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Soil properties modulate the effect of different carbon amendments on growth and phosphorus uptake by cucumber plant

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Abstract

Aim of study: Phosphorus (P) is a non-renewable, limited and strategic resource, inefficiently used in agriculture. Organic carbon (C) supply to soil can stimulate microbial activity increasing the mobilization of soil P thus improving its uptake by crops. This work aimed at investigating the effect of different C amendments on P uptake and how may differ depending on soil properties and P fertilization.

Area of study: Soils used in this study were collected in SW Spain.

Material and methods: An experiment with cucumber was performed involving three factors: (i) C amendment (cellulose, glucose, citric acid and control without amendment), (ii) soil type (Vertisol and Alfisol), and (iii) P supply (unfertilized, and mineral phosphate in form of KH_2PO_4).

Main results: Cellulose or glucose provided the highest P uptake by plants in soils independently of the treatment. Cellulose and glucose addition was effective increasing dry matter (DM) in the Alfisol. Citric acid application decreased the development of aerial parts and roots, and P uptake in soils compared with other sources. In the Alfisol, increased P uptake with cellulose was associated to an increased concentration of low molecular weight organic acids, which seemed to be related to microbial activity.

Research highlights: Organic amendments affect microbial activity, and P mobilization mechanisms are associated to microorganisms. This explain the improvement of P supply to plants with amendments; these effects, however, are modulated by soil properties and consequently vary depending on soil type.

Additional key words: vertisol; alfisol; enzymatic activities; cellulose; organic anion

Abbreviations used: CA (citrate ascorbate); CB (citrate bicarbonate); CBD (citrate bicarbonate dithionite); DM (dry matter); DTPA (diethylenetriaminepentaacetic acid); LMWOA (low molecular weight organic acids); PNP (p-nitro phenol); SOC (soil organic carbon); SOM (soil organic matter)

Authors' contributions: Conceived, designed and performed the experiments, and analyzed the data: AMGL, RR and AD. Wrote the paper: AMGL. Critical revision of the manuscript for important intellectual content, and coordinated the research project: AD. All authors read and approved the final manuscript.

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Introduction

Phosphorus (P) is an essential nutrient for life, and crucial for many relevant physiological and biochemical processes in plants (Simpson *et al.*, 2011). It is a major limiting nutrient for crops in many agricultural lands, in part associated to the low portion of total P being available to plants as a result of its rapid precipitation and adsorption in soils (Delgado & Scalenghe, 2008). After applied as fertilizer, P is easily and strongly retained by the soil solid phase resulting in low fertilizer recovery by plants, usually in the range 10–25% of applied fertilizer (Sun *et al.*, 2012). It is assumed that P is limiting for crop yields in around 40% of the world's arable lands (Balemi & Negisho, 2012). However, high P rates and the low P recovery by crops usually lead to P surplus from fertilizers or manures which accumulates in

soils (Withers *et al.*, 2001). A significant portion of this stored P surplus does not remain available to plants (Delgado & Schalenghe, 2008).

Microorganisms and plants have developed strategies for P acquisition from soils. These strategies involve the exudation of compounds able to hydrolyse organic P –phosphatases– or to solubilize poorly soluble metal phosphates or adsorbed phosphate on the solid phase –low molecular weight organic acids (LMWOAs) (Dakora & Phillips, 2002; George *et al.*, 2011; García-López *et al.* 2021). Among the LMWOAs, the citric and oxalic acids are reported to be among the most common root and microbial exudates (Macías-Benítez *et al.*, 2020). These P mobilization strategies in the rhizosphere increase P availability to plants (Menezes-Blackburn *et al.*, 2016). Citric and oxalic acids may also destabilize soil organic matter (SOM), thus promoting the cycling of organically bound soil P. In addition, LMWOAs may act as a source of energy for soil microorganisms, which may also affect the P cycle and its availability to plants (García-López *et al.*, 2015).

Agricultural practices may enhance soil P bioavailability by releasing it from the soil solid phase (Zhu *et al.*, 2018). Organic amendments may increase microbial activity thus triggering microbial mechanisms for P acquisition. Beside this, organic compounds may interact with the P cycle increasing its bioavailability. Different sources of organic C have been studied, such as LMWOAs (Kpomblekou-A & Tabatabai, 2003; Wang *et al.*, 2016), humic substances (Delgado *et al.*, 2002; Chen *et al.*, 2004), lignin, crop residue, manure and biochar (Jenkinson, 1971; Zimmerman *et al.*, 2011). Effects on microbial communities and activities explain that cellulose, which does not interact with P geochemistry in soil (*e.g.* through inhibition of adsorption or precipitation), increases P uptake by plants (Moreno-Lora *et al.*, 2019). However, C sources applied to the soil may act differently depending on: their composition, environmental conditions such as climatic conditions, soil type, nutrient availability in soil, and plant and microbial attributes (Bastida *et al.*, 2013; Razanamalala *et al.*, 2018).

