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Improving the short-term efficiency of rock phosphate-based fertilizers in pastures by using edaphic biostimulants

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Abstract

Background: The use of reactive rock phosphate (RP) in acidic soils as a phosphate (P) source for pastures and crops presents attractive economic advantages with respect to soluble phosphate. However, some studies have demonstrated that the short-term (1-year) efficiency of RP, compared with that of water-soluble P, is relatively poor. This fact penalizes not only the yield and quality of the earlier harvests, but even the whole final yield when the crop is affected by some abiotic or biotic stress at the beginning of the cycle. In the present study, we investigated the ability of new edaphic biostimulants to increase the short-term efficiency of RP-based fertilizer as a P source for pastures cultivated in acid soils. To this end, we have granulated rock phosphate with two edaphic biostimulants: tryptophan and a heteromolecular organic complex formed by humic acid and tryptophan through iron bridges, and compared their short-term P fertilizer efficacy with that of single superphosphate and rock phosphate.

Results: Soil incubation studies showed that the heteromolecular complex humic acid–tryptophan and Tryptophan were able to increase soil CO_2 production compared with native soil, rock phosphate, and superphosphate. Likewise, the presence of humic acid–tryptophan complex and Trp significantly increases plant-available phosphate compared with rock phosphate, up to levels similar to those of superphosphate. Plant (ray grass)–soil–pot studies showed that rock phosphate/(humic acid–tryptophan) formulation yielded values for both ray grass dry matter production and shoot P concentration, clearly higher than those of rock phosphate and rock phosphate/tryptophan. In addition, the results associated with rock phosphate/(humic acid–tryptophan) were similar to those for superphosphate, after 3 months of cultivation.

Conclusions: Taken together, these results showed the suitability of the use of specific humic acid-based edaphic biostimulants to improve the short-term effect of rock phosphate fertilizers as a phosphate source for pastures cultivated in acid soils.

Keywords: Edaphic biostimulants, Rock phosphate, Phosphate fertilizer, Pastures, Plant-available phosphate soil microbial activity, Humic acid, Humic acid-based heteromolecular complexes

Background

Water-soluble phosphate (P)-based fertilizers, mainly single superphosphate (SSP) and triple superphosphate (TSP), are the main sources of P used for cultivated pastures, mainly in alkaline soils, but also in acidic soils [1, 2]. However, in pastures (and also other crops) cultivated in acidic soils, the direct application of rock phosphate (RP) (granule or powder)—without previous reaction with sulfuric and/or phosphoric acids—may be a suitable, less-expensive, alternative to water-soluble P fertilizers [1]. In addition, the slow solubilization of RP in acidic soils may also contribute to decrease environmental risks, such as the eutrophication of surface waters [3].

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However, although some authors have reported that the efficiency of RP as a P source for pastures cultivated in acid soils is as effective as that of SSP or TSP in long-term studies (3–4 years) [1, 4, 5], most studies have shown that RP efficiency is normally lower than that of water-soluble P fertilizers, with this fact being likely related to the lower short-term (1-year) efficiency of RP as a source of plant-available P with respect to that of water-soluble P (SSP or TSP) [1].

Historically, researchers have tried to improve plant-available P release rates from RP using several strategies. Some authors have prepared partially acidulated RP, or RP mixed with water-soluble P (TSP, SSP, or ammonium phosphates) [6–11]. Recently, other strategies included the use of stabilized phosphorus-solubilizing microorganisms (PSMs) [12–16], or plant growth-promoting rhizobacteria (PGPR) [17–19] along with or without RP.

Very recently, several studies have shown that specific enzyme-based hydrolyzed compost and protein residues, named edaphic biostimulants, are able to significantly increase the activity of most soil enzymes [20, 21]. This action was accompanied by increases in the biodegradation of many organic contaminants and xenobiotics [21]. In this line, Renella et al. [22] showed that an edaphic biostimulant based on the heteromolecular complex between a sedimentary humic acid (HA) and tryptophan (Trp) (HA–Trp) was able to significantly increase the activity of several enzyme families (phosphatases, organic hydrolases, and proteinases) in different soil types. In fact, previous studies had shown the stimulant action of Trp on the growth of soil cultivated plants, likely through the promotion of the biosynthesis of auxin in both the rhizosphere and plants [23, 24]. However, the presence of Trp complexed by the HA supra-structure assures and enlarges the in vitro biostimulant action of Trp by delaying fast Trp degradation, thus promoting a more sustained effect [25].

