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Influence of phosphorus-solubilizing compounds on soil P and P uptake by perennial ryegrass

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Abstract Found throughout the world, phosphorus (P)-fixing soils have long been studied for their effect on the availability of P fertilizers for crop production. It is known that organic acids in the rhizosphere affect P solubilization and uptake, although effectiveness has been shown to vary with acid, crop, and soil characteristics. Regardless, commercial products have been developed that include some formulation of organic acids intended to increase P solubility and uptake. Thus, the objective of this research was to evaluate the ability of the commercial P-solubilizing products Avail (maleic-itaconic copolymer) and P Miner (organic acid-based product) to maintain a greater pool of plant-available P for uptake by perennial ryegrass (*Lolium perenne* L.). Greenhouse experiments were conducted with P fertilization (0, 17, 34, and 50 kg P ha^{-1}) and P solubilization materials in an incomplete 4×5 factorial design. Treatments were added to a soil with a high P-fixing capability and perennial ryegrass planted. Collected data included plant dry matter and P content, and acid (Mehlich-I) and labile (CaCl$_2$) extractable soil P concentrations. Results are not definitive, but they indicate that both Avail and P Miner have the potential to improve available forms of P in the soil that are available to ryegrass. In these greenhouse trials, the application of P Miner at four times the recommended rate (480 kg ha^{-1}) resulted in highest labile and acid-extractable P, while application of Avail and P Miner at the recommended rate (120 kg ha^{-1}) resulted in the highest P uptake.

Keywords Phosphorus fixation · Mehlich-I extractable phosphorus · Soil phosphorus · Phosphorus solubilization

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Introduction

Highly weathered soils found in tropic and subtropic regions often have extremely high P-binding capacities (Chikowo et al. 2010). These soils often bind P to the point that it is rendered unavailable to the plant (Fageria and Baligar 2001). Iron and aluminum hydrous oxides are common in weathered soils and largely responsible for P sorption (Menon et al. 1995). Additionally, amorphous and poorly crystalline hydrous oxides have highly reactive surfaces that form strong surface complexes with P (Kuo 1990). It has been demonstrated that the availability of applied P is a function of both reversible adsorption reactions and long-term precipitation reactions (Borling et al. 2004; Siddique and Robinson 2004; Shafqat and Pierzynski 2010). Phosphorus fixation, and the immediate non-availability of that P for the crop, has long been managed via application methods, such as placement in a band (Alessi and Power 1980; Stecker et al. 2001) or by regular fertilization to meet soil test recommendations (Malhi et al. 2009).

Plants and microorganisms release protons, OH^{-}, CO$_2$, and organic acids to solubilize poorly soluble inorganic P (Rodriguez et al. 2006; Marschner et al. 2011). In addition, microorganisms are also known to release various phosphatase and phytase enzymes to mineralize organic P (Rodriguez et al. 2006). Furthermore, mycorrhizal fungi are also known to increase plant P uptake by expanding exploration of soil through fungal hyphae and transfer of acquired P to the plant in exchange for carbon (Marschner et al. 2011). However, these mechanisms may be limited in a soil dominated by high iron and aluminum oxides that strongly bind P (Cleveland et al. 2002), such as those found in northern Alabama (Senwo et al. 2003; Shaw 2007).

Interest in reducing P fixation or increasing P solubility for crops and turf has resulted in the development of P-solubilizing treatments for fertilizer and soil. Marketed as

products to enhance P availability, the materials are either added directly to soil or impregnated on P fertilizer. One product currently on the market is Avail® (SFP, Leawood KS). This product is a water-soluble maleic-itaconic copolymer (patented from a family of dicarboxylic copolymers) that is added directly to fertilizer prior to application. It is designed to prevent P from sorbing to soil constituents allowing it to remain in a plant-available form for several months. Another product is P Miner®, developed by Floratine (Collierville TN), which is a patent pending material with a confidential formulation that is described by the company as follows: "P Miner focuses on six organic acids known to play a role in P release—citric, fumaric, glycolic, piscidic, aldonic and malonic acid." Of these products, only Avail has been evaluated for its efficacy in the field. Karamanos and Puurveen (2011) and McGrath and Binford (2012) found that it did not enhance P fertilizer efficiency in wheat (*Triticum* spp.) and corn (*Zea mays* L.), respectively; however, neither of these soils were considered to have a high P-fixing capacity.

Previous research demonstrates the ability of organic acids to potentially solubilize P for plant use (Strom et al. 2002), with effectiveness varying due to organic acid type (Harrold and Tabatabai 2006; Andrade et al. 2007; Wang et al. 2008; Oburger et al. 2011), soil type (Feng et al. 2004; Palomo et al. 2006), and soil factors such as pH (Palomo et al. 2006). Citrate is frequently cited as one of the most effective in solubilizing P (Oburger et al. 2011; Feng et al. 2004; Andrade et al. 2007). Although effective in enhancing P availability, the effectiveness of the citrate varied with the form of the citrate (citric acid versus potassium citrate) and soil pH, suggesting that organic acids may not universally enhance P uptake (Palomo et al. 2006). In other work, application of oxalate significantly increased rhizosphere P mobilization, while application of citrate had no effect (Khademi et al. 2010). The ability of various organic acids to desorb P is highly variable, with additive effects when various organic acid anions (citrate, malate, and oxalate) were combined as mixtures (Oburger et al. 2009). While there is some scientific evidence for the efficacy of organic acids to improve P availability, the efficacy of commercial products to solubilize P for plant use is largely unknown.