On this ground, it can be assumed that strategies for incorporating organic C to soil may improve P mobilization thus promoting an increased P uptake by plants from soils and fertilizers. This will contribute to a more efficient use of this valuable resource in agriculture. However, for accurate recommendations, it is necessary new insight on the factors that may affect the potential benefits of organic amendments on P availability to plants. Therefore, this study is focused on evaluating the effectiveness of organic amendments, which are expected to generate strong short-term changes in the turnover of SOM, on the P uptake by plants. Attention will be paid to P mobilization strategies associated to microorganisms or plants, such as hydrolytic enzyme activities and LMWOAs exudation. The study will be performed with and without P fertilization to assess the effects under different P supply conditions, and with two soils differing widely in properties to evaluate how these properties may affect potential benefits of organic amendments on P uptake by plants.

Material and methods

Soil sampling and characterization

Soils used in this study were collected from topsoil layer (0−10 cm depth) in SW Spain (Fig. S1 [suppl]. The first soil (37°24′07″N, 5°35′10″W) is classified as Vertisol (Chromic Haploxerert) and the other soil (37°43′34.00″ N, 5° 4′43.08″ W) is classified as Alfisol (Typic Haploxeralf) according to the Soil Taxonomy (Soil Survey Staff, 2014). These soils were chosen by their contrasting physicochemical properties and their similar P availability level as estimated by the Olsen P (Olsen *et al.*, 1954).

The soils were air-dried, passed through a < 2 mm sieve, homogenized and stored before the experiment. The initial chemical properties of the bulk soils are displayed in Table 1. SOM in our soil samples were determined by dichromate oxidation (Walkley & Black, 1934). Extraction with DTPA according to Lindsay & Norvell (1978) was performed, and Fe was determined in the extracts by atomic absorption spectrometry. Iron and P fractionation was performed following the scheme of Ruiz *et al.* (1997) with modifications, involving the sequential use of five extractants, namely: (i) 0.1 M NaOH + 1 M HCl (NaOH+HCl, $Fe_{NaOH+HCl}$ and $P_{NaOH+HCl}$), (ii) 0.27 M Na citrate + 0.11 M $NaHCO_3$ (CB, Fe_{CB}, and P_{CB}), (iii) 0.2 M Na citrate + 0.05 M ascorbate at pH 6 (CA, Fe_{CA}, and P_{CA}), (iv) 0.27 M Na citrate + 0.11 M $NaHCO_3$ + 2%Na dithionite (CBD, Fe_{CBD}, and P_{CBD}) and (v) 1 M HCl (HCl, Fe_{HCl} and P_{HCl}). These sequential extractions were performed at 25 ºC, using soil:extractant ratio of 1:40. The extraction time was 16 h except in the last extraction with HCl in step (v), which lasted 1 h. In this fractionation scheme, NaOH+HCl extractable corresponds to Fe-Al phosphates and adsorbed P; CB extractable Fe and P, mostly correspond to easily releasable Fe and P (adsorbed or precipitated as soluble compounds) and P reabsorbed from the previous step. Most Fe and P associated with poorly crystalline oxides is extracted by CA. Crystalline oxides not solubilized in these steps can be dissolved by using a stronger reductant (CBD). In the last step, P is assumed to be extracted from pedo- and lithogenic apatite fraction. All chemical extractions were carried out in triplicate, using polypropylene flasks. Iron in the extracts was determined by atomic absorption spectrometry, and P concentration in the extracts was determined colorimetrically according to Murphy & Riley (1962).

