adsorption following the addition of iron salts (e.g., FeCl₃, FeCl₂, FeNO₃)₃ has been reported in several agriculture and forestry waste-products, including coconut coir pith [43], wood particles [44], Sphagnum peat [45], and Sphagnum moss extract residue [46]. Previous research has proposed that ligand exchange is the mechanism by which P is adsorbed to metal-charged organic materials, where H₂PO₄⁻ replaces a surface OH⁻ group formerly attached to the metal in the organic matter-metal complex [43,47,48]. This sorption process, which is similar to P complexation by Fe oxides, occurs rapidly, and the resulting inner sphere complex between the metal and phosphate ion is strong and typically not easily reversible [49]. However, Gerke [50] asserts that P associated with solid-phase humic-Fe complexes is more accessible to plants than P bound to Fe oxides, as citrate and oxalate root exudates more effectively mobilize P associated with humic-Fe complexes than that with Fe oxides. Additional research is needed to confirm the P sorption mechanism in FSB and the availability of sorbed P to plant species commonly produced in pine bark-based container substrates.

Relative to the control, FSB treatments reduced P leaching by 29 to 96 mg/container in Experiment 1 (depending on FSB volume and Fe rate) and 26 mg/container in Experiment 2. In an outdoor nursery production setting, the mass of P that leaches during a single growing season from a 3.7 L planted container filled with a CRF-amended pine bark-based substrate has been shown to range from 7 to 237 mg (depending on the study) although 30 to 100 mg/container is more commonly reported [4,15,16,51–53]. Accordingly, an FSB layer

representing 25% or 50% of the total substrate volume with as little as 0.6 or 0.3 kg·m⁻³ Fe, respectively, has the capacity to retain most of the P that would otherwise leach out of the container. In addition, had the experiment duration been extended beyond 15 weeks, the FSB in the V1R3, V2R2, and V2R3 treatments likely would have continued to adsorb P, given that leachate P concentrations from those treatments at week 15 were still significantly less than leachate P concentrations of the control.

In Experiments 1 and 2, the efficacy of FSB to reduce P leaching was highest at the beginning of the study and decreased with time, indicating that sorption sites were becoming increasingly saturated with P. This pattern of decreasing removal efficiency of the adsorbent (FSB) aligns with that of a breakthrough curve, in which the effluent concentration of a solute passing through a vertical column of adsorbent is plotted over time, often revealing a sigmoidal curve (Patel, 2019). In container-based nursery production, greater P adsorption efficiency of FSB early in the growing season could be beneficial in preventing relatively high P amounts that leach from the substrate during the initial “watering-in” of container plants [54].

4.2. Effect of FSB Volume and Fe Rate on P Leaching

In Experiment 1, increasing the FS rate within a given FSB volume decreased P leaching without leaching excessive Fe (i.e., <5% of applied Fe leached from any treatment), suggesting the pine bark was not likely saturated with Fe, even in the R3 treatments. Therefore, amending pine bark with >5.9 kg·m⁻³ FS (>1.2 kg·m⁻³ Fe) may further increase P adsorption. However, as FS rate increases, so does the osmolarity of the water residing in the pine bark substrate. Unpublished preliminary research [55] showed that EC values in saturated media extracts of pine bark amended with 0.3, 0.6, or 1.2 kg·m⁻³ Fe from FeSO₄·7H₂O, the same rates used in Experiment 1 of the current study, were 1.6, 2.7, and 3.3 mS·cm⁻¹, respectively. The optimal EC range in saturated media extracts for substrates used in container-based production is 2.0 to 3.5 mS·cm⁻¹ [36]. Thus, adding more than 5.9 kg·m⁻³ FS to pine bark substrate without flushing the substrate with fresh water to lessen the salt load could result in osmotic stress, especially in salt-sensitive plant species. On the other hand, if the FSB layer is in the bottom of the container, away from the roots of the newly transplanted plant, the salinity of the FSB, even if initially >3.5, will not likely affect the plant as the FSB layer is flushed via routine irrigation.