Phosphorus is important for crop production and is considered the third most important plant nutrient, behind nitrogen and potassium. Phosphorus is often applied via fertilizers with 2-1-2 or similar ratios and in organic amendments and/or waste products (Vietor et al. 2004). Often, years of P application have resulted in P accumulation at the soil surface, with resultant concerns about environmental quality as accumulated P may move in runoff (Shuman 2004; Torbert et al. 2005; Soldat and Petrovic 2008). Soil P accumulation has led to state-by-state regulation of P fertilization, often in lawn fertilization, with some states eliminating or restricting applications of P in critical watersheds (Maguire et al. 2009; Steinke et al.

2009). Reduced application of P will eventually result in a demonstrated need for P by lawn grasses, especially in sandy soils or in soils with a high P-fixing capacity, as long shown in field crop production systems such as corn (*Z. mays* L.) (Davis et al. 1996; Eijk et al. 2006). Products that can improve the effectiveness of P fertilizers or solubilize existing soil P are needed in production systems to overcome tighter regulations against P fertilization. Perennial ryegrass is an excellent test crop for the evaluation of P-solubilizing products because: (1) it grows rapidly in a wide range of environments, (2) it responds to added P, and (3) it is a crop that is in widespread turfgrass and pasture use in both cool- and warm-season climates.

The objective of this research was to evaluate the ability of the commercial P-solubilizing products Avail and P Miner to maintain a greater pool of plant-available P for uptake by perennial ryegrass. It is hypothesized that the organic acid-based P Miner commercial product will increase biomass and total P content of ryegrass by solubilizing P. In order to evaluate this, the effect of P-solubilizing compounds on P availability in the soil was evaluated using two P availability indices. The dilute salt solution method represents labile P, which is the portion of soil P that is in equilibrium with the solid-phase P under the conditions prevailing in the soil. This fraction is a very small fraction of available soil P, but readily accessible to plant roots (Kuo 1996). The double acid method, which is also referred to as Mehlich-I, extracts a much larger fraction of available P. While it is not suitable for soils with high cation exchange capacity or appreciable contents of calcium carbonate or rock phosphate (Kuo 1996), it is a common availability index for soils in the southeastern USA. In addition, P uptake was determined by P concentration in the shoot and total P uptake by shoots per pot. Lastly, the effect of P on root and shoot biomass was also evaluated.

Materials and methods

Experiment design

Greenhouse experiments were conducted at the Auburn University Plant Science Research Center greenhouse facility located in Auburn, AL. The greenhouse experiments were conducted with ambient light only in the period January through May 2011. A Decatur soil (Fine, kaolinitic, thermic Rhodic Paleudult) was selected for the experiments because of its relatively high P-fixing capability due to aluminum and iron sesquioxide content. The soil (0–15 cm surface layer) was collected from a long-term continuous cotton (>15 years) (*Gossipym hisutum* L.) field located on the Tennessee Valley Research Station in northern Alabama. Background soil tests revealed that soil pH (1:1 soil/water)

was 6.0 and Mehlich-I extractable nutrients were 12.3, 283, 336, and 1,920 kg ha^{-1} for P, K, Mg, and Ca, respectively (Mehlich 1953). These values were considered "very low" for P and "very high" for K. In addition, total Fe and Al were 1,793±825 and 10,676±1,280 mg kg^{-1}, respectively, using the EPA 3050A microwave digestion procedure (US-EPA 2007), and citrate dithionite extractable Fe and Al were 2.34±0.3 and 0.26±0.03 % (mean ± standard deviation), respectively, using the Soil Survey Laboratory Methods Manual (Burt 2004). This method is thought to represent the total "free" iron oxides, which may also include small contributions from water-soluble, exchangeable, and organically bound Fe (Loeppert and Inskeep 1996). In addition, this method has been used to estimate poorly ordered Al phases, high-surface area crystalline Al phases (e.g., gibbsite), and perhaps Al contained in crystalline and noncrystalline Fe oxyhydroxide phases (Bertsch and Bloom 1996). Basically, 0.75 g of <2 mm, air-dry soil, 0.4 g sodium dithionite, and 25 mL of 0.57 M sodium citrate were mixed overnight at room temperature at 200 oscillations min^{-1}. Samples were removed from the shaker, allowed to settle, and centrifuged for 15 min at 3,000×g. Solutions were diluted appropriately, and Fe and Al were determined using atomic absorption spectroscopy in the clear solutions. Results are consistent with that reported by Shaw (2007) and Senwo et al. (2003).