Table 1. Main soil properties

Soil properties [a]	Soil	
	Vertisol	Alfisol
Sand (g kg^{-1})	50	421
Silt (g kg^{-1})	250	241
Clay (g kg^{-1})	700	338
SOC (g kg^{-1})	6.2	5
ACCE (g kg^{-1})	11	21
pH	7.7	7.9
EC (dS m^{-1})	0.17	0.18
CEC	36	12
Fe DTPA (mg kg^{-1})	34	21
Olsen P (mg kg^{-1})	6.6	7.1
$P_{NaOH+NaCl}$ (mg kg^{-1})	2.0	12.9
P_{CB} (mg kg^{-1})	32	84
P_{CA} (mg kg^{-1})	149	39
P_{CBD} (mg kg^{-1})	87	55
P_{HCl} (mg kg^{-1})	224	14
P_{total} (mg kg^{-1})	494	204
$Fe_{NaOH+NaCl}$ (g kg^{-1})	0	0
Fe_{CB} (g kg^{-1})	0.60	0.14
Fe_{CA} (g kg^{-1})	1.13	2.02
Fe_{CBD} (g kg^{-1})	11.94	13.86
Fe_{HCl} (g kg^{-1})	3.64	1.01
Fe_{total} (g kg^{-1})	17.31	17.04

[a] SOC, soil organic carbon. ACCE, active calcium carbonate equivalent. EC, electrical conductivity. CEC, cation exchange capacity. DTPA, diethylenetriamine penta-acetic acid. CB, citrate bicarbonate. CA, citrate ascorbate. CBD, citrate bicarbonate dithionite.

Experimental design and growing condition

A pot experiment with cucumber (*Cucumis sativus* L. cv Tropico) was performed following a completely randomized design with four replications and three factors: (i) two different soils, Alfisol and Vertisol; (ii) C amendment, involving four treatments with glucose, cellulose, citric acid and control without C, and (iii) P supply, with an unfertilized control, and KH_2PO_4 as P source at the rate of 40 mg P kg^{-1} and (iii). The P rate used (40 mg P kg^{-1}) was intended to mimic the usual P rate for many crops in P-poor soils. Addition of glucose, cellulose and citric to soil was carried out at a rate of 80 mg C kg^{-1}, split in three applications (after transplanting, and one and two weeks after the first application,) to avoid loss due to drainage and rapid consumption of the C source by microorganisms. For this purpose, three different solutions were prepared and 6.66 mL of each of them (13.88 mM of glucose, 14 mM of citric acid at pH 6 and 14.13 mM cellulose) was carefully applied to 250 g of soil together with the irrigation solution. This C rate was in the range of those used by Menezes-Blackburn *et al.* (2016) (0–10 mmol kg^{-1} LMWOAs) in order to simulate the cases of high organic acids exuding plants (such as *Lupinus* spp.) and the possible cumulative effect of continuous LMWOAs root exudation. Since the amount of C applied was lower than the expected C biomass in soil (García-Orenes *et al.*, 2010), increased microbial activity without significant alteration of community structure could be expected (Blagodatskaya & Kuzyakov, 2008).

Cucumber seeds were germinated in a layer of perlite irrigated with deionized water, during 14–15 days. After that, plants were transplanted into 350 mL pots (15 cm height × 5.5 cm diameter polystyrene cylinders, one plant per pot) containing 0.25 kg of air-dried soil. Plants grown under controlled conditions chamber with a photoperiod of 14 h d^{-1} at a light intensity > ~ 300 µmol m^{-2} s^{-1}, a temperature of 25°C (day) and 23°C (night), with a photoperiod of 16 h. Plants were daily irrigated with a P free-Hoagland solution: a concentrated nutrient solution containing (all concentrations in mmol L^{-1}): $MgSO_4$ (2), $Ca(NO_3)_2$ (5), KNO_3 (5), KH_2PO_4 (1), KCl (0.05), H_3BO_3 (0.009), Fe-EDDHA(0.02), $MnCl_2$ (0.0023), $CuSO_4$ (0.0005), $ZnSO_4$ (0.002), and H_2MoO_4 (0.0005) was applied twice per week. A 10 fold dilution of this solution was applied the other days of the week. At the end of the experiment, a total of 150 mL of the concentrated nutrient solution and 250 mL of the diluted solution were applied per pot. The pH of the nutrient solution ranged between 5.5 and 6. This fertilization scheme allowed supplying enough nutrients without salinization risk in soils.

Plant and soil analysis

After 42 days, the aerial part of the plants was harvested, and roots separated from soil; plant material was dried at 65 ºC for 48 h (until constant weight was reached), weighted, and ground to pass through a 1-mm sieve prior to mineralisation. An aliquot of 0.25 g was then mineralised in porcelain crucibles in a furnace at 550 ºC for 8 h. After that, 10 mL of 1 M HCl was used to dissolve ashes, and the resulting solution heated at 100 ºC for 15 min. In this digest, P were determined by colorimetrically according to Murphy & Riley (1962). Certified plant material was also analysed to assess complete recovery of the nutrient by this procedure.

