Soil Carbon and Phosphorus Pools in Field Crop Rotations in Pampean Soils of Argentina

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In temperate cropping systems, a better understanding of soil C and P transformations is pertinent to evaluate crop management consequences in the medium term. Six-year cropping systems experiments consisting of corn (*Zea mays* L.)–double-cropped wheat (*Triticum aestivum* L.)/soybean [*Glycine max* (L.) Merr.] (two sites) and corn–soybean–double-cropped wheat/soybean (two sites), with (Fp) or without P added (Wp), were evaluated in the Pampean soils of Argentina. The objectives were (i) to quantify the effects of continuous P fertilization on the size and vertical distribution of C and P in total organic C (TOC) and particulate organic matter (POM) fractions and (ii) to evaluate the POM-P fraction as a potential indicator of crop P availability. Annually, an average P rate of 34 kg P ha^{-1} was applied in Fp plots. The TOC, total P (TP), POM-C, and POM-P were measured in the 0- to 5-, 5- to 10-, and 10- to 20-cm soil depths at the end of the 6-yr period. Phosphorus fertilization increased TOC from 0.5 to 2.5 g C kg^{-1} and TP from 24.1 to 77.4 mg P kg^{-1} in the 0- to 20-cm depth. The POM-C and POM-P fractions were more sensitive than TOC or TP to P fertilization, increasing by 14 and 47%, respectively, in the 0- to 20-cm depth under continuous P addition. The greatest differences in POM-C and POM-P among treatments occurred in the surface soil layer. A significant relationship was found between POM-P and corn P uptake at anthesis.

Abbreviations: Fp, phosphorus fertilizer treatment; POM, particulate organic matter; SOM, soil organic matter; TOC, total organic carbon; TP, total phosphorus; Wp, treatment without phosphorus fertilizer.

Soil organic matter (SOM) constitutes a significant part of the global terrestrial C (Haile-Mariam et al., 2008), N, and P pools. The determination of SOM alone is not a proper soil quality and soil sustainability index; nevertheless, it is an essential factor to support agricultural production in cropland areas all across the world (Bezdicek et al., 1996). Several reports have found that some SOM fractions are better indicators of soil quality than SOM taken as a whole (Cambardella and Elliott, 1992; Six et al., 2000, 2002; Cambardella et al., 2001; Fabrizzi et al., 2003). For that reason, several studies have separated the SOM by chemical dispersion or physical separation of the soil, or a combination of both. The resulting fractions include labile fractions associated with the sand-size particles of the soil and partially protected fractions within aggregates associated with the silt- and clay-size particles (Stevenson and Elliott, 1989; Janzen et al., 1992; Beare et al., 1994; Six et al., 1998). The organic C of the labile fractions is supposed to have more rapid mineralization rates than the nonlabile fractions (Tiessen and Stewart, 1983; Dalal and Mayer, 1986; Gregorich et al., 1995).

Particulate organic matter is one of the labile intermediate compounds in the SOM continuum and is supposed to be more sensitive to changes in management practices than total SOM (Cambardella and Elliott, 1992; Gregorich and Carter, 1997; Cambardella et al., 2001).

Particulate organic matter can account for much more than 10% of the soil C (Carter et al., 1994; Gregorich et al., 1995). In addition, it is correlated with microbial growth rates and to biologically active C. The enrichment of nutrients in POM fractions suggests that this is a fraction where biological processes are

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concentrated (Janzen et al., 1992; Balesdent and Balabane, 1992; Schwenke et al., 2002). Nitrogen cycling from POM and its contribution to the N nutrition of agricultural crops have been well documented (Gregorich et al., 1995; Wander et al., 1998; Phiri et al., 2001; Wander, 2004). Both C and N associated with the POM fraction (POM-C and POM-N, respectively) were fair estimators of the potential N mineralized under different cropping systems (Willson et al., 2001). Moreover, POM-C was related to the uptake of N by crops and to the retention of N fertilizer by the soil (Nissen and Wander, 2003). For P nutrition, labile (Halm et al., 1972; Hedley et al., 1982) and soluble organic P (Dalal, 1979; Sekhon and Black, 1969), among others, were proposed as potential soil fertility indicators. In accordance, in a greenhouse experiment, Adepetu and Corey (1976) found that soil P derived from the mineralization process was highly related to the P taken up by corn. Little information is available on the relative contribution of the POM pool to P availability, although published reports have proposed that POM is a key component of the P cycle (Maroko et al., 1999; Vanlauwe et al., 2000, Ha et al., 2008). Several researchers have studied the role of P in POM but without relating this fraction to plant nutrition (Salas et al., 2003, Ha et al., 2007, 2008; Hao et al., 2008).