The experiment design was an incomplete 4×5 factorial of P rate and P-solubilizing treatments. Phosphorus was applied as triple superphosphate (TSP) at four rates of 0, 17, 34, and 50 kg P ha^{-1} (0, 30, 60, and 90 lb P$_2$O$_5$ acre^{-1}) with one of five P-solubilizing treatments: (1) none, (2) P Miner at the manufacturer's recommended rate (1×), which equaled to 0.1 g per pot (120 kg ha^{-1}), (3) P Miner at 0.2 g per pot (2×; 240 kg ha^{-1}), (4) P Miner at 0.4 g per pot (4×; 480 kg ha^{-1}), and (5) Avail preapplied to TSP at 0.25 %, which was 0.002 L kg^{-1} of fertilizer. Thus, the Avail treatments were confounded in that every increase in P fertilization also resulted in an increase in Avail. Additionally, an Avail treatment without P addition was not possible to conduct because Avail is a fertilizer treatment and not a soil treatment. Thus, the study was nearly a complete 4×5 factorial with a total of 19 treatments in the study. Each treatment was replicated three times. Destructive sampling of the study was performed at 4, 8, and 12 weeks after fertilization, which resulted in a total of 171 pots. The entire experiment was repeated twice.

The experiment unit (pot) was 10 cm in diameter and 15 cm tall. Each pot was filled to within 2.5 cm of the pot rim with ground and sieved Decatur soil. The same weight of soil (approximately 1,200 g air-dried soil) was placed in each pot to achieve an approximate bulk density of 1.25 g cm^{-3}. Pots were watered to and maintained at volumetric water content of approximately 20 % (~−100 kPa). Treatment amendments, except for P Miner treatments, were

mixed into the top 2.5 cm of soil. The P Miner treatments were mixed with 20 mL distilled water prior to pouring evenly over the soil surface. The same volume of water was applied to all other pots following treatment amendments. Twenty seeds of "Marshall" annual ryegrass were seeded in the top of each pot and lightly watered until emergence. Once the experiment was initiated, all pots were watered with a P-deficient Hoagland solution to supply 4.9 kg N ha^{-1} week^{-1} and a total of 59 kg N ha^{-1} for the 12 week experiment period (Hoagland and Arnon 1950).

Data collection and analysis

The experiments were conducted for the following periods: run 1 was initiated on 13 Jan 2011 with harvests occurring on 9 Feb (week 4), 9 March (week 8), and 6 April (week 12). Run 2 was initiated on 17 Feb 2011 with harvests conducted on 16 March (week 4), 28 March (week 6), 12 April (week 8), and 9 May (week 12). The extra harvest at week 6 was added in the second run because the ryegrass was growing rapidly, and the additional harvest was needed to control top growth. At each sampling, except for run 2 week 6, plants were removed from the pot. The total number of plants was counted, and ryegrass roots and shoots were separated, washed, dried (48 h at 60 °C in a forced-air drier), and weighed. Top growth was then analyzed for tissue P content, and P uptake was calculated using the dry weight of harvested material. Soil samples were collected for subsequent analysis (described below). In run 2, the additional harvest of shoot growth at week 6 was also analyzed for P content. At this week 6 sampling, top growth was clipped to an 8 cm height, and the harvested dry matter was collected, weighed, dried, and analyzed for P content. Shoot tissue P content was determined using standard dry combustion of plant material in a muffle furnace at 450 °C followed by dissolution in 10 mL of 1 N nitric acid and evaporation using a hot plate (Owen 1992). The residue was dissolved in 0.1 N nitric acid and filtered (Whatman #40, Whatman Laboratory Division, Springfield Mill UK) prior to analysis with an inductively coupled plasma (ICP) spectrometer (Spectro Ciros CCD side on plasma, SPECTRO Analytical Instruments Inc., Kleve Germany). Phosphorus uptake was determined by multiplying the tissue P concentration by the shoot biomass of the plant per pot for each destructive sampling date. Shoot biomass and total P uptake reported in weeks 8 and 12 include contributions from week 6; data per pot were added together prior to statistical analysis.

Phosphorus availability in the soil was evaluated based on labile P and acid-extractable P. Labile soil P was evaluated by extraction of 5 g soil with 50 mL 0.01 M CaCl$_2$. Samples were shaken for 1 h, centrifuged to settle soil, filtered through a 0.45 μm filter, and analyzed by ICP spectroscopy for P. Acid-

extractable soil P was evaluated using the Mehlich-I (double acid) extractant, which is the standard soil extractant for soils in the Southeast USA (Southern Regional IEG 1983). Briefly, 5 g of dried and sieved soil were shaken with 20 mL mixture of hydrochloric (0.5 N) and sulfuric acid (0.25 N) for 5 min (Mehlich 1953). The samples were then filtered with Whatman #40 filter paper or equivalent and analyzed for P using ICP spectroscopy.

Data were analyzed using generalized linear model (GLM) procedures of SAS (SAS Institute 2011). If significant using GLM, least significant differences among treatments and factors were determined using least significant difference analysis. For all statistical analyses, alpha was set at the 0.05 level.

Results and discussion

Extractable soil P was often affected by the interaction of P rate and P-solubilizing additive, regardless of the strength of the soil extractant ($CaCl_2$ or Mehlich-I) (Table 1). Because the factor "Run" was significant and because the interaction of P rate and P solubilizer was almost always significant, soil data will be discussed within those interactions. In comparison, there were few significant and consistent interactions between P rate and P solubilization treatment on plant properties (Table 1). Thus, general trends only will be discussed.