In this framework, the aim of our study is to investigate whether the granulation of micronized RP along with (HA–Trp) complex is able to increase the short-term fertilizer action of RP by improving P solubilization rates in the rhizosphere. Our working hypothesis was that the potential increase in soil microbial activity, related enzyme activities, and local pH acidification in the physical rhizospheric environment surrounding RP granule triggered by HA–Trp might increase the rate of P mobilization from water-insoluble P in RP to the soil solution, thus enhancing short-term RP fertilizer efficiency. With this aim, we have investigated the effect of a granulated fertilizer made from RP and coated with (HA–Trp) (RP/(HA–Trp)) and corresponding control treatments including RP coated with Trp alone (RP/Trp), RP, and SSP, on the early yield and P leaf concentration (1–3 months) of ray grass (*Lolium perenne*) cultivated in pots containing an acidic soil. These studies were complemented by soil incubation experiments carried out in order to evaluate the differential action of all treatments on soil microbial activity and potentially plant-available P.

Methods

Physico-chemical features of RP, SSP, HA, and Trp

The RP and SSP granulated samples (average size distribution 90 % between 2 and 4 mm of granule diameter) were obtained from Timac Agro Fertilizer plant in Lodosa (Spain). Samples of granulated RP/Trp and RP/(HA–Trp) were obtained by coating RP granules with a solution of vegetal oil containing talc and the edaphic biostimulant, while RP and SSP were coated with vegetal oil and talc. The concentration of P expressed as P_2O_5 of the different fertilizers were RP (29 %), RP/Trp (29 %), RP/(HA–Trp) (29 %), and SSP (17 %). In the case of RP-based fertilizers, P is not soluble in water (it is mainly apatite), while SSP contains water-soluble P (mainly monocalcium phosphate).

HA–Trp heteromolecular complex was obtained by reaction of potassium-iron humate and Trp at pH 6 and room temperature as described in [25].

The leonardite HA employed for the preparation of HA–Trp complexes was extracted, purified, and characterized as described in [26, 27]. Elemental analysis revealed that the average chemical composition of HA was 51 % C, 1.2 % N, 2.6 % H, and 45.2 % O. Regarding the distribution of the different functional C-types, ^{13}C NMR studies indicated that HA contained 32 % alkyl C, 9 % O-alkyl C, 43 % aromatic C, 13 % phenolic C, and 16 % carbonyl C. Concerning the size distributions of the different humic samples, the HPSEC study showed a main peak with a maximum corresponding to an apparent MW of 2.3×10^4 Da, a shoulder corresponding to an apparent MW of 5.8×10^3 Da, and a third minor peak related to a fraction with average apparent MW of 1.1×10^3 Da.

Samples of Trp (99.9 %) were obtained from Timac Agro Spain.

Physico-chemical features of the acid soil employed in soil incubation and soil–plant studies

An acidic soil from Egozkue (Navarra, Spain), with low potential plant-available P concentration, was used in the experiments. Egozkue is a small village placed in the north of Navarra. Soils in Egozkue are mainly acidic and poor in organic matter; pluviometry is around 1500 l per year and day/night winter temperature is 10 °C on average. The soil was air-dried and sieved at 2 mm. The final sample was analyzed using Spanish-official analytical methods [1, and references therein] (Table 1). Briefly,

Table 1 Physico-chemical features of Egozkue soil

Conductivity (μS cm^{-1})	24.0
pH	5.60
Extractable P (mg kg^{-1})[a]	4.02
K (mmol kg^{-1})[a]	1.20
Mg (mmol kg^{-1})[a]	3.50
Ca (mmol kg^{-1})[a]	17.4
Na (mmol kg^{-1})[a]	2.50
Fe (mmol kg^{-1})[b]	0.49
Mn (mmol kg^{-1})[b]	0.35
Cu (mmol kg^{-1})[b]	0.003
Zn (mmol kg^{-1})[b]	0.003
Mo (mmol kg^{-1})[b]	Under detection limits
Organic Matter (g kg^{-1})	0.10
Total $CaCO_3$ (g kg^{-1})	0.000
Sand (%)	15.0
Silt (%)	44.0
Clay (%)	41.0

[a] Mehlich-1

[b] DTPA

soil particle size composition was determined by densitometry (Bouyoucos method); total N was determined by LECO CHN elemental analyzer; K, Mg, Ca, and Na were extracted with a 40 mM HCl and 10 mM H_2SO_4 solution (Mehlich I extractant) and further analyzed using ICP-OES; micronutrients (Fe, Cu, Zn, and Mn) were analyzed following extraction with a solution of DTPA 5 mM [28] using ICP-OES; organic matter was determined by dichromate oxidation method [29]; total carbonates were measured with a Bernard calcimeter method. The pH and electrolytic conductivity were measured using specific electrodes in water (1:2.5 soil/water ratio). Molibdate was analyzed in the DTPA-water extract using ICP-OES.