Reduced-tillage and fertilization practices can result in improved soil physical conditions and retention of SOM (Paustian et al., 1997; Needelman et al., 1999; Six et al., 2002), but these effects are not usually found in the short term. For example, Eiza et al. (2005) observed an increase in SOM after 9 yr of continuous N application, but this effect was not found at the beginning of the experimental period (Studdert and Echeverría, 2002). There are a few reports, however, that have shown an effect of continuous P fertilization on changes in the organic nutrient fractions in the soil (Motavalli and Miles, 2002; Blake et al., 2003).

In terms of soil depth influence, a greater increase in POM as a result of management practices was reported by several studies in the surface soil layer (0–5 cm) compared with the adjacent deeper soil layer (5–20 cm). Similar results were reported for the N content in the POM fraction, which tends to accumulate and concentrate in the surface soil layer due to reduced-tillage and fertilization practices (Wander et al., 1998; Mrabet, 2002). Furthermore, reduced-tillage systems are characterized by greater aggregates stability in the topsoil layer (0–5 cm), which protects the soil from degradation, resulting in higher crop productivity and, consequently, greater residue input (Mrabet, 2002). No information is known about the P content in the POM (size) fraction and its vertical distribution in the soil profile; however, a close relationship is expected to be observed in the POM-P fraction associated with the POM-C pool. Knowing more about the soil C and P distribution at the topsoil layer (0–20 cm) and the relationship between the different soil depths might increase our ability to manage POM dynamics.

From a cropping systems viewpoint, differences in SOM levels are associated with the quantity and quality of residues returned to the soil. It is known that SOM has a great impact on soil physical conditions, soil erosion, water retention, and nutrient cycling (Haile-Mariam et al., 2008). Management practices that promote sustainable crop production such as reduced tillage, increased residue input, and the application of organic and inorganic fertilizers (among others) could not only lessen the loss of C, but even more, they might ameliorate soil C and N levels (Paustian et al., 1997; Flach et al., 1997). Conversely, there is considerably less information related to the quantity and distribution of other nutrients in the SOM pools, especially in those intermediate labile fractions such as POM. This pool could act as an organic source of nutrients for plant nutrition in high-yielding cropping systems.

Current methods to predict soil organic P availability to plants have failed to provide accurate predictions useful for soil fertility diagnosis; for example, organic fractions measured through the method of Hedley et al. (1982) produced uncertainty about the availability of soil P (Campbell et al., 1996; Zhang and MacKenzie, 1997; Motavalli and Miles, 2002; Blake et al., 2003). Moreover, conventional soil P tests based on the solubility of different P compounds might not reflect the potential contribution of organic P to the soil available P (Maroko et al., 1999; Phiri et al., 2001; Motavalli and Miles, 2002). For example, the traditional Bray method (Bray and Kurtz, 1945) preferentially extracts the P forms associated with the labile inorganic pools. In several soils of the Pampean Region (Argentina), the accuracy of the Bray index is variable, even in soils in which the soil test analysis resulted in low (insufficient) values. One hypothesis to explain this fact could be that the Bray method does not quantify the labile organic soil P (Suñer et al., 2002; Ciampitti, 2009). In cases like this, a soil test that includes labile organic P appears to be a promising tool to improve soil P availability tests (Sharpley et al., 1987; Möller et al., 2000). In that sense, the P contained in the POM fraction could be a parameter of the organic P availability (Ha et al., 2007, 2008; Hao et al., 2008).

Knowledge of changes in C and P associated with the POM fraction could provide valuable information about the pattern of release and immobilization of C and P from the SOM pool. The objectives of this study were: (i) to quantify the effects of continuous P fertilization on the size and vertical distribution of C and P in SOM and POM fractions; and (ii) to evaluate the POM-P fraction as a potential indicator of P availability for crops.

MATERIALS AND METHODS

The Pampas region is located in east-central Argentina. The climate of the region is temperate and the regional mean temperatures of the coldest and warmest months are 15 and 25°C, respectively. The rainfall regime is humid in the eastern part of the region and semiarid in the western part. Most common soils are Typic Argiudolls and Typic Hapludolls. Before the introduction of agriculture, the Pampas region included humid temperate prairies in the northeast and dry grassland steppes with a moderate continental climate in the west and southwest.