Labile ($CaCl_2$) and acid-extractable (Mehlich-I) soil phosphorus analyses

The effect of increasing rates of P fertilizer on labile and extractable soil P is shown for each P rate in Figs. 1 and 2, respectively. When no P fertilizer was added, P Miner at the highest rate of addition ($4\times$, 480 kg ha^{-1}) increased labile P in five of the six sampling weeks (three sampling events in run 1 and three sampling events in run 2) when compared to other P Miner treatments and the no P-solubilizing control treatment (Fig. 1a). Adding P Miner at $1\times$ or $2\times$ rate (120 or 240 kg ha^{-1}) sometimes also increased labile P, with a positive effect measured during three and two of the six sampling events, respectively. There was no consistent effect of sampling event with the response to P Miner addition. With the addition of 17 kg ha^{-1} P fertilizer, similar responses were observed, although there was no consistency among sampling weeks. However, when P fertilizer rate increased to 34 and 50 kg ha^{-1}, the $1\times$ and $2\times$ rates of P Miner were more

Table 1 Analysis of variance for plant (shoot dry weight, root dry weight, tissue P concentration) and soil (labile P using $CaCl_2$ extract and acid-extractable P using Mehlich-I extract) in a greenhouse pot study to evaluate the effects of P rate (0, 17, 34, and 50 kg P ha^{-1}) and P-solubilizing treatments (Avail®, P Miner $1\times$ (120 kg ha^{-1}), P Miner $2\times$ (240 kg ha^{-1}), and P Miner $4\times$ (480 kg ha^{-1}) and no P-solubilizing treatment) on P availability in perennial ryegrass conducted in 2011. Experiment was repeated in time as run 1 and run 2

	Run 1			Run 2		
	Week 4	Week 8	Week 12	Week 4	Week 8	Week 12
Shoot dry weight (g)	Pr > F			Pr > F		
P rate	0.69	0.004	0.0001	0.48	0.001	0.001
P amendment	0.75	0.87	0.0003	0.007	0.18	0.65
P rate × P amendment	0.32	0.60	0.57	0.02	0.02	0.22
Root dry weight (g)						
P rate	0.63	0.61	0.23	0.07	0.04	0.87
P amendment	0.92	0.51	0.02	0.0007	0.06	0.49
P rate × P amendment	0.56	0.38	0.95	0.10	0.24	0.58
Tissue P concentration (mg kg^{-1})						
P rate	0.26	0.0001	0.0001	0.0001	0.0001	0.0001
P amendment	0.45	0.0001	0.22	0.04	0.76	0.02
P rate × P amendment	0.96	0.02	0.42	0.21	0.19	0.18
Total P uptake (µg pot^{-1})						
P rate	0.002	0.0001	0.0001	0.001	0.0001	0.0001
P amendment	0.23	0.004	0.31	0.01	0.661	0.362
P rate × P amendment	0.54	0.49	0.75	0.37	0.14	0.03
Labile P (mg kg^{-1})						
P rate	0.0001	0.0001	0.005	0.0003	0.35	0.0002
P amendment	0.005	0.0001	0.0001	0.0001	0.0001	0.007
P rate × P amendment	0.02	0.06	0.005	0.07	0.17	0.004
Acid-extractable P (mg kg^{-1})						
P rate	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
P amendment	0.0001	0.004	0.0001	0.0001	0.0002	0.41
P rate × P amendment	0.09	0.54	0.0004	0.02	0.18	0.04