Soil incubation studies for the evaluation of plant-available P in soil

Treatments

Five repetitions for each treatment and a control without P treatment were used. The test consisted of SSP, RP, and RP complemented with two different edaphic biostimulants incorporated during the granule coating process. The two edaphic biostimulants considered in the study were as follows:

Trp, a precursor of auxin biosynthesis in soils and plants (1 % in the formulation with RP);

(HA–Trp), a heteromolecular complex of HA and Trp through electropositive bridges [25] (2:1 of HA:Trp ratio). The final dose of (HA–Trp) in the formulation was 3 %.

Control treatments included native soil without any treatment (blank, B), soil plus RP, and soil plus SSP. Preliminary studies conducted in our laboratory showed that control treatments including the soil plus Trp or (HA–Trp) without RP at concentrations equivalent to those involved in RP/Trp and RP/(HA–Trp) treatments did not present results different from those of the control without any treatment concerning soil-related parameters and plant growth rates. For this reason, we have not included these controls (soil plus Trp or (HA–Trp), without RP) in data presentation and further discussion. The concentration of P applied to the soil in the experiments is described below.

Soil incubation experiments for the evaluation of plant-available P in soil

A mixture of 100 g of soil and 7 g of perlite was placed in 150-mL plastic pots. The different treatments were added to the pot and the content was intensively mixed. A fertilizer rate of 150 mg P kg^{-1} soil was used for all treatments and positive and negative controls. A control without added P was also used (B). Treated soil samples were homogenized and supplied with type I de-ionized water to reach soil field capacity, which was previously determined by moistening a soil column and allowing it to drain freely. Pots were closed and allowed to stand at ambient temperature in the dark. Samples corresponding to five replications were taken after 10, 20, and 30 days from the onset of the treatments and air-dried for analysis. Pots were opened every day to avoid microbial life inhibition and anaerobic processes.

Analysis of plant-available P fractions in control and treatments

The total potentially plant-available P in samples of incubated soil was evaluated using the anion-exchange resin-extractable P [30, 31]. Soil samples were taken for analysis after 10, 20, and 30 days from the onset of treatments. The amount of P desorbed by an anion-exchange resin was determined using the method of Sibbesen [30] with slight modifications. The resin used was 20–50 mesh Dowex 1 × 4 anion-exchange material in chloride form. An amount of 0.6 g of air-dried soil was placed in a 50-mL plastic tube. A volume of 30 mL of de-ionized water and 2.2 g of resin, held in a nylon bag, were added to the soil ample. Following shaking at the maximum possible speed for 2.5 h, the resin bag was withdrawn, the soil suspension centrifuged, and the solution discarded. The nylon bag was then rinsed with water and P eluted with 30 mL of 0.5 M HCl with shaking at the maximum speed for 30 min. Another 30 mL of 0.5 M HCl was then added and shaking applied to complete P elution. The

two HCl solutions were filtered and analyzed by ICP-OES. This P fraction was designated plant-available P.

Soil incubation studies for the evaluation of soil microbial activity

Both the fertilizer treatments and the concentration of fertilizer used were similar to those employed for the evaluation of plant-available P, and described above.

Two different assays were carried out in order to measure microbial activity: the FDA method and CO_2 soil production [32, 33].

Soil incubations were carried out as described below.

Closed 0.5-L pots with a mixture of 100 g of soil–7 g of perlite and the different controls and treatments were irrigated with 43 g of water (at field capacity) and maintained at 25 °C. Samples for analysis were taken after 7, 14, 21, and 28 days. Two replicates for each treatment were used.

