Role of phosphorus type and biodegradable polymer on phosphorus fate and efficacy in a plant-soil system

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ABSTRACT

Phosphorus (P) is critical for crop production but has high nutrient use inefficiency. Tomato was grown in soil amended with five P-sources, used as-is or embedded within a biodegradable polymer, polyhydroxyalkanoate (PHA). Correlation analysis identified treatments that maintain plant growth, improve bioavailable soil P, and reduce P loss. Three performance classes were identified: (i) micro- and nano-hydroxyapatite, which did not increase bioavailable P, plant P-uptake, or change P in runoff/leaching compared to controls; (ii) monocalcium phosphate (MCP), dicalcium phosphate (DCP), calcium pyrophosphate nanoparticles (CAP), and PHA-MCP that increased P-uptake and/or bioavailable P but also increased P loss in runoff/leaching; and (iii) PHA-DCP and PHA-CAP, where increased bioavailable P and plant P-uptake were achieved with minimal P loss in runoff/leaching. In addition to identifying treatments that maintain plant growth, increase bioavailable P, and minimize nutrient loss, correlation plots also revealed that (i) bioavailable P was a good indicator of plant P-uptake; (ii) leached P could be predicted from water solubility; and (iii) P loss through runoff versus leaching showed similar trends. This study highlights that biopolymers can promote plant P-uptake and improve bioavailable soil P, with implications for mitigating the negative environmental impacts of P loss from agricultural systems.

Keywords: Biopolymer; Phosphorus management; Leaching; Run off; Correlation analysis; Nutrient use efficiency;

INTRODUCTION

Phosphorus is the second most required nutrient for plant growth,[1] both in terms of volume and physiological functions that include energy metabolism, phospholipid membranes, nucleic acids, and photosynthesis.[2] However, only 10-20% of the millions of tons of agrochemicals, including fertilizers, applied annually are used by plants.[3] Sustainably providing adequate P levels for crops is an ongoing challenge.[4] Consequently, increasing amounts of inorganic P[5,6] are added to soils as fertilizer.[2] By 2050, projected global P use will be 26,000 Gg/year.[7] Inadequate P levels due to low delivery efficiency and plant P-uptake[8] can be further compounded depending on soil and environmental conditions, negatively affecting plant performance.[9,10] Long-term P unavailability occurs due to P-fixation,[11] or the formation of insoluble phosphates with iron and aluminum oxides often found in acidic soils.[12] In areas with heavy rainfall and sandy soils,[13] there is significant P loss through leaching and runoff, with negative environmental consequences from eutrophication and decreased aquatic biodiversity.[8]

Taken together and considering that inorganic P mined as rock phosphate is a nonrenewable resource,[14] there have been efforts to produce more efficient P fertilizers that limit P losses, and nano-enabled agriculture has shown promise.[15] Hydroxyapatite (HA) is by far the most studied nano-scale P-source,[16] with many studies demonstrating improved efficacy over conventional P fertilizers in terms of plant biomass, P-uptake, yields, and/or reduced P in runoff, compared to conventional fertilizers.[16] These findings may be due to the extremely low water solubility of bulk HA,[17] although the greater surface area of nano-scale materials has the potential to increase P solubility, release rates, and soil mobility.[18]

Alternative P-sources have distinct advantages over the highly water-soluble P-sources that dominate modern conventional fertilizers, like ammonium phosphates (e.g. MAP and DAP) or superphosphates (e.g. SSP and TSP).[19] P-sources with lower water solubility, like rock phosphate, will typically lose less P in runoff and release P to plants more slowly.[20] Alternative P-sources can be produced more sustainably than DAP or TSP; for example, dicalcium phosphate can be extracted from hazardous wastes,[21] mining byproducts,[13] and lower quality P-containing ores,[22] which will be mined in the future as high quality deposits are exhausted.[23] Additionally, polyphosphates, like calcium pyrophosphate, are gaining popularity as fertilizers[24] because they provide slow-release P, prevent P-fixation, and improve availability of micronutrients (Fe, Mn, Zn) in calcareous soils.[25]

The efficiency of P-based fertilizers can be enhanced by integration into polymers that enable controlled delivery and tunable release.[26] Biodegradable polymers are often used as coatings[27] that release dry or pelleted fertilizers[28-30] based on conditions such as soil temperature and moisture, and the thickness and porosity of the polymer.[31,32] Alternatively, nutrients can be incorporated directly into polymers to create polymer composites,[29] wherein nutrient release is triggered by either biophysical-edaphic factors or biodegradation by native soil microbes. Biodegradable polymer composites have been shown to significantly reduce nutrient losses in runoff,[33,34] and can potentially improve plant nutrient uptake efficiency.[35] Our previous study showed the role of incorporating calcium phosphate (CAP) nanoparticles into a biodegradable polymer composite of polyhydroxyalkanoate (PHA), a highly biodegradable microbially-synthesized polymer[36] in modulating P loss in a tomato system.[33] Therefore, we sought to extend those findings by expanding the type of P sources to include monocalcium phosphate (MCP), dicalcium phosphate (DCP), nanoscale hydroxyapatite (HAN), microscale hydroxyapatite (HAM), in addition to CAP, in order to broaden the application of PHA in nutrient management. Monocalcium phosphate is the globally well-known P-fertilizer referred to as superphosphate. Dicalcium phosphate is primarily used as food additive and could thus be a form of P present in animal/human urine with the potential to leach into water bodies from sewage systems. The proactive recovery of dicalcium phosphate from ores for repurposing as fertilizer is an important topic of current interest.[22] Hydroxyapatite is a naturally occurring mineral form of calcium apatite that can also be synthesized artificially. However, hydroxyapatite suffers from low water solubility and is thus a less effective P-source as crop fertilizer. The advent of nanotechnology has led to a flurry of efforts on nanoscale P-sources, where hydroxyapatite is formulated at the nanoscale to increase P solubility and bioavailability, thereby improving hydroxyapatite's status as a crop nutrient source.[19] Notably, understanding the nanoscale effects must be done in comparison with micro or bulk-scale materials of similar chemistry. Hence, the inclusion of nano and micro scale hydroxyapatite in this study. Moreover, outside of nano-hydroxyapatite, few studies have compared the efficacy of alternative P-sources in conjunction with nano-enabled or coating strategies.[37]

P-sources were applied in both pure form ("as-is") and as part of a biodegradable polymer composite of PHA.[36] We reasoned that our studies would best be realized and articulated if we use the same crop (tomato), in order to eliminate crop species-dependent variability at this stage. Tomato performance under greenhouse conditions was measured through biomass and tissue element content, in addition to changes in total and bioavailable soil P. P lost through leaching and runoff was measured using soil columns, leachate from plant pots, and a runoff simulator. With this orthogonal design, we established correlations between both positive (e.g., P-uptake) and negative (e.g., P losses) performance metrics to identify optimal treatment strategies that enhanced plant and soil characteristics with minimum negative environmental impacts.

EXPERIMENTAL

A description of the key experimental methods; see SI for complete details of the synthesis, characterization and soil only biodegradation of polymer composites as well as the determination of soil P content.