Fig. 1 Mean labile soil P in a ryegrass greenhouse pot experiment treated with one of five P-solubilizing treatments (Avail, P Miner at 1× (120 kg ha^{-1}), P Miner at 2× (240 kg ha^{-1}), P Miner at 4× (480 kg ha^{-1}), and no P-solubilizing treatment) applied with **a** 0; **b** 17; **c** 34; and **d** 50 kg ha^{-1} P fertilizer). Experiment was duplicated in run 1 and run 2. Labile (CaCl$_2$ extractable) P was evaluated at 4, 8, and 12 weeks following treatment application during each run. Because Avail is a fertilizer treatment and cannot be applied alone, it is omitted from the figure. *Dissimilar letters* within each week indicate differences among P-solubilizing treatments with $\alpha = 0.05$
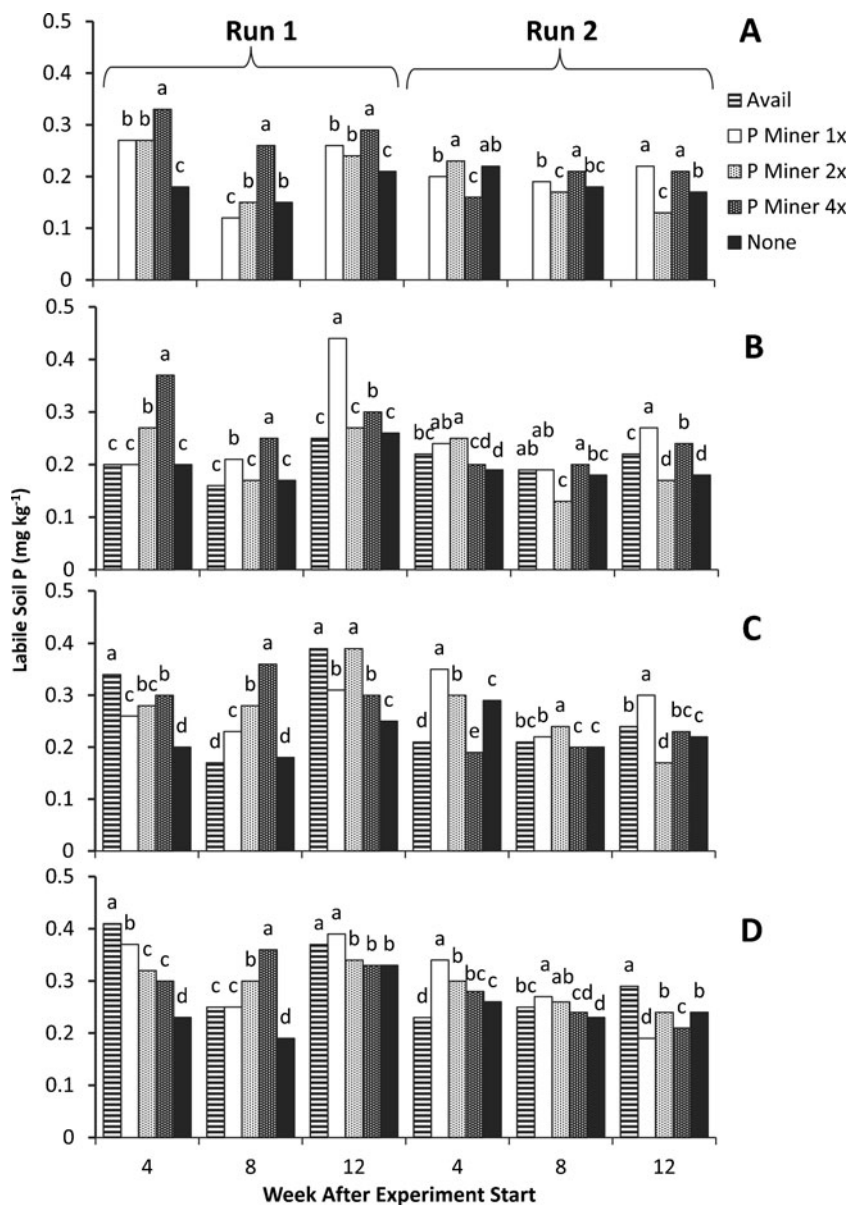

effective than the 4× rate. P Miner 1× increased labile P at six and five sampling events (out of six) and 2× increased labile P at five and four sampling events (out of six) for the 34 and 50 kg P ha^{-1} fertilization rate, respectively, while 4× increased in only three and two times (out of six). This trend was also found for the Avail treatments, which increased labile P in two, three, and five sampling events (out of six) for the 17, 34, and 50 kg P ha^{-1} fertilization rates, respectively. It is important to remember that increasing rates of P fertilization were confounded with increasing rates of Avail, as Avail was directly attached to the fertilizer. In general, as P fertilization rate increased, the average labile P increased.

When no fertilizer was added, acid-extractable soil P was often greater when P Miner was added at the lowest rate (1×; 120 kg ha^{-1}) compared to soil treated with no P Miner (Fig. 2a). This effect was observed in every sampling in

runs 1 and 2. Additions of P Miner at any rate increased acid-extractable P over that measured in the non-amended control in run 1 (Fig. 2a), but in run 2 P Miner at the two higher rates (2× and 4×; 240 or 480 kg ha^{-1}) did not affect acid-extractable P (or it was decreased) when compared to soil to which no P Miner had been added. With the addition of 17 and 34 kg ha^{-1} P fertilizer, P Miner at 1× was still the top performing P-solubilizing agent, which increased acid-extractable P in four and six of the six sampling weeks, respectively. P Miner 2× and 3× were about the same increasing in only two and three sampling weeks (out of six) at the 17 and 34 kg ha^{-1} P rates, respectively. However, at the highest P fertilization rate (50 kg ha^{-1}), P Miner 4× was the top performing treatment, which increased acid-extractable P at three of six sampling weeks, while 2× increased P in only two, and 1× in only one sampling week