Hydrolysis of fluorescein diacetate [3′,6′-diacetylfluorescein (FDA)] [32]: Two grams of air-dried soil was placed in a 125-ml Erlenmeyer flask. 50 ml of 60 mM sodium phosphate buffer, pH 7.6, and 0.50 ml of 4.9 mM FDA lipase substrate solution (20 mg FDA lipase substrate in 10 ml acetone) were added. After mixing, samples were incubated for 1 h at 25 °C. Then 10 ml of acetone was added to the suspension to stop FDA hydrolysis. Samples were filtered through Whatman No. 2 filter paper. The absorbance was measured on a spectrophotometer (HP-8453) at a wavelength of 490 nm.

Measurement of CO_2 release from soil by titration method according to Anderson [33]: A vial of 5 ml of 1 M KOH was placed in each closed 0.5-L pots. The alkali traps were changed and titrated at 7, 14, 21, and 28 days. Unreacted alkali in the KOH traps was back-titrated with 0.4 M HCl to determine the CO_2 release by microbial respiration.

Soil–plant experiments

Experiments were carried out in a greenhouse under controlled temperature and lighting conditions. A 24/18 °C day/night temperature regime and a relative humidity of 40–60 % were used.

Five replicates of 500 g of soil were blended with the different treatments: control without added P, SSP, RP, RP/Trp (Trp), and RP/(HA–Trp). In order to prepare the soil–fertilizer mixture, a Thermomix at maximum power for 5 s was used prior to the placement of the soil in plastic pots. Then, each soil–fertilizer mixture was carefully blended with 50 g of perlite and supplied with 10 seeds of ray grass (*Lolium* sp.) on pot surface (113 cm^2). The P addition rates used were 10, 30, and 50 mg P kg^{-1} soil. As for the other nutrients, 200 mg kg^{-1} soil N and 200 mg kg^{-1} K soil were added as urea and potassium chloride in order to complete macronutrient fertilization. Finally, 0.4 mg kg^{-1} soil of Mo as sodium molibdate was also added to prevent a potential deficiency of Mo. The shoots corresponding to each pot were consecutively harvested at the end of the month for 3 months after seed germination. Once analyzed for fresh matter, shoots were dried in an oven at 40 °C for 3 days to determine dry matter. Next, the dry shoots were homogenized in a mill and sub-samples attacked with HNO_3 and H_2O_2, and digested in a microwave oven, to determine P by ICP-OES as described in [27].

Statistical analyses

All experimental results were subjected to multiple pairwise comparisons between treatments, using Fisher's least significant difference (LSD) post hoc test in a one-way ANOVA method with the overall α-level set at 0.05.

Results and discussion

Association of RP with HA–Trp or Trp increased both soil-available P and soil microbial activity, compared with RP

Our results clearly show that SSP, RP/Trp, and RP/(HA–Trp) caused a prompt (after 10 days) and significant increase in the concentration of plant-available P in the soil with respect to RP and native soil (Table 2). This increase, however, was transient and decreased after 20 and 30 days to RP levels (Table 2). In principle, this decrease in plant-available P was expected as the absence of a sink for available P in the soil system (such as plant roots) leads to an increase in plant-available P in soil solution that, in turn, may cause a feedback inhibition of those processes and enzyme activities involved in the P mobilization mechanisms activated by these formulations [22]. In fact, some authors have reported that an increase in the concentration of P in water-soluble P was associated with a decrease in CO_2 soil production by an inhibition of soil microbial activity [34–36]. This fact was also linked to significant decreases in soil enzymatic activities, including those related to different types of soil phosphatases [37]. However, other mechanisms, such as phosphate precipitation or phosphate absorption, can also contribute to the plant-available P decline with time.

While the increase in plant-available P is easily explained for SSP since it has water-soluble phosphate, in the case of RP/Trp and RP/(HA–Trp) this fact has to be related to some kind of process leading to P solubilization from RP. These processes are normally related to changes in the activity of P-solubilizing microbiota [18]. Our studies showed that both Trp and HA–Trp associated with RP caused a significant increase in soil

Table 2 Time-course variation of soil plant-available P (mg kg^{-1}) measured by the resin method

Time/days	Treatments				
Days	B	RP	SSP	RP/Trp	RP/(HA–Trp)
10	3.7aA	19.7bA	33.6cB	46.9cB	33.2cB
20	11.5aB	24.2cA	21.7cB	16.0bcA	13.5bA
30	5.8aA	17.3bA	14.6bA	12.3bA	17.0bA