Treatments. Five P-sources were used. These included two nanoparticles, one microparticle, and two salts. Specifically, the P-sources were: amorphous nanoscale calcium pyrophosphate $Ca_2P_2O_7 \cdot H_2O$ (< 150 nm) purchased from Sigma (St. Louis, MO), abbreviated as CAP; nanoscale hydroxyapatite (<40 nm) purchased from Sky Spring Nanomaterials (Houston, TX), abbreviated as HAN; microscale hydroxyapatite (~5 µm) purchased from Sigma, abbreviated as HAM; monocalcium phosphate $Ca(H_2PO_4)_2$ purchased from Sigma, abbreviated as MCP; and dicalcium phosphate $CaHPO_4$ purchased from Sigma, abbreviated as DCP. PHA was purchased from Goodfellow Corporation (Coraopolis, PA) and chloroform was purchased from Sigma. All chemicals were used without further purification. P-sources were used as-is or embedded within a biodegradable polymer (PHA) composite; composites are indicated throughout the text with PHA- before the P-source (e.g., PHA-CAP). Composites were synthesized as previously described using solution blending.[33] Polymer composites were first digested in nitric acid at 115 °C before ICP-OES analysis to determine P content and attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) to assess the chemical bonding. Biodegradation of composites in soil over 49 days was analyzed. Aqueous dissolution of P was determined by immersion in water for 1, 2, 4, or 10 days, centrifugation to remove particulates, and ICP-OES analysis.

In total we studied 12 experimental treatments that include five phosphorus sources with and without PHA composites and two controls (soil only, and PHA only).

Greenhouse pot study and leachate collection. A greenhouse study was conducted to evaluate the impact of P type and PHA inclusion on agronomic and nutrient outcomes and leached P. Twelve treatments were established: six without PHA and six with PHA. The treatments were a soil-only control, pure PHA, MCP, DCP, HAN, HAM, CAP, PHA-MCP, PHA-DCP, PHA-HAN, PHA-HAM and PHA-CAP. In each treatment the amount of P-source or P-containing PHA polymer composite added to the soil was adjusted to ensure that the same quantity of P was used in each experiment (40 mg P present in each pot containing 220 g of soil). Tomato seeds (*Solanum lycopersicum*, Bonnie Best, Totally Tomatoes, Randolph, WI) were germinated in Pro-Mix BX potting soil (Premier Hort Tech, Quakertown, PA) in 36 cell (5.66 × 4.93 × 5.66 cm) plastic liners and grown for three weeks. Tomato seedlings (three weeks old) of uniform size based on height were transplanted into pots with different treatments as noted above. The soil used was a low-P soil from the Connecticut Agricultural Experiment Station Griswold Farm (soil pH 6.8, and sandy loam soil type); soil analysis showed 667 mg/kg total P (nitric acid and hydrogen peroxide digestion) and 1.47 mg/kg bioavailable P (modified Morgan extraction method); extraction details are below. Soil from the farm was sieved to remove debris and mixed with 10% of Pro-Mix BX potting soil. A mass of each P- treatment that contained 40 mg P was added to 2200g of soil. The P-treatment and the soil was then thoroughly mixed using a rotary mixer, before being divided equally into 10 pots (replicates). The pots were arranged in randomized block design and were placed on polystyrene weigh boats (Fisher Scientific, Inc., Waltham, MA) to facilitate leachate collection. Plants were irrigated regularly with 40 mL of deionized (DI) water to moisten the soil but not allow leaching (irrigated twice a day on hot days as required). Plants were fertilized every two weeks, and later, during the flowering phase, every week with phosphorus-free Hoagland solution at a rate of 30 mL per plant (10 mM KNO_3, 10 mM $CaNO_3 \cdot 4H_2O$, 2 mM $MgSO_4 \cdot 7H_2O$, 1 mM Fe-EDTA, 1 mM Hoagland microelement stock solution).[38] On select days, plants were overwatered with 100 mL DI water, and leachate from weigh boats was collected within approximately 10 min into pre-weighed 50 mL centrifuge tubes (Thermo Fisher Scientific, Rochester, NY). The tubes were weighed again to record the leached mass and volume. Leachate was collected on day 1 (day of transfer), 5, 9, 15, 23, 30, 40, and 49 and analyzed for P using ICP-OES. For this analysis we filtered the collected leachate through a Whatman filter no. 2, acidified

(5%) with 70% nitric acid and analyzed by ICP-OES to determine elemental phosphorus. All replicate plants across all treatments received the same volume of water or nutrient solution at each interval.

Plants were harvested 49 days after transplanting and were washed with tap water to remove soil. The fresh mass of the roots and shoots was determined, and the samples were stored in an oven wrapped in individual paper bags at 70 °C to enable determination of dry mass and element content. The harvested plant tissues were analyzed for P and other elements (Ag, Al, As, B, Ca, Cd, Ce, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, S, Sb, Se, Si, Ti, and Zn). Dried roots and shoots for each individual treatment were homogenized, weighed out in approximately 0.2 g portions, and digested using 3 mL of 70% nitric acid at 115 °C for 45 min. The digests were diluted with 47 mL DI water and the elemental composition was determined using ICP-OES.

Soil samples were collected from each pot (making sure to collect from a similar region near the roots from each pot) to determine total and bioavailable P determined with ICP-OES following a modified Morgan extraction method.[39-41]

Statistical analysis. A total of 10 replicate pots were established for each treatment in the greenhouse study. Replication for the plant-free leaching and runoff studies is noted above. Statistical differences between treatments were determined by a one-way analysis of variance (ANOVA) followed by a Post Hoc Tukey HSD with 95% confidence level. Differences were considered statistically significant when $p \leq 0.05$. Statistical analysis was conducted using RStudio computer software (Posit, Boston, MA).

P Leached in the Absence of Plants. A separate leachate collection experiment was conducted without the inclusion of the tomato plants. In this experiment, PVC columns, 1.5 inch in diameter and 6 inch in length, were filled with 140 g of low-P soil from the Connecticut Agricultural Experiment Station Griswold Farm used in the greenhouse study (see Figure S1). This soil was lightly compacted down to a uniform depth of 4 inch across all columns. At the bottom of each column, a piece of Fisher brand filter paper (Qualitative P8, Porosity: Coarse, Flow Rate: Fast) and a piece of wire mesh (200 mesh, plastic) were affixed with a SAE size 36 adjustable steel hose clamp. These additional layers were included to prevent sand or dirt particles from entering the leachate collection beakers. The column was then attached to a 60 mL plastic filter funnel with a layer of parafilm to ensure all leachate drained into the collection beakers. Identical treatment groups (12) to the plant study were included; three replicates were established for each treatment. Enough mass of each P type was added to reach the same respective soil to P ratios in the plant study. This required 25.5 mg of added P for all columns except the soil and PHA controls. P was manually mixed throughout the 140 g of soil before adding to each column.

An identical leachate collection timescale to the greenhouse study was used in this experiment. The ratio of added water to soil was also kept the same. On day 0, 127 mL of deionized water was added to the top of each column and allowed to pass through the soil over several hours into the beakers. After day 0, only 64 mL of deionized water was added on each day of watering. The columns were watered on days 5, 9, 15, 23, 30, 40, and 49. The total volume of leachate was measured in a graduated cylinder after each watering. From this leachate, 15 mL was collected into a 15 mL centrifuge tube. Collected samples were then centrifuged in a Thermo Sorvall Legend XTR centrifuge for 30 min at 4000 rpm. A portion (9.5 mL) of the supernatant was removed by pipette and acidified with 0.5 mL of 70% nitric acid. Samples were then analyzed for their P content by ICP-OES as noted below.