Field experiments were established on private farms that are associated with the Regional Consortium of Agricultural Experimentation

(CREA) experimental network. The Southern Santa Fe region of CREA is comprised of 12 groups of 10 to 15 farmers, located in the southern Santa Fe, southeastern Cordoba, and northern Buenos Aires provinces in the northern Pampas, Argentina (32°11′50.68″–34°11′55.04″ S and 63°17′43.70″–61°20′0.00″ W). The experimental network started in 2000 and comprises 11 private farms distributed across the central part of the Pampas. Each experimental location followed one of the following two crop rotations: three crops in 2 yr as corn–double-cropped wheat/soybean (C-W/S, five sites) or four crops in 3 yr as corn–full-season soybean–double-cropped wheat/soybean (C-S-W/S, six sites). Each crop phase was not present each year, nor were the two crop rotations replicated in each experimental site (Ciampitti et al., 2011). In addition, only the fertilizer treatments were replicated within each rotation. At each site, all crops were no-till seeded. For the present study, four farms from the network with contrasting soil types and soil management histories were selected (Table 1). Soil and plant measurements were performed in 2006 at the silking stage of the corn growing season (Ritchie et al., 1996).

General soil test analyses were performed when the experimental network was initiated (in 2000) (Table 1). Soil samples (20-cm depth) were collected before applying treatments, dried at 40°C, and sieved. For available P analyses, samples were sieved to 0.5 mm and the classic Bray and Kurtz (1945) analysis was performed. Soil pH was measured in a 1:2.5 soil/distilled water suspension using a precalibrated glass electrode (Thomas, 1996). Soil bulk density was determined according to the method of Grossman and Reinsch (2002). Micronutrients (diethylenetriaminepentaacetic acid; Gaines and Mitchell, 1979), B (hot H_2O extraction; Gupta, 1967), and cation (pH 7 NH_4OAc method; Chapman, 1965) determinations were performed. Gravimetric soil moisture was determined as the difference between the fresh weight and dry weight after drying at 105°C to constant weight. Soil texture was determined by the pipette method (Gee and Bauder, 1986) for each of the experimental sites. Soil properties, previous crops, and the continuous years under agriculture are shown in Table 1.

Table 1. Soil classification, previous history, and soil properties (0- to 20-cm soil depth) for the four experimental sites selected at the beginning of the experimental period (September 2000).

Soil property	Corn–double-cropped wheat/soybean rotation		Corn–soybean–double-cropped wheat/soybean rotation	
	La Marta	**San Alfredo**	**La Blanca**	**La Hansa**
Classification	Entic Haplustoll	Typic Argiudoll	Typic Hapludoll	Aquic Argiudoll
Continuous cropping, yr	40	8	6	+20
Previous crops	wheat/soybean	wheat/soybean	wheat/soybean	soybean
Bray-1 P, mg kg^{-1}	11.2	18.3	16.2	45.5
Total organic C, g kg^{-1}	12.1	19.8	13.3	12.2
Bulk density, g cm^{-3}	1.40	1.41	1.31	1.25
pH	6.3	6.0	6.6	5.5
Ca, mg kg^{-1}	1380	2200	1440	1520
Mg, mg kg^{-1}	252	252	240	192
K, mg kg^{-1}	936	663	741	663
Na, mg kg^{-1}	115	92	92	92
B, mg kg^{-1}	1.1	0.8	1.1	0.9
Cu, mg kg^{-1}	0.9	1.8	1.3	1.4
Fe, mg kg^{-1}	54	105	71	86
Mn, mg kg^{-1}	28	103	36	54
Zn, mg kg^{-1}	1.1	1.7	1.7	0.8
Clay, %	8.0	18.0	15.5	18.0
Silt, %	35.4	62.0	56.4	78.9
Sand, %	56.6	20.0	28.1	3.1

Treatments

At each site, the experimental design was a randomized complete block with three replications. The plots were 25 to 30 m in width and 65 to 70 m long. In the present work, the selected treatments were: (i) control without P application (Wp) and (ii) continuous P fertilization (Fp). Both treatments received additional N and S fertilization at the same fertilization rate. Fertilizer rates changed every year as a function of the crop nutrient requirement, potential yield (annual differences in temperature and precipitation), and soil nutrient supply. Macronutrients other than N, P, or S do not usually limit yields in these soils. The P rate was decided annually, according to the expected crop yield (which changed with the environment, mainly due to differences in precipitation), through the estimation of P removal. For each year and experimental site, a constant P concentration (calculated to 0% moisture content) was assumed for the different crops: 4.0, 5.0, and 7.0 g P uptake kg^{-1} of crop produced for corn, wheat, and soybean, respectively (Ciampitti and García, 2007). The final P rate for each crop was 10% higher than the estimated P removal. The goal of the fertilization rate was to obtain a slightly positive P budget to gradually build up soil P levels. For all crops, P was banded at planting time with a seed/fertilizer minimum tillage planter 5 cm below and to the side of the seed. The total amount of applied P was 204 kg ha^{-1} during the 6 yr of the experimental period at all sites except La Hansa, which received 184 kg P ha^{-1}. The P source was monoammonium phosphate (12–52–0, N–P_2O_5–K_2O). Further details about this fertilization network were provided by García et al. (2007).