Data followed by the same lowercase letter in each row or by the same uppercase letter in each column are not statistically different from each other (Fisher's test, $P < 0.05$). Capital letters are used for differences with time within each treatment. Lowercase letters are used for differences among treatments within each time

microbial activity compared with the native soil, SSP, and RP (Table 3). This fact was very clear when CO_2 soil production was evaluated, while the results for FDA hydrolysis were less conclusive (Table 3). These differences between methods have also been described by other authors, and seem to be related to the fact that FDA hydrolysis method only includes specific families of soil hydrolases [38–40] and, therefore, it can give an incomplete evaluation of soil microbial activity in some soil types. It is for this reason that traditional methods such as the analysis of soil CO_2 production might be more sensitive for the evaluation of whole soil microbial activity changes. In any case, these results suggest that the increase in soil microbial activity plays an important role in the P-solubilizing action of Trp and HA–Trp from RP.

Association of RP with HA–Trp, but not with Trp alone, produced a sustained increase in shoot dry matter production up to levels similar to those of SSP

The results clearly showed that the short-term action of RP on both shoot dry matter and P fertilizer efficiency is significantly lower than that of SSP for all harvests and higher P doses (30 and 50 mg kg^{-1}) (Fig. 1). In line with these results, shoot dry matter values for RP only presented a dose–response pattern with added P rates at the first harvest, while SSP maintained a dose–response pattern for all harvests (Fig. 1). These results confirm that our experimental model is adequate to investigate the main issues posed by the study.

As for the ability of the different fertilizers to enhance shoot dry matter production, although both RP/Trp and RP/(HA–Trp) presented very similar results to each other in soil incubation studies, HA–Trp—but not Trp alone—presented a beneficial action of the efficiency of RP to enhance shoot dry matter production when all harvests are considered (Fig. 1). In fact, even though RP/Trp caused a prompt and significant increase in shoot dry matter production at the first harvest and for the higher doses of P (30 and 50 mg kg^{-1}), this effect disappeared at the following harvests. Conversely, both SSP and RP/(HA–Trp) presented higher increases in shoot dry matter production than the control and RP for the second and third harvests (Fig. 1). Thus, the association of RP with (HA–Trp) caused a significant and sustained increase in shoot dry matter production compared with RP for all harvest times, with this increase being similar to that

Table 3 Time-course variation of FDA hydrolysis and CO_2 soil production

	μg FL g^{-1} h^{-1}			
	7 days	14 days	21 days	28 days
FDA hydrolysis				
B	1.88	2.76	3.71	4.08a
RP	1.80	2.73	3.59	4.02a
SSP	1.58	2.76	3.55	3.98a
RP/Trp	2.04	3.03	4.00	4.41c
RP/(HA–Trp)	1.71	2.74	3.70	4.15b
	mg CO_2 kg^{-1} soil			
	7 days	14 days	21 days	28 days
CO_2 soil production				
B	83.6	123	147	165a
RP	117	154	170	203a
SSP	81.4	128	152	178a
RP/Trp	96.8	154	187	229b
RP/(HA–Trp)	123	167	202	240b

Data followed by the same lowercase letter in each column are not statistically different from each other (Fisher's test, $P < 0.05$)