Before beginning the experiment, 10 seeds of cucumber were analysed for P content. The P content in the seeds was subtracted from the total content of P in to standardize the measurement of total P uptake by plants (expressed as mg of P per plant) (Marcante *et al.*, 2016).

The activity of soil β-glucosidase was determined at the end of experiment as the amount of PNP (p-nitrophenol) formed from PGN (4-nitrophenyl-β-D-glucopyranoside),

according to Eizavi & Tabatabai (1988). This enzyme activity is highly sensitive to changes in soil properties, including organic matter supply (de Santiago *et al.*, 2009) and is considered a basic indicator related to soil microbial activity involved in the C cycle (Stott *et al.*, 2010). Alkaline phosphatase activity was measured as the enzyme involved in the P cycle, according to Tabatabai & Bremer (1969). LMWOAs were extracted from rhizospheric soil by shaking an amount of 5 g of sample with 5 mL of NaOH (0.1 mol L^{-1}) at 4 Hz during 1.5 h (Baziramakenga *et al.*, 1995; Radersma & Grierson, 2004). After shaking, suspensions were centrifuged at 10,000 g for 10 min and the supernatants were filtered through Whatman 42 filter paper. The filtrate from each NaOH extract was acidified to pH 2–3 with H_2SO_4 (1 mol L^{-1}) and the supernatant passed through a filter of 0.45 µm pore size. Organic anions were separated by high performance liquid chromatograph (HPLC) Varian ProStar 410 HPLC instrument furnished with a C18 column (Varian, 250 mm × 34.6 mm, 8 µm particle size), using isocratic elution with 98 % of H_2SO_4 (5 mmol L^{-1}) at pH 2 plus 2 % methanol at 0.8 mL min^{-1} as carrier solution and a 20 µl injected volume. Organic anions were detected at 215 nm, using a Varian 486 photo-diode array detector. Individual standard solutions of acetic, oxalic, citric, malic, fumaric and succinic acid, all from Sigma (Barcelona, Spain), were also used. Soil pH after harvest was also measured.

Statistical analyses

A General Linear Model procedure (GLM) in Statgraphics Centurion XVI (StatPoint Technologies, 2013) was performed with three-way analysis of variance to assess the effect of soil, P fertilizer and C source. Normality and homoscedasticity were checked according to the Shapiro-wilk test and Levene test, respectively. Potential transformations were performed if required to fully meet these criteria. When the effect of a factor was significant, means for each factor level were compared via Tukey's test ($p < 0.05$), except when the interaction between factors was significant. In the case of significant interactions, the effect of main factors cannot be assessed and only the interaction can be discussed since the effect of one factor depends on the level of the other (Acutis *et al.*, 2012). Linear regression was performed, using the same software mentioned above.

Results

Soil properties

The Alfisol showed a higher content in total Fe oxides than the Vertisol (Table 1). Crystalline Fe oxides were dominant in both soils, but the content was higher in the Alfisol (13.86 g kg^{-1}). No significant differences were observed in the available P status assessed with the Olsen P, with an average value of 6.85 mg kg^{-1} (Table 1). The P recovered in the fractions corresponding to the most readily desorbable P (NaOH+CB; Table 1) was greater in the Alfisol (96.9 mg kg^{-1}) than in the Vertisol (34 mg kg^{-1}). Carbonate content was higher in the Alfisol (21 g kg^{-1}) than in the Vertisol (11 g kg^{-1}) (Table 1). Both soils differed widely in their clay content, Vertisol is a clayish soil with a clay content of 700 g kg^{-1}, meanwhile this content in the Alfisol was 338 g kg^{-1}

Dry matter and total P uptake by plant

The type of soil significantly affected root dry matter (DM). Moreover, the phosphatase activity measured after harvest, the total amount and P concentration in shoots, and the plant P uptake also differed between both soils (Table 2). Total plant DM was higher in the Alfisol

Table 2. Analysis of variance (*p* values) of the different variables studied in cucumber plant tissues (*Cucumis sativus* L.) and in the growth substrates