Aqueous solubility of P. A water-only dissolution experiment was conducted to compare the relative solubility of P amongst the different forms of P used (CAP, HAN, HAM, MCP, DCP) as-is and when they were present in PHA composites. Sets of PHA polymer composites and P-sources were immersed in water over a period of 1, 2, 4, and 10 days. For this experiment, the mass of phosphorous in each P-source and polymer composite sample was held constant at 1.16 mg. For each PHA-P composite the P loading was such that this 1.16 mg of P was incorporated within 50 mg of PHA to form a polymer composite. Each P-source and P-containing PHA polymer composite was added to a centrifuge tube containing 15 mL water. These samples were placed inside of a New Brunswick Scientific Innova 4000 incubator shaker at 150 rpm and 28 °C. Samples were subsequently removed from the incubator at selected time intervals and allowed to settle overnight. Three replicate samples were removed at each time point. Twelve mL of supernatant was then removed by pipette from each sample and centrifuged for 30 min at 4000 rpm. A portion of the supernatant (9.5 mL) was acidified with 0.5 mL of 70% nitric acid and analyzed for P by ICP-OES as described below in the “Phosphorus and other elemental analysis” section. By considering the mass of the P-source added, the percentage of phosphorus in the salt by mass, and the volume of water in each experiment, the concentrations determined from ICP-OES allowed the total mass of dissolved P generated from the P-sources and polymer composites to be calculated at each time point.

Runoff simulator. A runoff simulator was constructed based on a previous design using plastic housing to create a slight angle (theta = 4) that would allow water to runoff of a soil pan into a runoff collection bin (see Figure S2 for image). The housing held a stainless-steel tray with a depth of 0.5 inches. At the top of the plastic housing, a cut-out allows a plastic tray to be inserted to simulate rainfall. Holes (1/8-inch) were drilled in the plastic container in a rectangular array and were stuffed with plastic mesh to simulate droplets instead of a steady downpour when water was added to this top tray. To fill the bottom tray, 750 g of the low-P soil from the Connecticut Agricultural Experiment Station Griswold Farm was placed and gently pressed flat until it reached the rim of the soil tray in order to best simulate runoff. Each of the 12 treatments studied were manually added to the top of the soil, taking care to evenly disperse the treatment across the surface area of the soil-filled tray. For each P-containing treatment, 0.1 mg of P was used per g of soil (which corresponded to approximately 3-4 g of polymer composite); the same loading used in the greenhouse study. For the PHA control, 3.3 g of pure PHA was added since this was a mass similar to the polymer composite treatments. After the prepared soil trays had been added to the simulator, water was added to the perforated plastic container in 500 mL quantities causing water to drip onto the soil below; a total of 1.5 L water was used in each rainfall simulation. The simulator was positioned next to a sink, where another plastic container was positioned to catch the runoff from the simulator. The runoff from each 500 mL treatment was collected, the volume was recorded, and the liquid was then transferred to a secondary container. These samples were allowed to sit for five days to allow particles to settle and any salts/composites to adequately dissolve before aliquots were analyzed for P by ICP-OES. After each experiment, the soil pan and simulator were thoroughly washed with soap and water and allowed to dry. Each individual treatment was run in triplicate.

RESULTS AND DISCUSSION

Characterization of polymer composites:

Each treatment, except for the soil only and PHA control is described hereafter to indicate the P-source and the presence or absence of PHA. Thus, CAP refers to a treatment where amorphous nanoscale calcium pyrophosphate was used as is, while PHA-CAP refers to a treatment where CAP was embedded in a PHA polymer composite. The P-containing polymer composites contained approximately 2% weight P (Table S1). The one exception was PHA-MCP, which did not form a true composite, likely because of the insolubility of hygroscopic MCP in chloroform, resulting in large clumps of MCP poorly incorporated within the PHA. ATR-FTIR spectra of polymer composites show characteristic absorption bands associated with PHA in all of the as-synthesized (day 0) samples (Figure S3).[42] The inclusion of the P-sources is not discernible due to the overlap of the absorption bands in the C-H deformation and C-C and C-O-C stretching regions from 1300-1000 cm^{-1}, which are found in PHA, with the P-O and P=O stretching regions from 1300-1100 cm^{-1}, which are characteristic of the P-sources used here.

Figure S3 also shows photographs of polymer composites biodegraded in soil over 49 days and corresponding ATR-FTIR spectra. For all polymer composites, the primary spectral feature altered during biodegradation is the C=O (ester) absorption band at approximately 1720 cm^{-1}, which undergoes near complete attenuation by Day 49. This is consistent with microbial enzyme-driven biodegradation of PHA, which begins with cleavage of the PHA ester bond by extracellular PHA-depolymerases.[43] Figure S3 shows that the PHA biodegradation process is not affected by P inclusion, and biodegradation proceeds at approximately the same rate for all polymer composites. It should be noted that we used thin films to characterize and visualize the biodegradation process, since direct biodegradation measurements in our plant experiments were precluded due to the inability to extract the finely milled composites from the soil. The milled polymer composites used in the plant experiments are expected to fully biodegrade over the time course of the plant studies (49 days) due to the increased surface area.

Plant growth:

Figure 1(A) shows representative tomato plants treated with PHA and non-PHA amendments after 49 days of growth. The dry root biomasses for all 12 treatments are shown in Figure 1(B). The dry root biomass of the soil-only (average 0.95 g; ranging from 0.8-1.1 g) and

PHA control (average 0.88 g; ranging from 0.8-1.2 g) treatments were statistically equivalent. Dry root biomasses for the PHA and non-PHA P-treatments were not statistically different than the controls (Table S2). The influence of PHA on each P-source was also statistically insignificant, except for MCP, where the average dry root biomass of MCP and PHA-MCP were 1.2 and 0.84 g, respectively. Figure 1(C) shows the average dry shoot biomass for each of the 12 treatments, which followed a trend similar to the root biomass. Thus, the average dry shoot biomass of soil-only and PHA control treatments were statistically equivalent, and for PHA and non-PHA P-treatments, shoot biomasses were not statistically different from the controls (Table S2). The influence of PHA on each P-source was also statistically insignificant, again with the exception of MCP, with an average dry shoot biomass of 5.5 g for MCP and 4.0 g for PHA-MCP. In summary, the various P-treatments exhibited a negligible influence on root and shoot growth, with plant biomass being statistically equivalent across all twelve treatments. Montalvo D. et al showed that soil properties can influence shoot biomass production, with nano- and micro-hydroxyapatite treated plants producing equivalent biomass as controls and TSP in an Oxisol soil.[44] It should be noted that although there was not a statistical difference between the biomass measured in the presence or absence of PHA, a general trend of reduced biomass with PHA is discernible. This difference could become statistically significant in longer timescale experiments (e.g. field studies).

P loss in leachate/runoff:

The primary downside of using P-containing fertilizers is P loss from soil through runoff or leaching, leading to negative environmental consequences.[45,46] Leachate was collected from plants as one measure of P loss; Figure 2(A-F) shows the amount of P in leachate collected on pre-selected days throughout the 49-day plant growth period. The P concentration was determined by ICP-OES and combined with the leachate volume to determine the mass of P lost from the soil of five representative replicate pots on each pre-selected day for non-PHA, PHA P-treatments as well as the soil only and PHA controls. In contrast to the biomass data, leached P differed significantly between non-PHA and PHA P-treatments. In general, the extent of P leached was determined primarily by the P-source rather than the presence of PHA. This can be seen by comparing the y-axis scales for Figures 2(A)-(F), which vary considerably between treatments but are impacted far less by the presence of PHA within each plot. However, the magnitude of P released was reduced for some PHA P-treatments, most notably PHA-DCP (Figure 2B). Specifically, the total mass of P leached over 49 days was PHA-MCP (2.9 ± 0.52 mg) > MCP (2.2 ± 0.28 mg) > DCP (0.78 ± 0.06 mg) > CAP (0.59 ± 0.06 mg) > PHA-CAP (0.58 ± 0.02 mg) > PHA-DCP (0.44 ± 0.01 mg) > HAN (0.28 ± 0.02 mg) > PHA-HAM (0.20 ± 0.02 mg) > PHA-HAN (0.18 ± 0.01 mg) > HAM (0.13 ± 0.01 mg) > soil-only control ≈ PHA control (0.11 ± 0.01 mg).