For TOC, TP, POM-C, and POM-P analysis, soil samples were collected at the 0- to 5-, 5- to 10-, and 10- to 20-cm soil depths when corn silks were emerging. At the same time, corn aerial biomass was collected to estimate the plant P accumulation. For this purpose, six consecutive representative plants from each treatment were cut at the stem base, individually chopped to a fine consistency, and dried to a constant weight at 60°C to determine the aboveground vegetative biomass. The P content of the plant was determined colorimetrically in a HNO_3 and $HClO_4$ digestion (Johnson and Ulrich, 1959, p. 26–78). The P accumulated in the corn plants was calculated multiplying the dry matter content and P concentration of each treatment, and it was expressed as kilograms P per hectare.

The POM fraction was calculated using the Elliott and Cambardella (1991) procedure, but changing the solution of $(NaPO_3)_6$ to NaCl, as suggested by Salas et al. (2003), to facilitate dispersion of the soil aggregates. Briefly, 10 g of moist soil was placed into 50-mL plastic containers and mixed with 30 mL of a 0.05 mol L^{-1} NaCl solution. The soil suspension was shaken for 15 h in a reciprocal shaker at 150 rpm, after which it was passed through the 53-μm sieve and rinsed with distilled water until a clear solution was obtained. The material retained on the sieve, a mixture of POM and sand, was dried at 50°C for 24 h. This material was homogenized, weighed, and ground by hand using a porcelain mortar and pestle. The soil slurry that passed through the 53-μm sieve contained the mineral-associated organic matter (MAOM). The values obtained were corrected for the mineralogical composition of the soil texture (silt + clay). The POM-C and POM-P were determined by difference between TOC and TP contents, and C and P associated with the MAOM fraction (MAOM-C and MAOM-P, respectively) for each nutrient, in the measured fractions:

$$\text{POM-C} = \text{TOC} - \left[\frac{\text{MAOM-C}(\text{clay\%} + \text{silt\%})}{100}\right]$$

$$\text{POM-P} = \text{TP} - \left[\frac{\text{MAOM-P}(\text{clay\%} + \text{silt\%})}{100}\right]$$

All laboratory analyses were done in triplicate. Inorganic phosphate in the slurry was determined colorimetrically using the molybdate–ascorbic acid procedure (Murphy and Riley, 1962). Total organic C was measured by dry combustion (Nelson and Sommers, 1982). A POM subsample of 250 mg was also used for HNO_3–$HClO_4$ digestion and TP colorimetric analysis.

The soil parameters were determined for the 0- to 5-, 5- to 10-, and 10- to 20-cm soil depths. The values for the 0- to 20-cm soil depth were determined as

$$\begin{aligned}\text{Value}(0\text{–}20\text{ cm}) = {} & [\text{value}(0\text{–}5\text{ cm})0.25] \\ & + [\text{value}(5\text{–}10\text{ cm})0.25] \\ & + [\text{value}(10\text{–}20\text{ cm})0.50]\end{aligned}$$

The proportion of the C associated with POM relative to the TOC was determined as the ratio between POM-C and TOC:

$$\frac{\text{POM-C}}{\text{TOC}} = \left[\frac{\text{POM-C}(\text{g C kg}^{-1})}{\text{TOC}(\text{g C kg}^{-1})}\right]100$$

The proportion of the P associated with POM relative to the TP was determined as the ratio between POM-P and TP:

$$\frac{\text{POM-P}}{\text{TP}} = \left[\frac{\text{POM-P}(\text{mg P kg}^{-1})}{\text{TP}(\text{mg P kg}^{-1})}\right]100$$

The proportion of C associated with POM relative to the P associated with POM was determined as the ratio between POM-C and POM-P:

$$\frac{\text{POM-C}}{\text{POM-P}} = \frac{\text{POM-C}(\text{g C kg}^{-1})}{\left[\text{POM-P}(\text{mg P kg}^{-1})\right]/1000}$$

For mass C and P calculations, element concentrations (g kg^{-1}) were obtained directly from chemical analyses. Carbon and P masses in genetic horizons (g m^{-2}) were calculated from the thicknesses (0–20-cm soil depth) and bulk densities of the horizons (Table 1):

$$M_C = C\rho_f T(10{,}000\text{ cm}^2\text{ m}^{-2})(0.001\text{ kg g}^{-1})$$

where M_C is the element mass per unit area (g m^{-2}), C is the element concentration (g kg^{-1}), ρ_f is the field bulk density (g cm^{-3}), and T is the thickness of the soil layer (20 cm).