In Experiment 1, increasing the FSB volume within the same FS rate resulted in a reduction in P leaching. However, treatments with the same FS mass per container, whether in the bottom 4 or 7.5 cm of pine bark (e.g., V1R2 and V2R1), leached similar amounts of P throughout the study. Thus, in the range of FSB volumes and FS rates used in this study, P retention was controlled predominantly by the total amount of Fe available for P adsorption per container rather than where the FS is distributed within the bottom half of the container. This trend should not be extrapolated to FSB volumes greater than half the container volume, as substrate in the upper portion of the container can become dry and hydrophobic and may result in the fertilizer solution channeling through the substrate before P can be adsorbed [56].

4.3. Effect of FS Form and Dolomite on P Leaching

In Experiment 2, the absence of a treatment effect on the linear relationship between total P and dissolved P, the slope of ≈1 (0.962), and small y-intercept (0.154) suggests that practically all the P draining from the containers was dissolved P. Accordingly, particulate P species, including those associated with Fe, were a minor contributor to the total P mass leaching from the containers. Shreckhise et al. [22] came to a similar conclusion when comparing total and dissolved P concentrations in leachate from pine bark substrate fertilized with CRF and amended with a soluble micronutrient fertilizer or dolomite or both, citing the following equation: TP = 0.266 + 1.083(dissolved P) (R² = 0.98).

In Experiment 2, the form of FS (granular or liquid) that was mixed into the pine bark when creating the FSB had no effect on P leaching, suggesting that there was ample time and

substrate moisture for the granular FS to dissolve and liberate Fe to subsequently adsorb to the substrate particles prior to the first irrigation. Adding dolomite to the FSB raised leachate pH but did not affect the efficacy of the FSB to adsorb P. This result was unexpected, given that increasing the adsorbent pH results in deprotonation of organic functional groups which increase in the number of negatively charged surface sites that repel phosphate ions [57]. Indeed, in Fe-charged Pahokee or Sphagnum peat, increasing the solution pH decreased P adsorption [45,58]. In this study, one possible explanation for the absence of a pH effect on P adsorption is that the addition of dolomite [$\text{CaMg}(\text{CO}_3)_2$], in addition to increasing pH, also increased the concentration of CO_3^{2-} which has been shown to out-compete phosphate for adsorption sites in Fe-loaded okara (byproducts of soybean milk production; [59]). Thus, the greater number of P adsorption sites in the dolomite-amended FSB resulting from the higher pH may have been offset by the increase in competition with CO_3^{2-} . Adsorption envelopes for FSB with varying concentrations of CO_3^{2-} would provide further insight into the relationship between pH, CO_3^{2-} , and phosphate.

4.4. P in Post-Experiment Pine Bark

The positive linear relationship between insoluble P and insoluble Fe measured in the substrate is evidence that the adsorbed P was likely associated with Fe-pine bark complexes. The molar ratio of PO_4 (i.e., insoluble P \div 0.3261) to insoluble Fe in the FSB layer of R1, R2, and R3 treatments, regardless of FSB volume, was 0.15 to 0.16. Similarly, Morris and Hesterberg [60] observed a PO_4 :Fe molar ratio of ≈ 0.15 in Fe^{2+} - or Fe^{3+} -amended peat ($1200 \text{ mmol}\cdot\text{kg}^{-1}$) at pH 6.8 after equilibrating in 400 to $500 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ PO_4 .

The concentration of soluble P in the bottom layer of the post-study substrate decreased with an increasing Fe rate. This is in line with results reported by Handreck [61], who showed that DTPA-extractable P and Fe concentrations in pine bark substrate decreased and increased, respectively, as the applied FS rate was increased from 0 to $2 \text{ kg}\cdot\text{m}^{-3}$. Lower soluble P concentrations in the FSB layer of V2 vs. V1 treatments is likely the result of the V2 FSB layer having more Fe per container and, thus, more P adsorption sites. The strong effect of FSB volume on soluble P concentrations in the top layer of substrate can be explained by evaporation at the substrate surface resulting in fertilizer salts accumulating in the upper portion of the substrate profile [62]. Hence, P fertilizer salts accumulating at the substrate-air interface is expected to have a greater effect on the average P concentration in the substrate pulled from the upper 6 cm (V2) of substrate than that pulled from the upper 9.5 cm (V1). The higher soluble P concentrations in the top 1500 cm^3 ($16 \text{ }\mu\text{g}\cdot\text{cm}^{-3}$ P) versus bottom 500 cm^3 ($11.5 \text{ }\mu\text{g}\cdot\text{cm}^{-3}$) of the control is evidence that soluble P concentrations increased with increasing substrate height, despite no FSB being present in the bottom of the container.