Figure 2(A-F) shows the P release profiles for both the non-PHA and PHA P-treatments as well as the soil only and PHA controls. PHA-DCP exhibited slower and reduced P loss compared to DCP. Conversely, PHA-MCP did not show reduced P loss compared to MCP, which is consistent with our observation that PHA-MCP did not constitute a functional composite and is actually a mixture of PHA and MCP. For HAN and HAM, the effect of PHA inclusion is more difficult to discern because the quantities of P leached are low and comparable to the two unamended controls. For CAP, the presence of PHA altered the rate, but not the overall amount, of P loss. In the absence of PHA, 0.4 mg P was leached on day 1 and nearly all of the leached P was released during the first 15 days. Conversely, PHA-CAP only leached 0.1 mg P on the first day but continued to lose P throughout the entire growth period. By day 49, the total P lost by CAP and PHA-CAP was equivalent at 0.58 and 0.57 mg, respectively. Figure 2(G) demonstrates that, importantly, the cumulative P leached from the soil for each of the treatments correlates with the water solubility of each P-source as determined experimentally. This highlights that relative solubility of P-sources is a valid predictor of leached P from soil, regardless of PHA presence.

Figure S4 shows P lost in leachate from soil-only columns, which were designed to mirror the studies described in Figure 2(A-F), but without the inclusion of plants. A comparison of Figures 2(A-F) and S3(A-F) shows a high degree of similarity between the two data sets in the trends of leached P across treatments. The only notable difference is CAP and PHA-CAP, where the column leachate studies show a significant decrease in the total leached P for the PHA-CAP compared to CAP. Thus, for most of the amendments, plant presence did not significantly impact P lost through leachate. This suggests that laboratory-based column studies could be used as a high-throughput system to rapidly screen soil amendments to determine P release profiles while still being a valid predictor of more complex greenhouse and field studies.

The relative solubilities of the different P-sources are generally in line with the solubility products of the respective bulk calcium phosphates, with MCP having the highest solubility (Figure 2(G)).[47] The low solubility of HAM is consistent with previous studies showing that the relatively low solubility of hydroxyapatite (HA) as compared to P-salts, such as MCP and DCP, is retained for microscale HA particles.[48] In contrast, there are a number of studies which have demonstrated the benefit of using nanoscale HA (i.e. HAN) for improving crop yield, as compared to traditional P-based fertilizers such as MCP,[49] where the benefits of using HAN have been linked to an increased P solubility. However, these previous studies have typically used laboratory-synthesized HAN, while commercial HAN particles (nominally 40 nm) were used in this study. The discrepancy suggests that any nano-scale properties of HAN are sensitive to the method of preparation and/or the form of the material, such as the aggregation state.

For comparison, runoff was used as a second measure of P loss. Figure 3(A) shows the mass of P lost in runoff using the runoff simulator (Figure S2). Figure 3(B) shows the correlation between the total P lost as runoff and the P lost as leachate. With the exception of CAP, there is a high degree of correlation between runoff and leachate data, with a general trend of decreased P loss when P-sources are incorporated into PHA. Thus, although P loss in runoff is a more complex, less well-defined process than leachability, involving additional factors such as soil erosion, particle size, and the affinity with which P-containing species bind to soil particles, the correlation observed in Figure 3(B) suggests that P leachability is a good predictor of P loss in runoff for the same treatment. However, as previously noted,[50] the methods for determining P loss in runoff are not standardized and will likely be highly sensitive to the experimental design, such as the angle of soil tilt and volume and size of water droplets used to simulate rainfall, and these results would likely vary using simulators of different design. Thus, while our data shows that similar qualitative trends exist for P runoff and leachate among the 12 different treatments, it is not possible to make a quantitative determination of how much P would be lost as runoff vs leachate. Moreover, there is a significantly larger degree of error associated with the measurement of run off as compared to leachate (compare the error bars in Figure 3(B) between the horizontal and vertical axes), a reflection of the intrinsic difficulty in being able to reproduce the rainfall and the soil packing between different experiments.

Correlation Analysis:

In this study we have assessed a significant number of different agronomic and nutrient outcomes, including plant biomass, plant P-uptake, and bioavailable P, associated with the 12 treatments encompassing five P-sources (MCP, DCP, HAN, HAM, CAP) used as-is or embedded in a biodegradable polymer (PHA) as well as the soil only and PHA controls. As a means to better contextualize the data and compare the effectiveness and relative advantages and disadvantages of the different treatments, a correlation analysis approach was employed, where the potentially positive outcomes of each treatment are plotted against negative environmental metrics, specifically expressed as P lost in leachate or runoff. The negative environmental metrics represent lost P that is not accessible to plants and could enter surface waters and promote eutrophication, toxic algal blooms, and other undesirable impacts.[51] The decision to consider leached P and P lost in runoff separately in these correlation analysis plots is conservative and reflects our inability to know the extent to which these two P loss processes are quantitative metrics, particularly for runoff. However, trends within each P loss process can be meaningfully compared among the 12 treatment strategies, including controls. Figure S6 shows the first of these correlation analysis plots, rendered for the total (root and shoot) dry plant biomass. Analysis of Figure S6 reflects the data shown in Figure 1, notably that different P-sources, the presence of PHA, and the amount of P lost in leachate and runoff do not influence biomass.

Plant P-uptake:

In contrast to biomass, the plant P-uptake data highlight the enhanced P accumulation that occurs for nearly all of the P-containing treatments as compared to the soil only and PHA controls. Figure 4 shows the total shoot (A) and root (B) P content for each treatment. Figure 4(A) shows that in the shoots of P-amended plants, the lowest P content was with HAM and HAN, with values ranging from 790-1000 mg/kg (3.84-5.26 mg) and 1200-1500 mg/kg (6.76-8.73 mg), respectively, while the shoot P content in the MCP, DCP, and CAP treatments ranged from 2000-2200 mg/kg (10.37-12.54 mg), 2400-2700 mg/kg (11.86-14.36 mg), and 1800-2400 mg/kg (9.81-13.50 mg), respectively. For all P-sources, the presence of PHA did not significantly impact shoot P content (see Table S2). Figure 4(B) shows that root P content was lowest in the HAM and HAN treatments, with values ranging from 600-170 mg/kg (0.40-1.08 mg) and 670-1400 mg/kg (0.95-1.46 mg), respectively. Root P content was 70-250% higher in the MCP and DCP treatments, with values ranging from 1600-2000 mg/kg (1.34-2.71 mg) for MCP and 1800-2600 mg/kg (2.49-3.48 mg) for DCP. The presence of PHA significantly increased the root P content with CAP by 73%, from 1.3 to 2.2 mg. However, the presence of PHA did not result in significant differences in the root P content of the other treatments.