Source of variation	Plants								Growing media		
	Dry matter			P concentration		Total P			β-glucosidase	Alkaline phosphatase	LWMOA[a]
	Shoots	Roots	Total	Shoots	Roots	Shoots	Roots	Uptake			
Soil (A)	**0.0000**	**0.0318**	**0.0000**	**0.0021**	0.4375	**0.0373**	0.4375	**0.0391**	**0.0000**	0.0000	**0.0000**
P source (B)	**0.0000**	**0.0028**	**0.0000**	**0.0000**	0.3372	**0.0000**	0.3372	**0.0000**	0.3352	0.0441	0.6807
C amendment (C)	**0.0000**	**0.0221**	**0.0000**	0.2887	0.5045	**0.0471**	0.5045	**0.0224**	0.1198	0.6232	0.1122
A × B	0.3141	0.2678	0.5586	0.8277	0.5634	0.1458	0.5634	0.2086	0.0552	0.2274	0.7987
A × C	**0.0006**	0.1818	**0.0012**	0.3102	0.7979	0.2404	0.7979	0.2853	**0.0007**	0.7680	**0.0009**
B × C	0.1628	0.7997	0.1913	0.2881	0.8287	0.2955	0.8287	0.3152	0.3214	0.0907	0.4378
A × B × C	0.1020	0.2718	0.0976	0.5461	0.5053	0.7678	0.5053	0.7011	0.0244	0.6623	0.7656

[a] LWMOA, low molecular weight organic acid. *p* values < 0.05 indicate a significant effect of the source. Significant effects in bold

(1.9 g plant^{-1}) than in Vertisol (1.5 g plant^{-1}), these differences being mainly associated to the shoot DM (Table 3). Phosphorus uptake was also higher in the Alfisol than in the Vertisol (Table 3). The effect of C amendment on shoot DM and on total plant DM differed across soils, as revealed by the significant interaction between the two factors (Table 2). In the Alfisol, amendment with glucose led to the highest plant DM values, while differences in the Vertisol were not significant (1.15 g plant^{-1}) (Fig. 1). Overall, citric acid led to the lowest plant DM. Glucose and cellulose promoted the highest plant P uptake while the worst results corresponded to the application of citric acid (Table 3).

Soil enzymes and LMWOAs

LMWOAs in soils after harvest were affected by the interaction between C amendment and soil (Table 2). The accumulation of LMWOAs in the Alfisol was highest with cellulose. Conversely, in the Vertisol the effects of this amendment were worse than those observed with other treatments (Fig. 2). Citric acid and glucose increased LMWOAs concentration in the Vertisol.

Significant interactions between the three factors were only observed for the β-glucosidase activity (Table 2). This activity increased as result of cellulose and citric acid application in the Vertisol soil without P fertilization. On the other hand, in the Alfisol, citric acid without P fertilization led to the lowest β-glucosidase activity (Fig. 3). In the Alfisol without P supply, organic amendments decreased β-glucosidase activity relative to the control without amendment.

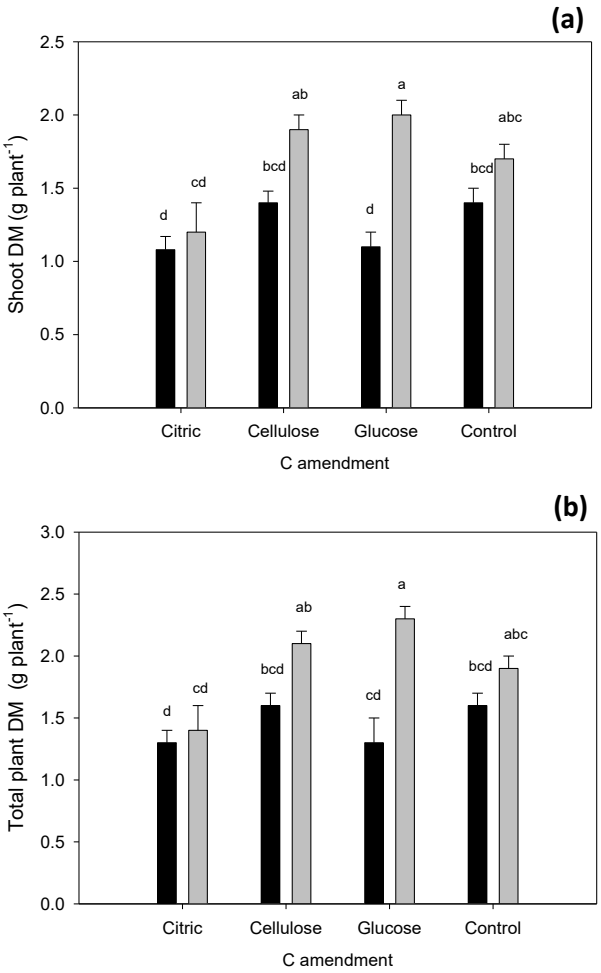

Figure 1. Total plant biomass (a) and shoot biomass (b) of cucumber plants grown in Vertisol (black) or Alfisol (grey) amended with different organic compounds. DM, dry matter. Error bars indicate standard error (n=8).