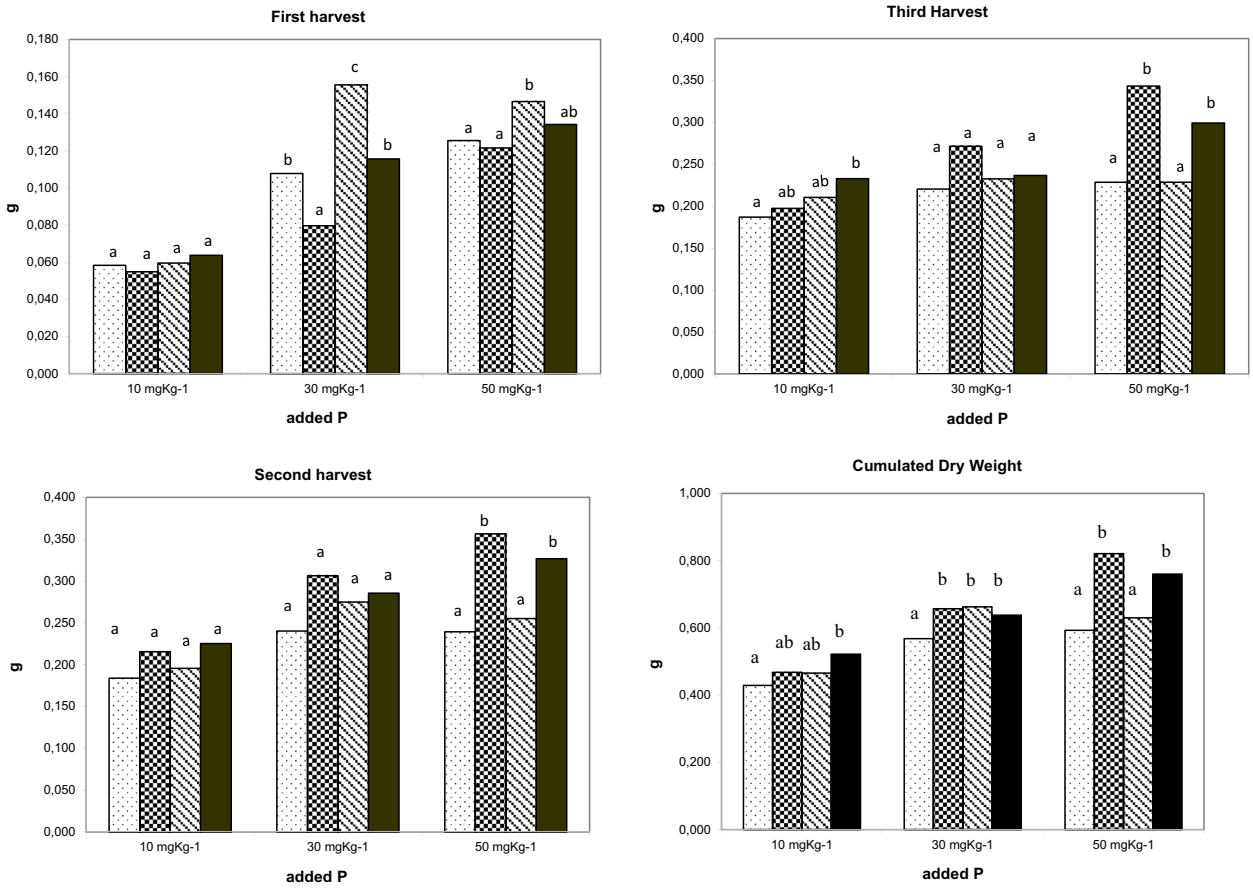

Fig. 1 First, second, and third harvests as well as accumulated shoot dry matter production (g per pot; average of five replications) for RP *dotted bar*, SSP *gray shaded bar*, RP/Trp *cross-lined bar*, and RP/(HA–Trp) *black shaded bar*. *Columns* followed by the *same lowercase letter* are not statistically different from each other (Fisher's test. $P < 0.05$)

caused by SSP (Fig. 1). These results were clearer for the highest dose of P added to the soil (50 mg kg^{-1}) (Fig. 1). Differences between RP/Trp and RP/(HA–Trp) might be a consequence of an effect of HA on RP solubilization either directly or through an increase in soil microbial activity. However, preliminary studies using P fertilizers containing 1 % HA did not show any effect on both P bioavailability and shoot growth in several soil types [41]. In some way, these results were expected since the concentration of P applied to soil (50 mg Kg^{-1}) involved the soil application of very low concentrations of HA (lower than 40 mg kg^{-1}). However, these studies showed that when HA was applied forming stable and soluble complexes with Zn or Cu, a clear increase in plant growth was observed resulting from an improvement in the plant uptake of both micronutrients [42]. Although in our study we do not use humic metal complexes, some action of HA in RP fertilizers improving micronutrient plant nutrition cannot be ruled out. Another factor influencing the differential effect of HA–Trp compared with Trp alone might result from the presence of Fe in HA–Trp complexes. However, taking into account that the amount of plant-available Fe in Egozcue soil is quite high (Table 1), it is rather improbable that the Fe added with HA–Trp in RP/(HA–Trp) treatment can cause significant changes in plant growth. Finally, another possible explanation for these results may relate to some type of synergic action of HA and Trp when applied together. In fact, Trp complexation in HA supramolecule might delay its conversion in indolacetic acid (IAA) (and, therefore, IAA degradation) by favoring a slow release of Trp to the rhizospheric environment [23–25]. This hypothesis might also contribute to explain the results obtained, but further experiments using this experimental model are needed in order to establish its validity.