Figures 4(C) and (D) show correlation analyses of plant P-uptake (root plus shoot) on the y-axis, and on the x-axis, P lost through (C) leachate or (D) runoff; replicate values are shown for each treatment. Analysis of Figure 4(C) reveals three broad classes of treatments. The first class, shown within the blue oval in the bottom left of Figure 4(C), represents treatments that produced little or no measurable increase in P-uptake as compared to the two controls, but at the same time did not increase P lost in runoff or leachate. This group includes HAM, PHA-HAM, PHA-HAN, and to a slightly lesser degree, HAN. The extremely low water solubility of these treatments results in minimal P losses but can also reasonably be assumed to reduce the amount of P available for plant uptake. The second group, also captured within the blue oval, corresponds to treatments that produced measurable increases in P-uptake, but where this positive effect is counter-balanced by a corresponding increase in P lost as leachate. This class includes MCP and PHA-MCP. The third class of treatments, highlighted within the red oval in the upper left of Figure 4(C), corresponds to treatments which enhanced P-uptake compared to the controls, but did so without significant increases in P losses. These treatments that provide the greatest benefits to plants with the least negative environmental impacts include CAP, PHA-CAP, PHA-DCP, and DCP.

A comparison between Figure 4(C) and (D) reveals that although the data is somewhat more scattered in Figure 4(D), the same three broad classes of treatment strategies are still evident. The two treatments whose classification changes between Figures 4(C) and (D) are CAP and DCP. For CAP this is a consequence of the significantly greater amount of P lost in runoff (Figure 3(A)) as compared to leachate (Figure 2(C)), causing CAP to be classified differently in Figure 4(D) as a treatment that produces measurable increases in P-uptake, but where this positive effect is counter-balanced by a corresponding increase in the P lost in runoff. For DCP, the relative increase in the P lost in runoff as compared to leachate causes DCP to have a less defined classification in Figure 4(D).

P availability in soil:

Figure 5(A) shows the total soil P after the plant harvest, as determined by nitric acid digestion and ICP-OES analysis.[52] The soil-only and PHA controls contained approximately 190 mg of total P. All other soils containing non-PHA and PHA P-treatments had a significantly higher total P content (~240 mg), corresponding to the amount of added P, with the exception of PHA-MCP, which was statistically equivalent ($p > 0.05$) to the controls. The amount of bioavailable P in the soil after plant harvest was determined by a modified Morgan extraction method, a preferred extraction method for the Northeast region particularly when ICP-OES is used to analyze P (Figure 5(B)).[39,40] The bioavailable P in the soil-only and PHA controls was 2.5 mg (11.2 mg/kg) and 2.3 mg (10.6 mg/kg), respectively, which was anticipated considering the soil used in this study was chosen for its low P content. For the P-containing treatments, the amount of bioavailable P was greatest in PHA-MCP (8.6 mg) ≈ PHA-CAP (7.3 mg) ≈ PHA-DCP (6.3 mg) > MCP (5.0 mg) ≈ DCP (4.4 mg) > CAP (3.4 mg) ≈ PHA-HAM (3.3 mg) ≈ PHA-HAN (2.8 mg) > HAN (2.3 mg) ≈ HAM (1.7 mg). Importantly, the bioavailable P in PHA-MCP, PHA-DCP, and PHA-CAP (statistically equivalent) was significantly greater than MCP, DCP, HAN, HAM, CAP, PHA-HAN, PHA-HAM, and the controls.

Figure 5(B) shows that the amount of bioavailable P in all PHA P-treatments was greater compared to non-PHA treatments using the same P-source. Specifically, the presence of PHA increased the bioavailability of P in soil with PHA-CAP by 118.2%, PHA-HAM by 92.4%, PHA-MCP by 80.4%, PHA-DCP by 45.3%, and PHA-HAN by 19%, as compared to the respective non-PHA treatments. This effect is likely the result of changes in the microbial community that accompany PHA biodegradation,[53-56] or through changes in soil properties triggered by either PHA alone or microbial activity, such as pH or moisture content, which could increase P bioavailability. The observation that an increase in bioavailable P was observed for PHA-MCP as compared to MCP is notable because PHA-MCP is likely a mixture of PHA and MCP rather than a composite. This suggests that the effect of PHA on bioavailable P could be achieved by adding a biodegradable polymer separately from the P-source but as part of the same treatment. It is important to note that the benefit of PHA in terms of increasing bioavailable P only occurs when a P-source is also added; there is no benefit in terms of increased bioavailability to the P already present in the soil, as shown by the soil-only and PHA controls in Figure 5(B).

When bioavailable P is correlated with P lost in leachate or runoff, the same three classes of treatments identified in Figure 4 are also observed in Figure 5(C) and (D), and the treatments within each class generally remain the same for both soil bioavailable P (Figure 5) and plant P-uptake (Figure 4). The two exceptions are DCP and CAP, of which neither increased soil bioavailable P to the extent needed to be grouped in the red oval, representing the most outstanding treatments, but instead falling into the category where treatment benefit on the plant and/or soil is offset by corresponding increases in P loss. Significantly, the two optimum treatments in terms of increasing both P-uptake in the plant and bioavailable P in the soil, with only minimal P loss in leachate/runoff, are PHA-CAP and PHA-DCP (Figure 4(C) and (D) and Figure 5(C) and (D)).

Figure 6 shows that there is a correlation ($R^2 = 0.61$) between bioavailable P and plant P-uptake across the treatments, with plant P-uptake being approximately double the bioavailable P in the soil. The two controls, as well as HAM, PHA-HAM, HAN, and PHA-HAN, are associated with relatively low levels of plant P-uptake and bioavailable P. Conversely, CAP, DCP, and MCP exhibit intermediate levels of plant P-uptake and bioavailable P, while PHA-CAP, PHA-DCP, and PHA-MCP provide the most favorable combination of elevated plant P-uptake and soil bioavailable P. Moreover, neither plant P-uptake nor soil bioavailable P appear to be solely related to the water solubility of each P-source.

Comparing across the ten P-containing treatments, those containing water-insoluble HAM and HAN provided limited benefit, regardless of the presence of PHA. Treatments containing the more soluble P-sources MCP, DCP, and CAP provided improved benefits to plants and soil, but with the drawback of greater P loss through leachate and runoff. This increased P loss was mitigated in PHA-CAP and PHA-DCP, where the ability of the P-sources to form true composites allowed for the full benefit of the PHA to be obtained in terms of both improved bioavailable P and reduced runoff. Conversely, PHA-MCP did not form a true composite due to the solution blending approach adopted in this study and did not benefit from the reduced runoff provided by PHA in the true PHA-CAP and PHA-DCP composites. The disparity in the utilization of added P between the different treatments is also dramatic. For the better performing treatments such as CAP, PHA-CAP, DCP, PHA-DCP, approximately 60% of the added P is utilized as either plant P-uptake or as an increase in bioavailable P. However, for treatments containing HAM and HAN, where plant P-uptake and bioavailable P were not significantly greater than the controls, less than 5% of the added P was utilized.

Correlation analyses have been shown to be an effective means to demonstrate the relationship between various treatment inputs (e.g. quantity of added fertilizer) and agronomic and nutrient outcomes (e.g. crop yield) or environmental impacts (e.g. runoff).[57,58] In the present study we have expanded this approach by comparing the positive agronomic and nutrient outcomes (e.g. P-uptake, bioavailable P) to their negative environmental impacts (i.e. P loss in leachate/runoff) across a range of treatments. Moreover, by considering these two types of outcomes on an equal footing, we have been able to identify PHA-DCP and PHA-CAP as the two strategies with the highest degree of efficacy, a conclusion which was not obvious from an analysis of the raw data alone.