Fig. 2 Mean acid-extractable soil P in a ryegrass greenhouse pot experiment treated with one of five P-solubilizing treatments (Avail, P Miner at 1× rate (120 kg ha^{-1}), P Miner at 2× rate (240 kg ha^{-1}), P Miner at 4× rate (480 kg ha^{-1}), and no P-solubilizing treatment) applied with **a** 0; **b** 17; **c** 34; and **d** 50 kg ha^{-1} P fertilizer. Experiment was duplicated in run 1 and run 2. Acid-extractable (Mehlich-I) P was evaluated at 4, 8, and 12 weeks following treatment application during each run. Because Avail is a fertilizer treatment and cannot be applied alone, it is omitted from the figure. *Dissimilar letters* within each week indicate differences among P-solubilizing treatments with $\alpha=0.05$
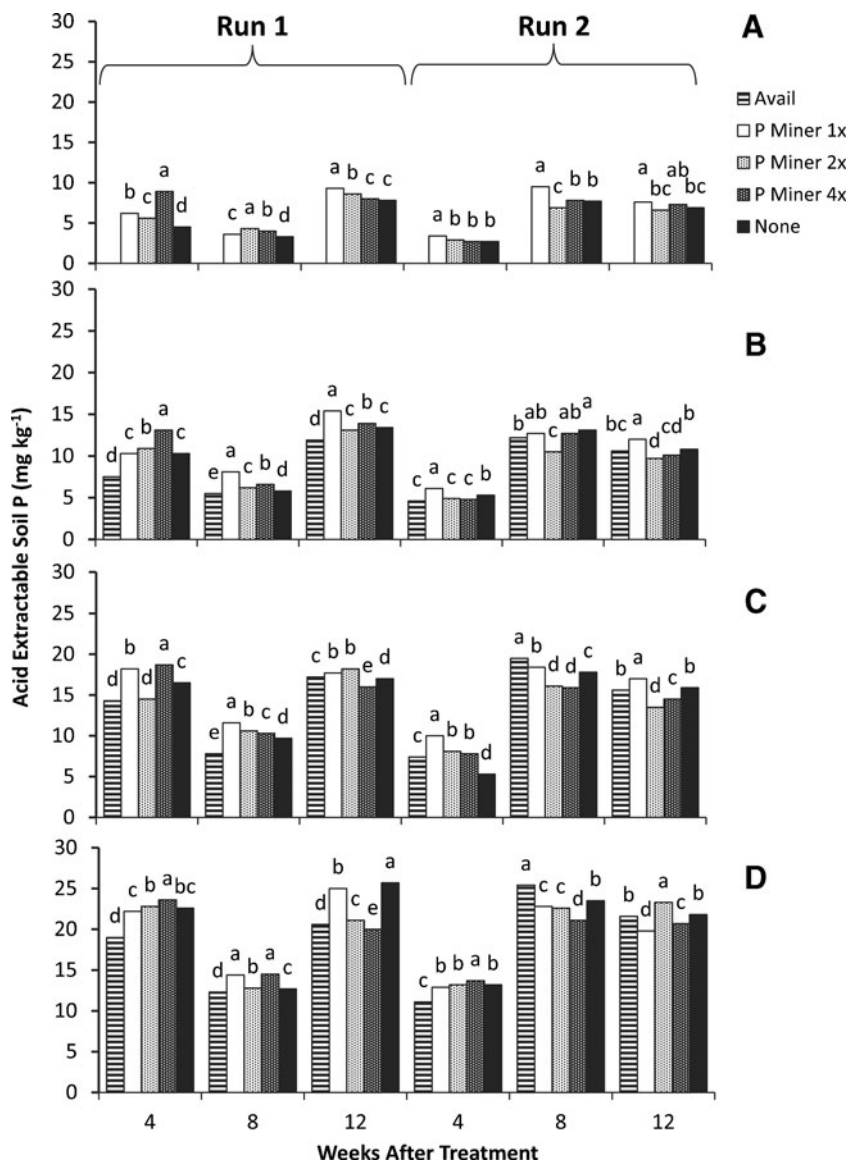
(out of six). Avail only increased acid-extractable P in two sampling weeks or less for all the P fertilization rates.

Overall, the addition of P-solubilizing amendments sometimes increased labile (CaCl$_2$ extractable) and acid-extractable (Mehlich-I) soil P, although effects were less apparent as P fertilization rate increased. In order to evaluate overall ability of P-solubilizing products, the percentage of success was determined. This is defined as the number of times the P-solubilizing treatment significantly increased labile or acid-extractable P beyond that measured in the control (no P solubilizer added) across all P rates and runs. Thus, for Avail, there were six sampling weeks with three P rates, or 18 total evaluations of its ability to increase available P. With the P Miner products, there were 24 sampling events, as the 0 kg ha^{-1} P treatment could also be assessed. Using this form of analysis, all P-solubilizing treatments increased labile P over the control more than 50 % of the time (Avail, P Miner 1×, P

Miner 2×, and P Miner 4× were 61, 79, 54, and 63 % of the time, respectively). Acid-extractable P increased in 22, 71, 38, and 46 % of the data collected for the Avail and P Miner 1×, 2×, and 4× treatments, respectively. Both labile P and acid-extractable P were increased most often with the P Miner 1× treatment. The P Miner 2× and 4× treatments were fairly similar and the next most effective, while the Avail treatment was effective least often, especially when evaluated using the acid-extractable method.

The influence of P-solubilizing products was more pronounced on the rapidly changing labile P than with acid-extractable P. This is not unexpected, as the acid-extractable P method removes considerable amounts of nonlabile P (Kuo 1990), which in this soil represented 92–99 % more P than the labile P method. Thus, treatment differences may be difficult to discern with the acid-extractable treatments if more labile forms of P are affected the most. The generally significant

interaction between P rate and P-solubilizing treatment on labile P and acid-extractable P indicates that P-solubilizing treatments are solubilizing P differently. Using labile P as an indicator of P-solubilizing ability, P Miner 4× was most successful a 0 and 17 kg P ha^{-1} fertilizer rates, while P Miner 1× and Avail were more successful with the higher P fertilizer applications. A greater response with added fertilizer indicates that products are increasing the solubility of the fertilizer rather than native soil P concentrations.