Taking into account that the effect of RP/(HA–Trp) on shoot dry matter production was consistent with the increase in soil microbial activity caused for this fertilizer in soil incubation studies (Table 3), this effect might be linked to some kind of secondary action of (HA–Trp) on

local soil features in granule environment resulting from the enhancement in soil microbiota activity, which in turn favor P solubilization from RP.

Regarding the relationships between shoot dry weight production and the concentration of P in the shoot, the values of shoot P concentration showed that RP/(HA–Trp) increased this parameter with respect to RP. In addition, these effects tended to be lower than those associated with SSP only at the first harvest (Fig. 2). The fact that the differences of shoot dry matter production between RP/(HA–Trp) and SSP were not different from each other led to the fact that the fertilizer efficiency of RP/(HA–Trp) and SSP was quite similar to each other and significantly higher than that of RP principally for the highest P dose (50 mg kg^{-1}) and third harvest (Table 4).

The fact that plants treated with RP/Trp have the highest concentration of P in the shoot for the second and third harvests probably derives from a concentration effect associated with the low production of shoot dry matter produced by this fertilizer (Figs. 1, 2). This fact was also reflected in the low values of RP/Trp fertilizer efficiency parameters (Table 4).

Finally, we did not observe clear differences between the efficiency of plant P utilization in the shoot for SSP and RP/(HA–Trp), although in both cases this parameter was higher than those for RP and RP–Trp, principally for the highest P dose (50 mg kg^{-1}) (Table 5). This fact indicated that plant metabolism was not differently affected by SSP and RP/(HA–Trp), thus suggesting that HA–Trp complex had not direct effect on plant metabolism at the doses associated with RP/(HA–Trp) fertilizer rates.

Conclusion

Summarizing, the results here described show that the association of RP with an organic complex formed by HA and Trp linked to each other through electropositive bridges (mainly, proton or metal, in this case Fe) avoided the short-term differences in agronomical efficacy