Table 2. Average values of some plant variables (*Cucumis sativus* L.) and phosphatase activity in the plant growth substrate

Source of variation[a]	Dry matter roots (g plant^{-1})	Alkaline phosphatase[b] (mg PNP kg^{-1} h^{-1})	P concentration		Total P		
			Shoots	Roots	Shoots	Roots	Uptake[c]
			(g kg^{-1})		(mg plant^{-1})		
Soil							
Vertisol	0.20±0.01 b	164±3 a	1.88±0.11 a	1.64±0.13	2.36±0.18 b	0.32±0.03	2.57±0.20 b
Alfisol	0.24±0.02 a	102±4 b	1.56±0.09b	1.49±0.09	2.72±0.23 a	0.35±0.03	2.96±0.24 a
P source							
+	0.25±0.01 a	123±7 b	2.13±0.08 a	1.64±0.13	3.44±0.15 a	0.40±0.03 a	3.73±0.16 a
-	0.19±0.02 b	140±7 a	1.31±0.08 b	1.49±0.10	1.65±0.12 b	0.27±0.02 b	1.81±0.13 b
C source							
Cellulose	0.25±0.02 a	133±9	1.69±0.11	1.55±0.19	2.75±0.26	0.38±0.05	3.02±0.28 a
Citric acid	0.17±0.02 b	128±11	1.78±0.18	1.57±0.15	2.19±0.35	0.26±0.04	2.34±0.38 b
Glucose	0.24±0.02 ab	129±10	1.81±0.15	1.74±0.12	2.82±0.32	0.40±0.04	3.10±0.35 a
Control	0.22±0.02 ab	136±9	1.59±0.16	1.38±0.18	2.40±0.23	0.31±0.04	2.59±0.25 ab

[a] Mean±standar error, n = 32 for soil, n = 32 for P source and n =16 for C source. [b] PNP, p-nitro phenol. [c] Estimated as the total P in aerial parts and roots minus P present in seeds. Averages were only calculated for variables that did not show significant interaction between the factors studied (soil type, P fertilization, and organic amendment). Means followed by different letters within a column are significantly different according to the Tukey test ($p < 0.05$) for each factor.

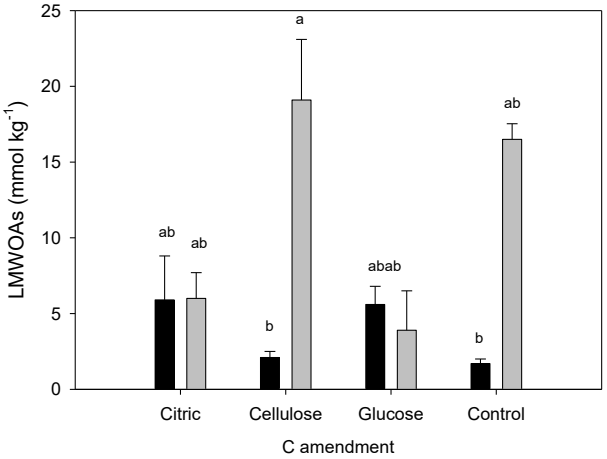

Figure 2. LWMOAs accumulated in Vertisol (black) or Alfisol (grey) rizhosphere amended with different organic compounds. Error bars indicate standard error (n=8).

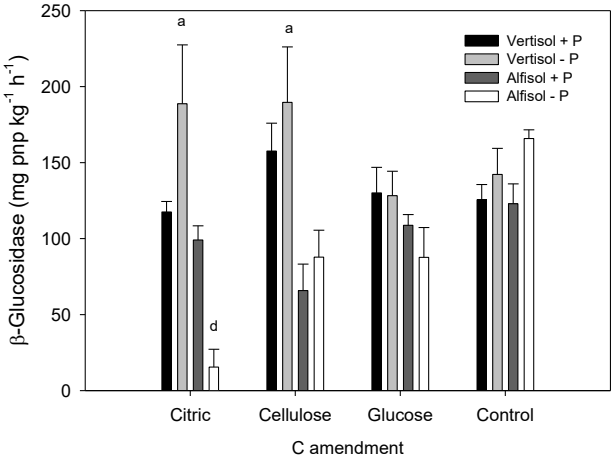

Figure 3. β-glucosidase activity in Vertisol and Alfisol amended with different organic compounds and P sources. Error bars indicate standard error (n=4).