In general, the organic acid-based P Miner product at the recommended rate was the most successful at increasing available P in the soil. It increased labile P 79 % of the sampling times and acid-extractable P in 71 % of the sampling times, regardless of P fertilization rate. Studies have shown that exudation of organic acids by microorganisms and plants increase P availability to the organism (Harrold and Tabatabai 2006; Palomo et al. 2006). Other studies have demonstrated that these organic acids can increase P availability (Feng et al. 2004; Hu et al. 2005; Andrade et al. 2007; Wang et al. 2008; Oburger et al. 2011) and reduce sorption/precipitation of added P (Andrade et al. 2003). Soil type is thought to play a role that determines the effectiveness of the solubilization (Palomo et al. 2006; Oburger et al. 2011). Oburger et al. (2011) found that organic acids were more effective in soils with medium to high amounts of anionic binding sites (e.g., Fe- and Al-oxyhydroxides) and that citric acid was more effective than malic, oxalaic, and malonic. Other studies have also shown the effectiveness of organic acids (e.g., citrate, malate) releasing P from iron oxides (Johnson and Loeppert 2006). Because the soils in this study had an appreciable Fe- and Al-oxyhydroxide content, it is feasible that the P-solubilizing compounds are indeed solubilizing P to some extent. Alternatively, the treatments may be stimulating microbial populations that are increasing P solubility through their own activities.

While data do not show a consistent top performing P-solubilizing amendment, they do show that the P-solubilizing P Miner product shows some promise to improve available P, even in this high P-fixing soil. Furthermore, the 100 % success rate of P Miner at the recommended rate (1×) when P fertilizer was not applied, suggests that this product may be useful in situations where P will need to be scavenged from soils because P may no longer be applied (such as when environmental regulations limit application of P). This effect needs continued study in field trials, as positive results observed here may be partly due to ryegrass roots filling and scavenging P from the entire volume of each pot. Furthermore, P Miner may be more effective in soils with a lower P-fixing capacity.

Plant tissue

The number of plants per pot did not differ by P rate or P-solubilizing treatment nor was there a significant interaction during all sampling weeks. Thus, data are not shown.

Ryegrass shoot dry weight ranged between 0.04 to 0.12, 0.20 to 1.51, and 0.75 to 3.00 g for weeks 4, 8, and 12 (runs 1 and 2 data combined; week 6 biomass was added to biomass collected during the destructive harvest). Shoot dry weight was not affected by P rate during week 4, but it did increase with increasing P rate during weeks 8 and 12 of both runs (Table 1). The P-solubilizing treatments increased shoot dry weight compared to the no P-solubilizing treatment in two of six sampling weeks or less, regardless of P fertilization treatment. Of these, Avail was the top performing P-solubilizing agent, which improved shoot biomass when compared to the control in two sampling weeks. All of P-solubilizing treatments increased shoot biomass compared to the control in week 12 of run 1.

Ryegrass root dry weight ranged from 0.36 to 0.45, 0.45 to 0.61, and 0.93 to 1.29 g for weeks 4, 8, and 12, respectively (runs 1 and 2 data combined). Root dry weight was increased by P rate in less than 1/3 of the sampling weeks, regardless of the P-solubilizing treatments, and this occurred during week 8 of run 2 (Table 1). P-solubilizing treatments were not generally effective in increasing root biomass. P Miner 4× and Avail increased root biomass in two of six sampling weeks, P Miner 2× increased in one of six sampling weeks, and P Miner 1× did not increase root biomass.

In general, the ratio of ryegrass root dry weight to shoot dry weight (root/shoot ratio) decreased with increasing P rate, which was significant in three of six sampling weeks (data not shown). In general, root/shoot ratio increased with time, regardless of P rate or P solubilization treatment. During run 1, the root/shoot ratio was not affected by P-solubilizing treatment, except in week 8, where all P-solubilizing treatments had lower root/shoot ratio than the no P-solubilizing treatment ($P=0.003$). During run 2, the root/shoot ratio differed among P-solubilizing treatments only in week 4, where P Miner was greater than the control ($P=0.001$). Typically, shoot growth is more inhibited than root growth when P is limiting to plant growth, which results in a higher root/shoot ratio. However, the root/shoot ratio is also affected by other factors such as water and availability of other nutrients (Marschner 1995). The effect of P availability on the root/shoot ratio was not conclusive. Only two of six sampling weeks showed significant differences among P-solubilizing treatments, and for those weeks, only one of the two weeks showed a decrease in the root/shoot ratio due to P-solubilizing treatments.

Ryegrass tissue P concentration increased with P rate in all sampling weeks (including week 6 additional shoot harvest) of both experimental runs (Table 1; data not shown). Reponses to the addition of the P-solubilizing treatments were mixed, and some cases were significant (Fig. 3). Increased tissue P, compared to the no P-solubilizing treatment, was found in a few treatments. Both P Miner 4× and Avail increased tissue P in two of eight sampling weeks