Although the current cost of PHA is relatively high, as global polymer markets shift in favor of biodegradable products it is reasonable to assume that the price of PHA and other biodegradable polymers will ultimately decrease.[59] Additionally, we are exploring other biodegradable polymers (e.g. starch, chitosan) for more sustainable and cost-effective composites. In terms of the overall treatment efficacy, PHA-CAP and PHA-DCP were identified as the optimum treatments but given the relative cost of the two respective P-sources, PHA-DCP would represent the most commercially viable option for future studies. Studies are underway to unravel the full benefits of these treatments under field conditions.

The treatment efficacy of different P-sources, including the impact of incorporating these P-sources into a biodegradable polymer (PHA), was assessed for tomato plants. This assessment was based on correlation analyses where positive agronomic and nutrient outcomes were compared with corresponding negative environmental consequences, the latter in the form of P lost in leachate or runoff. Metrics identified that plant biomass was independent of treatment type, but correlations existed for both P-uptake and bioavailable soil P. Commercial nano- and micro-hydroxyapatite were too insoluble to produce significant agronomic and nutrient benefits or lead to negative environmental consequences beyond those observed for soil-only or PHA controls. Alternatively, monocalcium phosphate (MCP), dicalcium phosphate (DCP), calcium pyrophosphate nanoparticles (CAP), and MCP-PHA polymer composites yield some agronomic and nutrient benefits in the form of P-uptake and/or increases in bioavailable P, but this was compromised by negative environmental impacts. PHA-CAP and PHA-DCP were identified as the two optimal treatments given increases in bioavailable P and increased P-uptake while P leaching and runoff losses remained comparable to unamended soils. This positive combination of treatment outcomes was ascribed to the solubility of CAP and DCP, combined with the benefits of increased bioavailable P and decreased P loss that occurred when these P-sources were incorporated into PHA. In general, these results demonstrate that biodegradable polymers can act synergistically with P-sources to increase the bioavailable P in soils and facilitate P-uptake. Also, amongst different treatments, P solubilities determined in water were useful predictors of P lost through leachate in plant studies. Moreover, the trends in P loss amongst the different treatments were similar for P lost as either leachate or runoff. More broadly speaking, correlation analyses where positive and negative outcomes associated with different treatments were compared on an equal footing, were an effective means to identify optimal treatment strategies, particularly in studies with large data sets.

SUPPORTING INFORMATION

Experimental details of PVC columns used for soil only leaching studies, polymer composite synthesis, characterization and soil only biodegradation experiments; determination of soil P content; runoff simulator photographs; plant and leachate P-content. (PDF)

ACKNOWLEDGEMENTS

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Figure 1

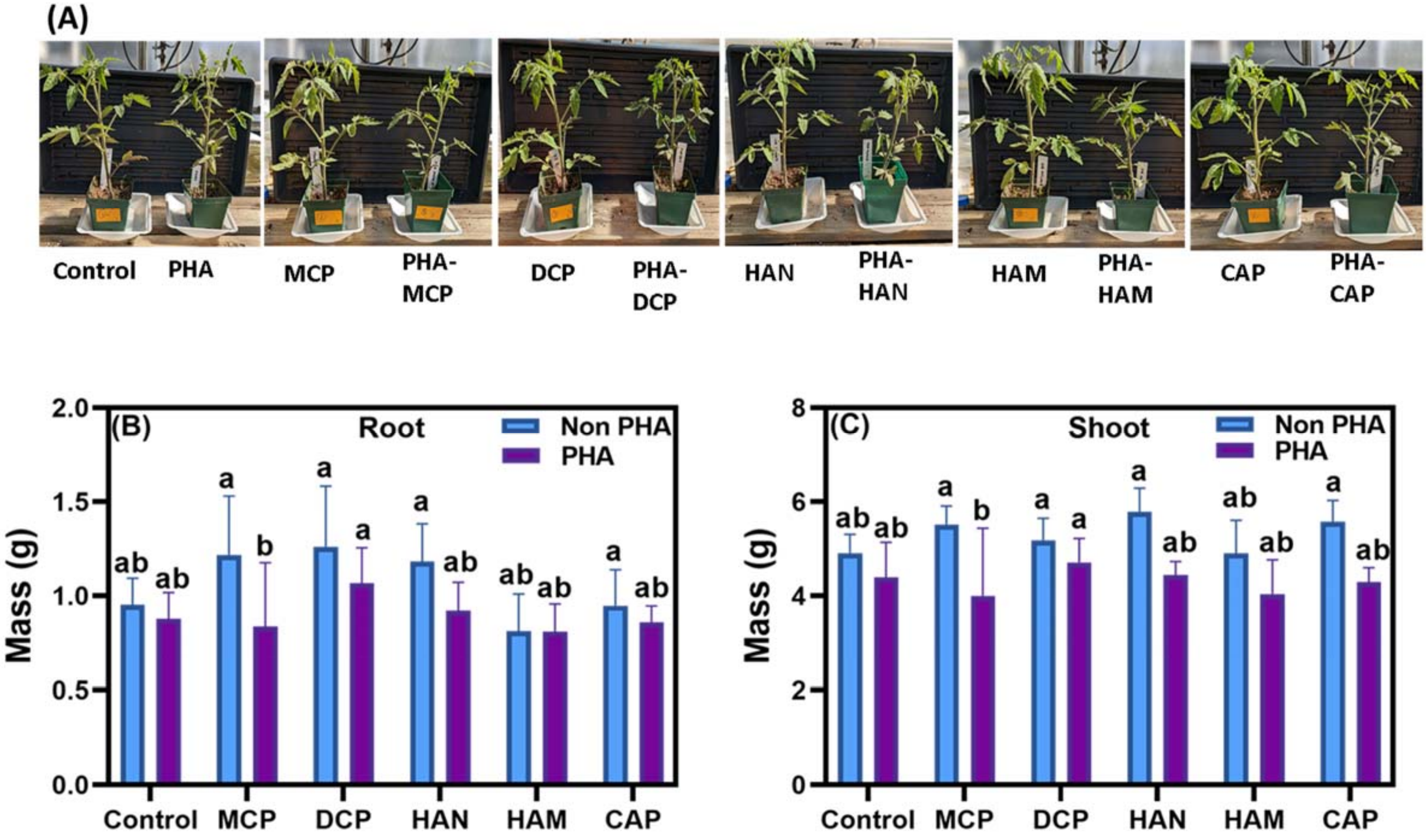

Figure 1. (A) Pairwise comparison of non-PHA and PHA -amended plants with different P- sources, (B) dry root biomass, and (C) dry shoot biomass, after 49 days of growth. Error bars show standard deviation of 10 replicates. Letters denote statistical differences as calculated by one-way ANOVA followed by Tukey's HSD multiple comparison test.