Phosphatase activity showed the highest values in the Vertisol with an average value of 164 mg PNP kg^{-1} h^{-1}. On average, P fertilization decreased phosphatase activity in soil by 12% relative to the non-fertilized treatment. However, no significant differences were shown for C treatments (Table 3).

Relationship between studied variables

In both soils, P uptake increased linearly with total plant DM (R^2 = 0.49; p<0.0001). Phosphatase activity increased linearly with β-glucosidase activity in soil (Fig. 4). In the Alfisol, P uptake decreased linearly with phosphatase activity (R^2= 0.21; p<0.01) (Fig. 5). In glucose-amended soils total plant DM was negatively correlated with phosphatase activity (R^2 = 0.52; p<0.005). Phosphatase and β-glucosidase activity showed positive relationships with oxalic acid in soil measured after harvest (exponentially, R^2 = 0.45, p<0.01; and logarithmically R^2 = 0.52, p <0.005; respectively).

Discussion

Both soils differed widely in their physicochemical properties, particularly in clay content and Fe oxides, which are relevant properties affecting P dynamics in soil and P uptake by crops (Recena *et al.*, 2016). The P mobilizing capacity of microorganisms in soil depends on microbial community composition and activity, P availability, and soil physic-chemical properties (Moreno-Lora *et al.*, 2019). All these factors may explain the differences in P uptake observed between plants growing in the two different soils (Recena *et al.*, 2015; García-López & Delgado, 2016).

Cellulose or glucose addition increased DM of plants growing in Alfisol and plant P uptake in both soils. Similar results were obtained by Moreno-Lora *et al.* (2019) with cellulose amendment. As reported by these authors, increased P uptake and DM with organic amendments were observed in the soil with the lower soil organic C (SOC) content.

Since cellulose and glucose are not expected to affect P dynamics in soils, potential benefits may be associated to changes in microbial activity or community composition. This suggests that P mobilizing capacity by rhizospheric microorganisms can be modified after application of a labile C compounds to soil. Several studies found that labile C substrates provided sufficient C and energy for microbial growth and activity compared to more recalcitrant C substrates. Furthermore, the addition of labile C subsequently facilitated production of enzymes able to degrade SOC (Nottingham *et al.*, 2009; Aye *et al.*, 2018), which likely cause organic P mineralization. This would explain the well-known effect of organic amendments on improving P uptake by plants grown in low organic C soils (Diacono & Montemurro, 2010). Nevertheless, organic amendments did not promote an increased β-glucosidase activity in the Alfisol soil (Fig. 3). Consequently, the positive effect on P uptake by plants does not seem to be related to an increased microbial activity. Although, an increased phosphatase activity to be related with β-glucosidase in this study (Fig. 4). However, LMWOAs increased with cellulose in the Alfisol (Fig. 2). This may contribute to an enhanced P uptake by releasing adsorbed or precipitated inorganic P and by increasing the hydrolysis of adsorbed organic P (García-López *et al.*, 2021). Phosphatase activity in soil solution may have contributed to increase P availability throughout the hydrolysis of organic P compounds desorbed by the action of LMWOAs (García-López *et al.*, 2021). The desorption of P forms might have been more pronounced in the soil with the

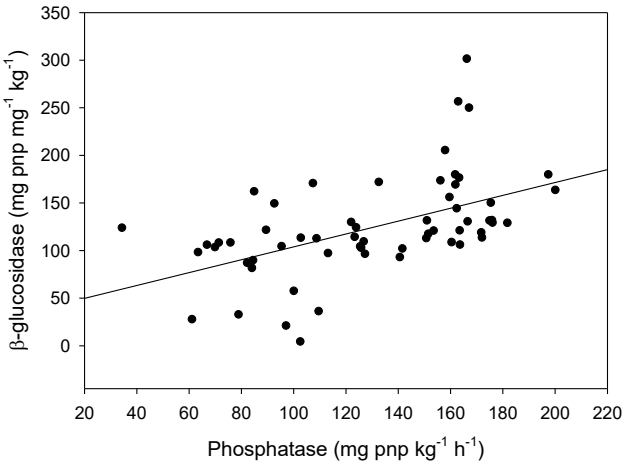

Figure 4. Relationship between β-glucosidase and phosphatase activity in rizhosphere Y=25.70 + 0.75X; R^2=0.30; $p < 0.0000$; n=64.