Fig. 3 Mean (**a**) tissue P concentration and (**b**) total P uptake in a ryegrass greenhouse pot experiment treated with one of five P-solubilizing treatments (Avail, P Miner at 1× (120 kg ha^{-1}), P Miner at 2× (240 kg ha^{-1}) rate, P Miner at 4× (480 kg ha^{-1}) rate, and no P-solubilizing treatment) applied with 0, 17, 34, or 50 kg ha^{-1} P fertilizer (data combined). Experiment was duplicated in run 1 and run 2. Data are missing from the P Miner 4× treatment at week 12 run 1. *Dissimilar letters* within each week indicate differences among P-solubilizing treatments with α=0.05
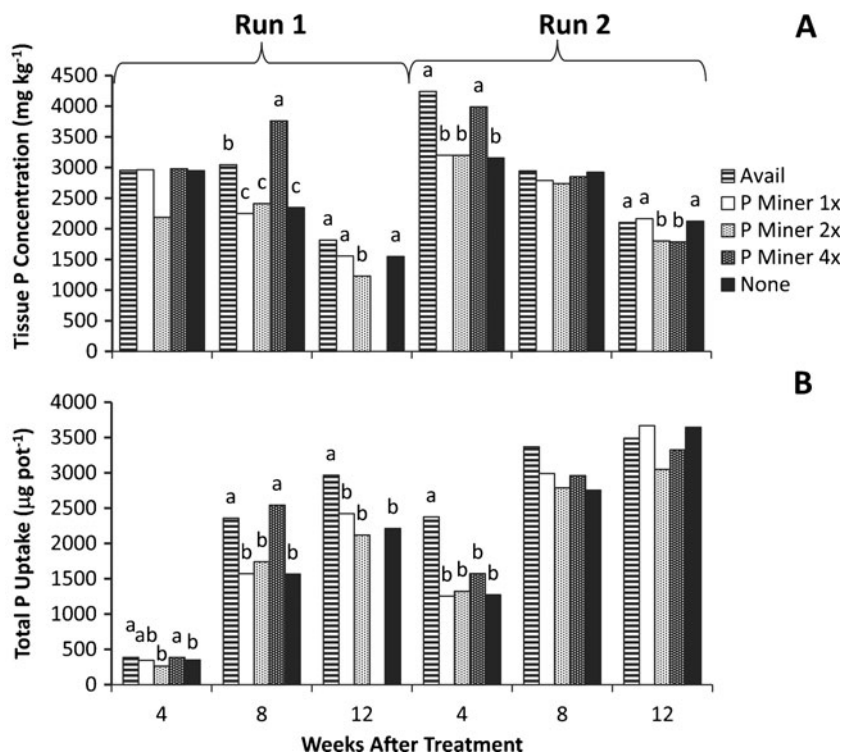
(includes two week 6 shoot-only harvests in run 2 for plants designated for week 8 and week 12 destructive harvest) (Fig. 3a). During week 8 (run 1), P Miner 4× and Avail treatments increased tissue P concentration by 60 and 30 % of the control treatment, respectively. Similar results were observed in week 4 (run 2) with 26 and 34 % increases, respectively. In week 12 (run 2), P Miner at 2× and 4× rates reduced tissue P concentration by approximately 15 % below all other P-solubilizing treatments including the control (Fig. 3a). Week 6 shoot P concentration data, which ranged between 1,513 and 5,250 mg kg^{-1} for the 8-week destructive harvest and 206 to 7,918 mg kg^{-1} for the 12-week destructive harvest, were not presented in Table 1 or Fig. 3 due to lack of difference among P-solubilizing treatments and simplification of data presentation.

Total P uptake of ryegrass increased with P rate in all sampling weeks of both experimental runs. Differences in total P due to P-solubilizing treatments were observed in many of the same sampling weeks as that observed with tissue P concentration, i.e., increases in weeks 8 (run 1) and 4 (run 2) (Fig. 3b). During week 8 (run 1), the P Miner 4× treatment increased total P uptake by >60 % of the control. Application of Avail resulted in the next highest total P uptake, which was >50 % of the control. During week 4 (run 2), total P uptake was 86 % greater in Avail-treated plants compared to the control.

Plant growth parameters (shoot and root dry weight, tissue P concentration, and total P uptake) were affected by P-solubilizing treatments in three of six sampling weeks

or less. Most often, tissue P concentration and total P uptake were greater than the control (no P solubilizer added) when treated with the Avail and P Miner 4× treatments. This was not consistent with labile and acid-extractable soil P results, which found the P Miner 1× to be the best treatment (especially at high rates of P); however, P Miner 4× was the next best treatment. The Avail treatment was deemed the least effective treatment based on soil P analysis but was one of the most effective at providing P to the plant. This may be due to rapid uptake of solubilized P to the plant or to some unknown effect of the maleic-itaconic copolymer soil P analytical methods.

Overall, results are not definitive, but they indicate that both Avail and P Miner have the potential to improve available forms of P in the soil that is available to ryegrass. Although P Miner 4× resulted in the highest labile and acid-extractable P, Avail and P Miner 1× treatments resulted in the highest P uptake. Results support a growing body of research that suggests that organic acids may improve P acquisition; however, there likely numerous factors affecting this ability. Factors such as microbial biodegradation (Strom et al. 2002; Oburger et al. 2009), soil properties (e.g., surface charge and pH; Ohno et al. 2011), degree of saturation and form of P (Oburger et al. 2011), and specific composition P-solubilizing organic acids are likely to contribute to their effectiveness. Additional field evaluation of these commercial products is needed, as is additional study to determine the exact nature and composition of the organic acids contained in these products.

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