Figure 2

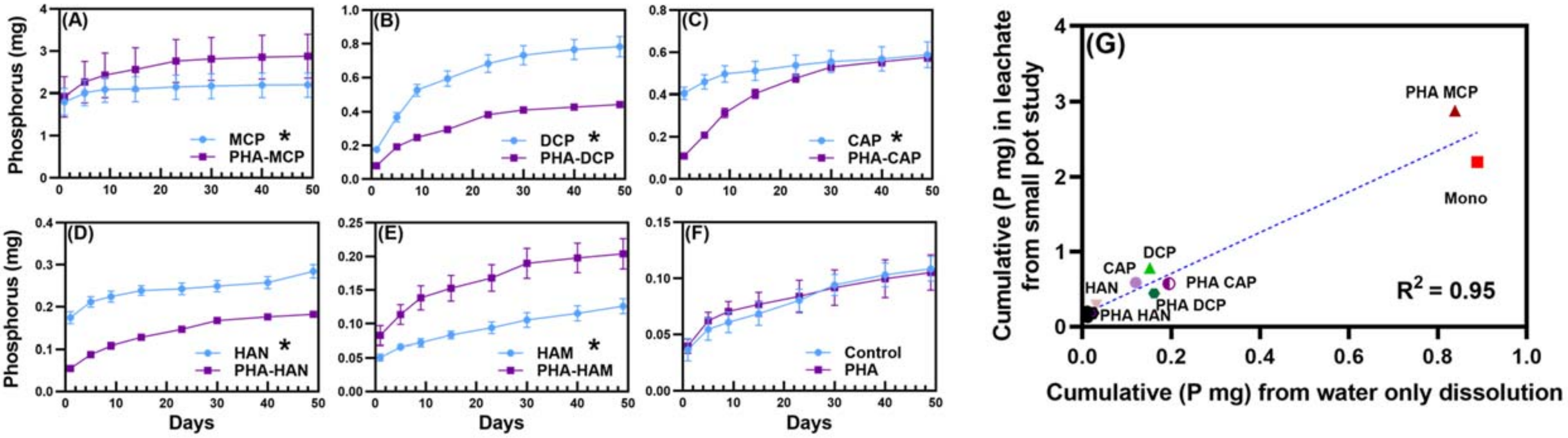

Figure 2. Cumulative P lost in leachate over 49 days of plant growth for each of the 12 treatments, shown in purple for treatments with PHA and in blue for treatments without PHA. (A) MCP and PHA-MCP, (B) DCP and PHA-DCP, (C) CAP and PHA-CAP, (D) HAN and PHA-HAN, (E) HAM and PHA-HAM, and (F) soil-only and PHA controls. Error bars show standard deviation of five replicates, asterisks denotes statistically different slopes ($p < 0.05$, calculated by Student's t-test paired two samples for means) of the linear regression line (Figure S3). (G) Correlation plot of P loss in cumulative leachate versus water- only dissolution.

Figure 3

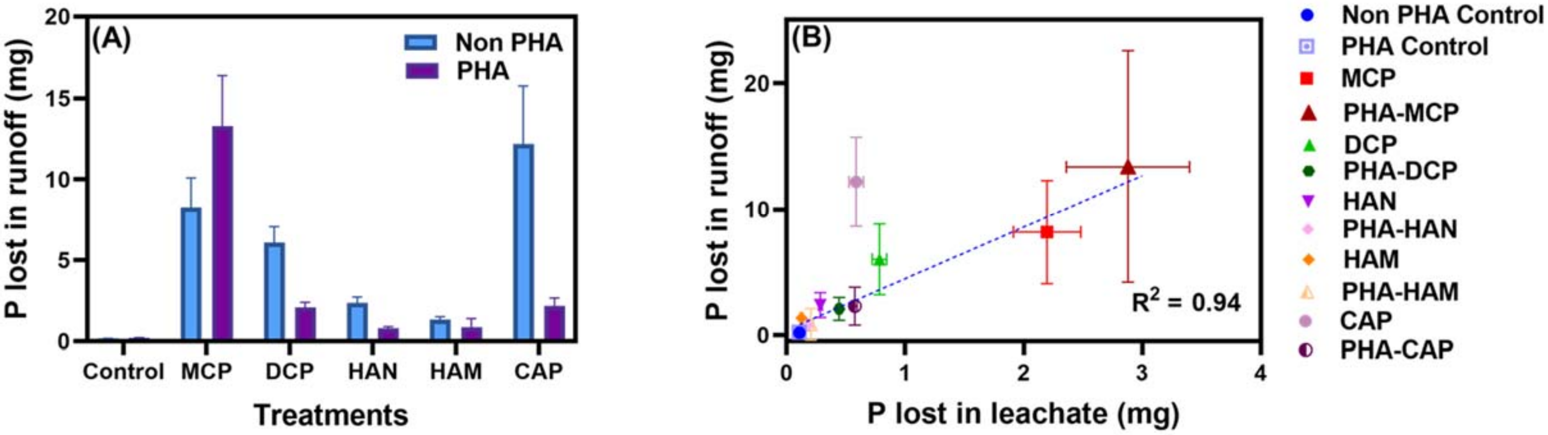

Figure 3. (A) Mass of P lost in runoff (mg) measured using a runoff simulator for each of the 12 treatments. Average P lost and error bars are shown on the basis of triplicate experiments. (B) Correlation between the P lost in runoff and P lost in leachate ; the correlation excludes the CAP data (see text for details).