In Experiment 2, the comparison between the water- versus DTPA-extractable P content in the bottom substrate layer showed that DTPA was superior at solubilizing P in the substrates amended with FS, whereas the two extractants solubilized similar amounts of P from the control substrate. Phosphorus concentrations in pine bark from water- and DTPA saturated media extracts have been shown to be strongly correlated with P availability to plants [61]. Given that our results indicate that water and DTPA were not equally effective at solubilizing P in pine bark amended with a relatively high rate of FS, the relationship between P availability to plants and the substrate-P extracted by water versus DTPA may need to be reevaluated in Fe-charged substrates.

4.5. Treatment Effects on Fe Leaching

Despite statistically significant differences in Fe amounts leached among treatments in Experiment 1, these differences likely have no biological significance. Compared to the amount of Fe applied per container (i.e., 150 to 1200 mg Fe depending on treatment), $<5\%$ leached from the V1 treatments and $\approx 1\%$ leached from the V2 treatments. Handreck [63] also observed rapid and strong adsorption of Fe by *Pinus radiata* bark substrate amended with $0.75 \text{ kg}\cdot\text{m}^{-3}$ FS, citing that Fe concentrations in water-based slurries containing

1000 cm³ bark decreased from 121 mg·L⁻¹ to 0.2 mg·L⁻¹ within 50 min. In this study, the lower leachate pH of the control (which was not amended with dolomite) compared to that of the FSB treatments (which were amended with dolomite) and the relatively high concentration of Fe (509 ± 144 sd µg·cm⁻³) inherently in the pine bark may explain why the control leached a similar amount of Fe as some of the FSB treatments. The strong influence of pH on Fe solubility was also evident in Experiment 2, where FSB without dolomite (pH 2.9 to 3.4) ultimately leached over 16× more Fe than FSB with dolomite (pH 6.3 to 7.0). This inverse relationship between pH and Fe solubility was also documented by Altland and Buamscha [64], who showed that Fe solubility in non-fertilized Douglas-fir bark increased exponentially with decreasing pH.

5. Conclusions

The objectives of this research were to investigate P and Fe leaching characteristics in response to FSB layer height, FS rate and form (i.e., granular versus liquid) used to formulate the FSB layer, and the presence of dolomite in the FSB layer. This research shows that a 4- to 7.5-cm-deep layer of FSB, representing 25% to 50% of the total substrate volume, respectively, in the bottom of nursery containers can substantially reduce P leaching from daily-irrigated nursery containers. A shallower FSB layer was equally effective at reducing P leaching compared to a deeper layer, as long as the layers had the same total mass of FS. A shallower FSB layer may be advantageous when transplanting small, salinity-sensitive plant species into nursery containers, as the greater initial distance between the tender roots and the FSB layer will allow routine irrigation or rainfall to rid excess salinity from FSB layer before the growing roots reach that layer. As P adsorption increased linearly with increasing insoluble Fe concentration in the pine bark, an optimal Fe mass per container for P adsorption could not be determined explicitly. The V1R3 and V2R2 treatments (596 mg Fe from FS per container) reduced the cumulative P leached relative to the control by over 70% in the first 6 weeks and over 50% after 15 weeks, indicating these treatments could reduce P leaching substantially for both short- and mid-term container-grown plants. While doubling the applied Fe per container (i.e., V2R3) further reduced P leaching, users will need to weigh the additional expense incurred when using higher FS rates. The FS form mixed into the pine bark to create the FSB had no effect on the efficacy of the FSB to sorb P, indicating growers and substrate manufacturers have flexibility when formulating FSB. The presence of 5.93 kg·m⁻³ dolomite and resulting higher pH in the FSB also had no detectable effect on P leaching, although, given the wide range of substrate pH values commonly used to grow nursery crops, a deeper investigation into the pH effect on P adsorption to FSB by conducting adsorption envelopes may be insightful.

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