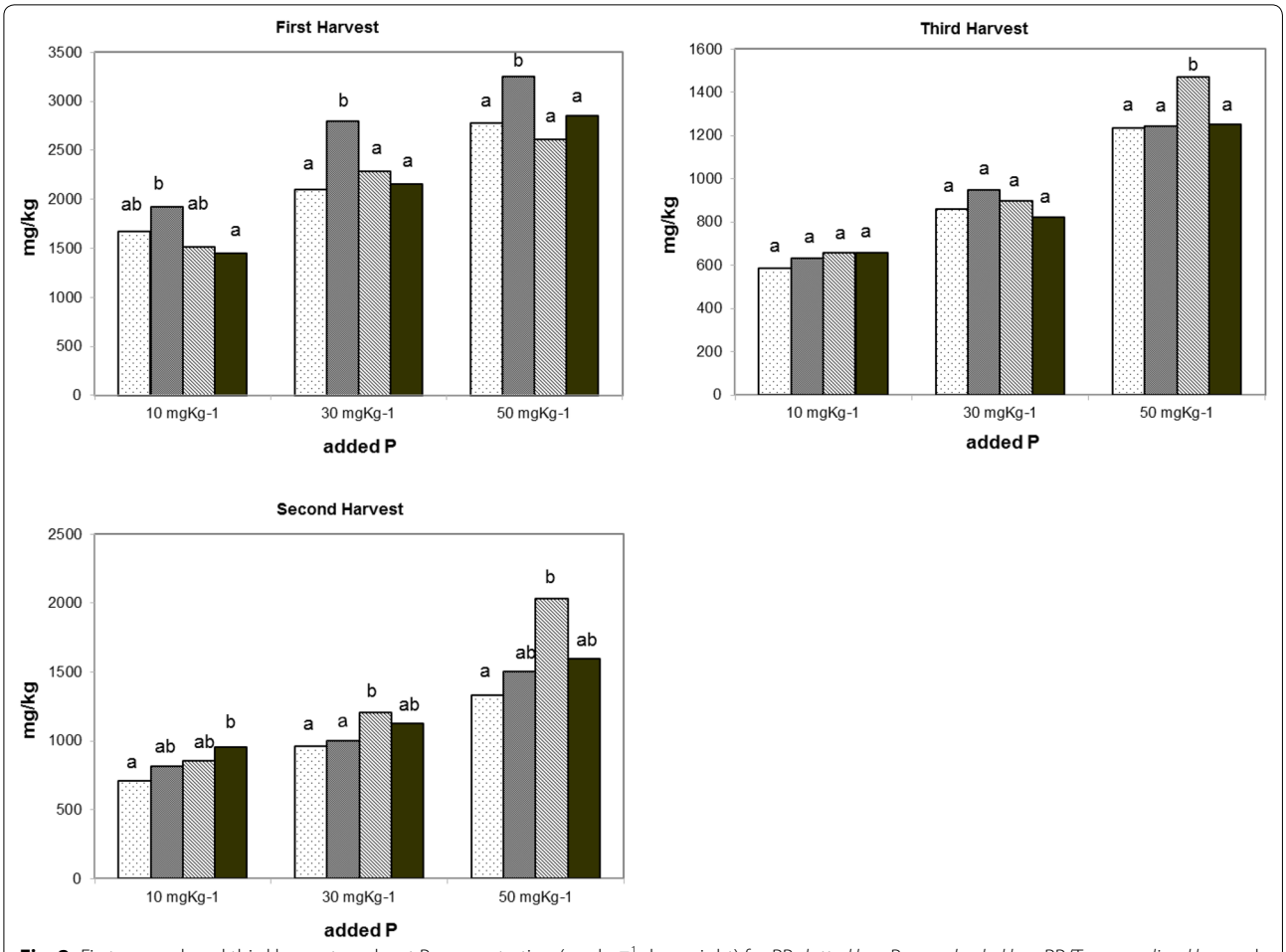

Fig. 2 First, second, and third harvests—shoot P concentration (mg kg^{-1} dry weight) for RP *dotted bar*, P *gray shaded bar*, RP/Trp *cross-lined bar* and RP/(HA–Trp) *black shaded bar*. *Columns* followed by the *same lowercase letter* are not statistically different from each other (Fisher's test. $P < 0.05$)

Table 4 Fertilizer efficiency index defined by [(dry matter production) × 100]/(added P to the soil)

Treatments	RP			SSP			RP/Trp			RP/(HA–Trp)		
Added P (mg kg^{-1} soil)	10	30	50	10	30	50	10	30	50	10	30	50
Harvest												
First	5.83	0.36	0.25	5.49	0.27	0.24	5.95	0.52	0.29	6.37	0.39	0.27
Second	18.37a	0.80c	0.48	21.56b	1.02d	0.71	19.58b	0.92d	0.51	22.53b	0.95d	0.65
Third	18.70a	0.73c	0.46d	19.73b	0.90d	0.69e	21.04b	0.77c	0.46d	23.29b	0.79c	0.60e

Data followed by the same lowercase letter in each row are not statistically different from each other (Fisher's test, $P < 0.05$)

Table 5 Plant P utilization efficiency index defined by [(dry matter production) × 10,000]/(shoot P concentration)

Treatments	RP			SSP			RP/Trp			RP/(HA–Trp)		
Added P (mg kg^{-1} soil)	10	30	50	10	30	50	10	30	50	10	30	50
Harvest												
First	0.35	0.51	0.45	0.29	0.28	0.37	0.39	0.68	0.56	0.44	0.54	0.47
Second	2.60	2.50	1.80b	2.65	3.06	2.37c	2.29	2.29	1.26a	2.35	2.53	2.04c
Third	3.21	2.56	1.85a	3.14	2.86	2.76b	3.21	2.60	1.56a	3.55	2.89	2.39b

Data followed by the same lowercase letter in each row are not statistically different from each other (Fisher's test, $P < 0.05$)

existing between RP and SSP, thus improving the starter action of RP-based fertilizers. This issue is very relevant because the main negative feature of RP as a P source for pastures cultivated in acidic soils is its poor short-term (1-year) effect in comparison with that of water-soluble P fertilizers, thus negatively affecting yield and quality [1]. However, the long-term action (3–4 years) of RP-based fertilizers as a P source for pastures cultivated in acidic soils may be even more efficient than that of water-soluble P fertilizers [1, 4, 5]. This fact indicates that both fertilizers (HA–Trp activated RP and SSP) may be rather complementary in a whole fertilization plan for pastures in acidic soils.

Authors' contributions

LF principally but also RB, OU, and JE have made the experimental work and contributed to MS preparation; RB, OU, JE, and PMA contributed to the experimental design; JCY contributed to the discussion of results; and JMG contributed to the design of the experiments, discussion of results, MS preparation, and final writing. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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