Figure 4

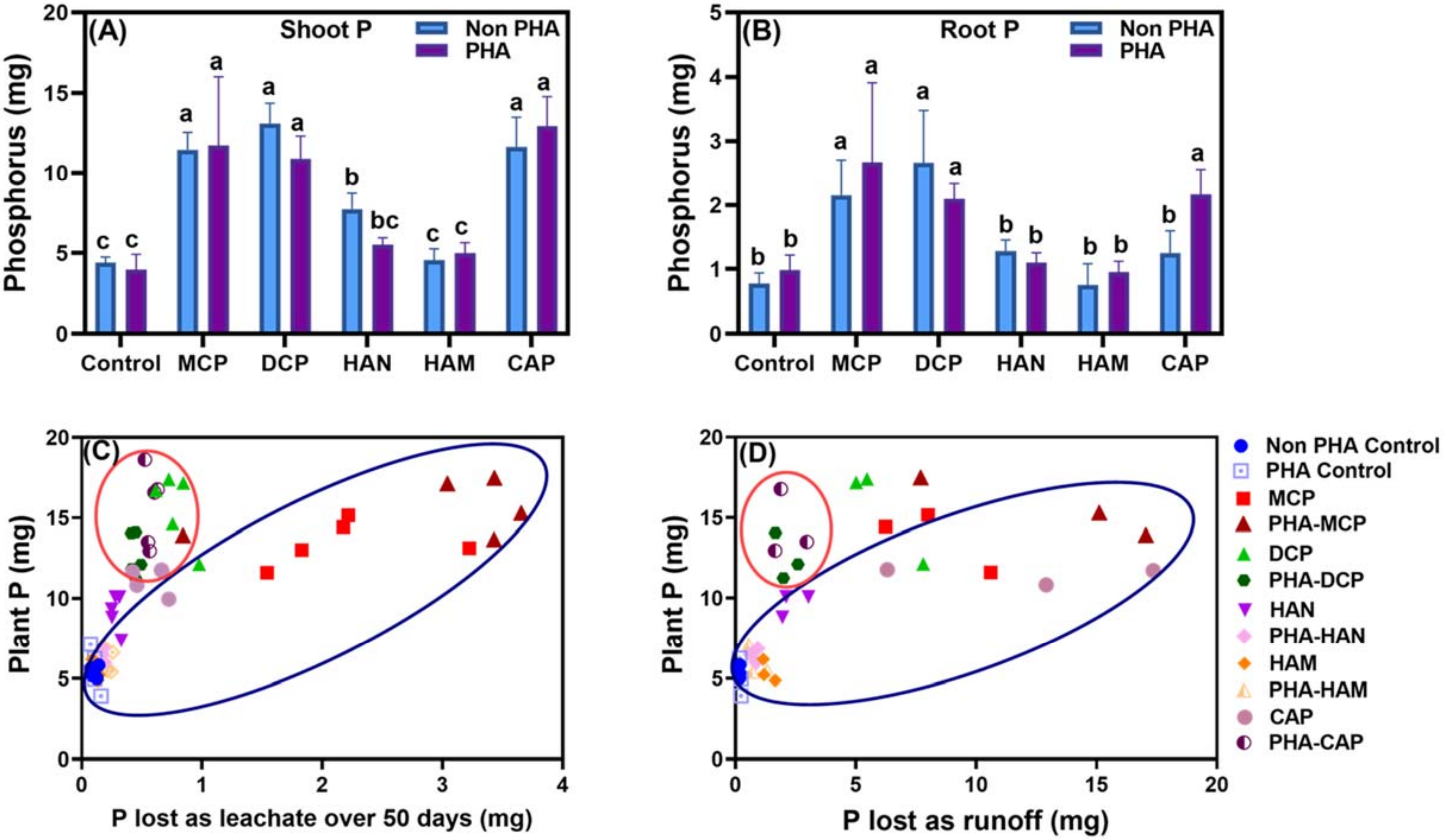

Figure 4. Comparison of different P-sources in PHA polymer composites and without PHA on P content in tomato (A) shoots and (B) roots. Error bars show standard deviation of ten replicates. Letters denote statistical differences calculated by one- way ANOVA followed by Tukey HSD multiple comparison test. Correlation analysis plots between the total plant P content after 49 days of plant growth and P lost as either, (C) leachate or (D) runoff; all replicate values are shown for each treatment. The blue and red ovals represent classes of treatments with different efficacies (see text for details).

Figure 5

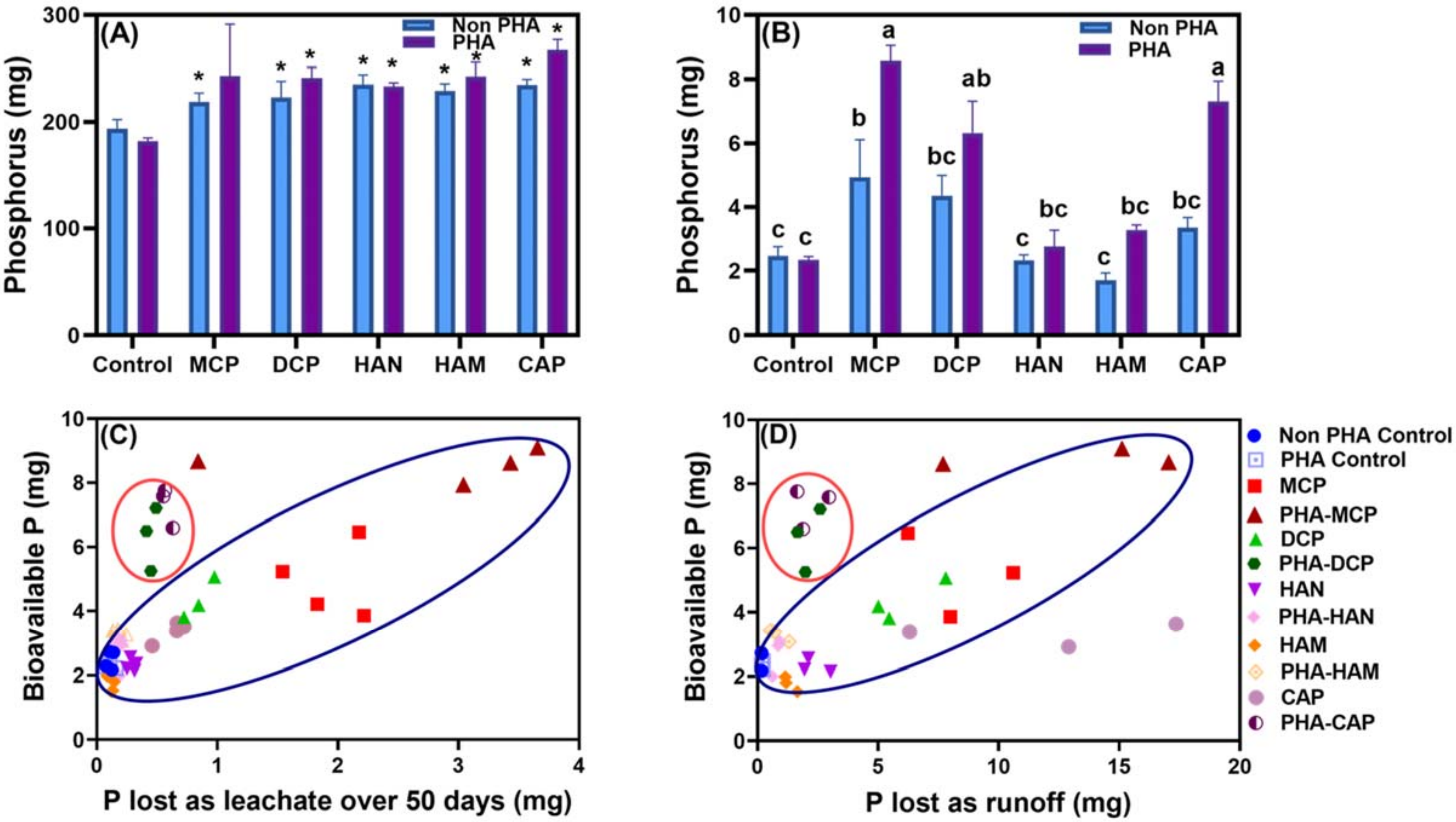

Figure 5. (A) Total P in soil harvested after plant growth; (B) bioavailable P determined using a modified Morgan extraction method and analyzed by ICP-OES. Asterisks denote statistical significance ($p < 0.05$) with respect to the non-PHA control, as calculated by Student's t-test. Correlation between the bioavailable P measured in the soil after 49 days of plant growth and P lost as (C) leachate or (D) runoff; all replicate values are shown for each treatment. The blue and red ovals encompass different classes of treatments (see text for details).

Figure 6

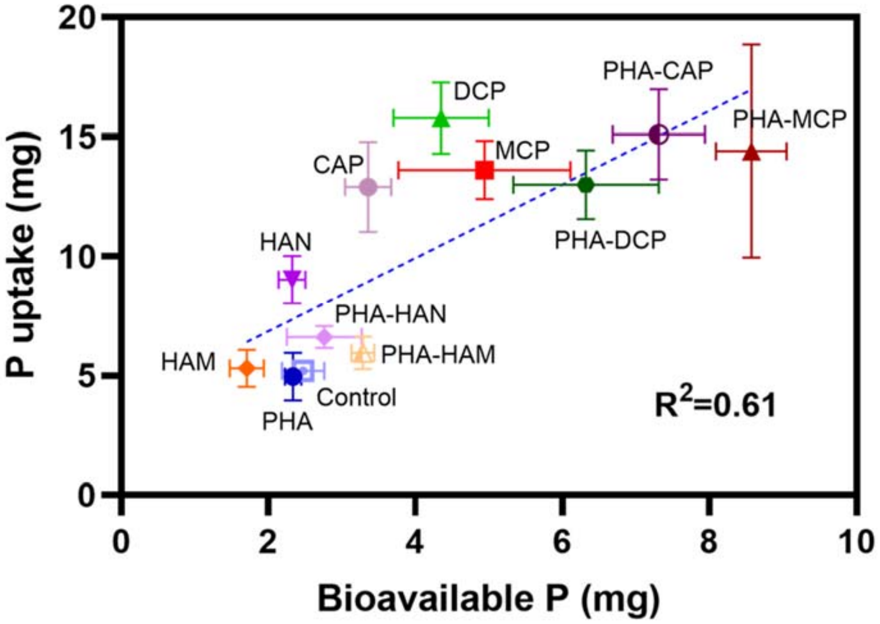

Figure 6. Correlation between the P uptake in the plant and the bioavailable P measured in the soil after 49 days of plant growth.

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