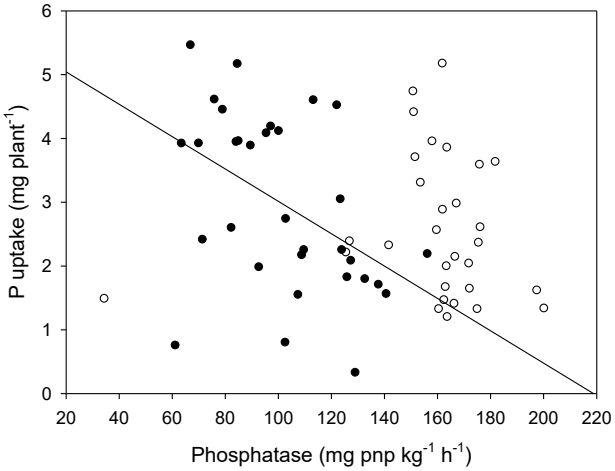

Figure 5. Relationship between P uptake by plants and phosphatase activity in the rizhosphere for both soils. Alfisol soil (full symbol) Y = 5.55 − 0.25X; R^2=0.21; $p < 0.0082$; n=32. In the Vertisol (empty symbols) the relationship was not significant.

highest Fe oxide content, *i.e.* the Alfisol. The positive correlation between the accumulation of LMWOAs and the β-glucosidase activity, suggest that the stimulation of microbial activity may lead to an increased desorption of P compounds. Thus, the benefits of organic amendments may be related, at least in part, to microbial mechanisms of P mobilization in the soil. The increased DM of plants grown in the Alfisol receiving organic amendments may be associated to an improved P nutrition. In the Vertisol, β-glucosidase activity increased with cellulose and citric acid application (Fig. 3), which suggests a stimulation of soil microorganisms. In this soil, the positive effect of the amendments on P uptake could be related to an increased microbial activity. However, in this soil the amendments did not increase phosphatase activity. As in the Alfisol, the accumulation of LMWOAs increased with glucose, which can contribute to explain the effect of this C source on P uptake by plants.

Beneficial plant-microbial interactions (such as phytohormone production by rhizospheric microorganisms) may explain the enhanced root development (Table 3), which was independent of soil type. A better root development likely contributed to an enhanced P uptake (García-López & Delgado, 2016). Differences between soils may be explained by the greater SOC and clay content of the Vertisol when compared with the Alfisol. Cellulose and citric acid are C sources readily available to soils microorganisms when compared with the endogenous SOM, thus enhancing microbial growth and activity. However, the high Fe oxide content of the Alfisol may decrease the activity of extracellular enzymes due to enzyme-mineral interactions. When adsorbed to mineral surfaces, soil extracellular enzymes likely lose their mobility and catalytic activity for SOM degradation (Servagent-Noinville *et al.*, 2000; Quiquampoix & Burns, 2007). This may contribute to explain the lack of a positive effect of these organic amendments on the indicators of microbial activity in the Alfisol. In addition, this increase in β-glucosidase activity in the Vertisol was more pronounced without P (Fig. 3) which is probably due to the important role of P availability on microbial activity and community structure. The lack of a positive effect of organic amendments on total DM yield in the Vertisol may be associated to a decreased P availability in the soil and P uptake when compared with the Alfisol. Thus, despite the enhanced P uptake, this does not overcome the P deficiency and is not reflected in an improved plant shoot development.

The decreased P uptake in the Alfisol with citric acid may be explained by the P immobilization in rhizospheric microorganisms or to a decreased LMWOAs content (Fig. 2). On the other hand, an increased LMWOAs concentration in soil is not necessarily due to an increased microbial activity as observed in the Alfisol, since plant roots also exudate LMWOAs as nutrient mobilizing strategy.

Dry matter yield decreased with phosphatase activity in the Alfisol. Other authors also showed negative relationship between acid phosphatase activity and efficiency of P uptake under phosphate deficit (Mc Lachlan, 1980; Yan *et al.*, 2001). Phosphatase release by plants and microorganisms may trigger under conditions of extreme P deficiency. On the other hand, an increased P availability induced by LMWOAs, may have had a negative effect on phosphatase activity.

In conclusion, cellulose or glucose additions were effective in increasing P uptake by plants in both soils, which seems related to changes in P mobilization mediated by microbial processes. The study emphasises the importance of soil properties to assess the effect of organic C amendments on P uptake by plants. These results are relevant for improving plant P nutrition and for achieving a more sustainable management of P fertilization.

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