Statistical Analysis

The validity of the assumptions of variance homogeneity and normal distribution of the TOC, TP, POM-C, and POM-P was analyzed. The data were analyzed using PROC MIXED (SAS Institute, 1996). The variables, experimental sites, and fertilization treatments were considered as fixed effects of the model, while blocks and all interactions were considered as random effects. If any variance component resulted in zero, this was removed to achieve a more harmonious model. Medians and all the significant interactions were obtained using the LSMEAN/PDIFF procedure. The best fixed parameters of covariance structure for each model were the Akaike Information Criterion and the Bayesian Information Criterion. In this analysis, experimental sites were randomly chosen (randomized factors) and our interest was to make inferences about rotations in each region.

At corn silking time, a relationship between the plant P concentration and the soil POM-P fraction was established. Model fitting was implemented with GraphPad Prism 5.01 software (Motulski and Christopoulos, 2003) using this (componentized) GraphPad equation:

$$Y_1 = I_1 + B_1 X + B_2 X^2$$

Model fitting was robust, with an R^2 value of 0.91 and a P value of 0.001.

RESULTS

Accumulated P removal by crops for the Wp treatment averaged 147.7 kg P ha^{-1}, with a minimum of 122.2 kg P ha^{-1} (La Hansa) and a maximum of 168.4 kg P ha^{-1} (San Alfredo). For the Fp treatment, the accumulated P removal averaged 176.7 kg P ha^{-1}, with a minimum of 141.2 kg P ha^{-1} (La Hansa) and a maximum of 204.9 kg P ha^{-1} (San Alfredo). Differences in P removal and soil P balance between fertilization treatments were significant for each crop rotation. More details about P rates, removal, and P balance were provided by Ciampitti et al. (2011).

Carbon Fractions

Total organic C showed a significant three-way interaction ($P < 0.01$) of site × depth × fertilization for both crop rotations (Table 2). The POM-C fraction was significant ($P < 0.05$) for site × depth interaction for the C-S-W/S rotation and for site × fertilization interaction for the C-W/S crop rotation (Table 2). Furthermore, single effects of fertilization and depth were significant for both C-S-W/S and C-W/S rotations (Table 2).

Phosphorous fertilization promoted the accumulation of TOC at all sites (Tables 3 and 4). At the end of the experiment, fertilized plots had, on average, 5% more TOC at 0 to 20 cm than unfertilized plots. The POM-C was more sensitive to P fertilization: in this case, the relative difference between fertilization treatments was 14% (Tables 3 and 4). For this parameter, the plots with continuous P fertilization averaged 4.8 g kg^{-1} and the plots without P averaged 4.3 g kg^{-1} in the C-S-W/S rotation in the 0- to 20-cm soil layer. Values for the C-W/S rotation averaged 5.4 g kg^{-1} in the fertilized plots and 4.7 g kg^{-1} in unfertilized plots in the top 20 cm. It is interesting to note that this site showed the highest initial TOC value (Table 1) and the greatest increase in TOC and POM-C after 6 yr of continuous fertilization. Even

Table 2. Analysis of variance for total organic C (TOC), total P (TP), C and P in particulate organic matter (POM-C and POM-P, respectively), and their relationships as affected by site, fertilization, and soil depth factors.

Parameter	TOC	POM-C	TP	POM-P	POM-C/TOC	POM-P/TP	POM-C/POM-P
			Corn–soybean–double-cropped wheat/soybean rotation				
Site (Si)	0.0002	0.0079	NS†	<0.0001	0.0051	NS	0.0001
Fertilization (Fe)	<0.0001	0.0118	0.0040	<0.0001	0.0212	<0.0001	<0.0001
Si × Fe	<0.0001	NS	NS	0.0023	NS	<0.0001	NS
Depth (De)	<0.0001	<0.0001	<0.0001	<0 .0001	<0.0001	<0.0001	0.0001
Si × De	<0.0001	0.0107	0.0011	0.0002	NS	0.0124	0.0351
Fe × De	<0.0001	NS	<0.0001	<0.0001	NS	<0.0001	0.0257
Si × De × Fe	0.0028	NS	<0.0001	0.0006	NS	0.0087	NS
			Corn–double-cropped wheat/soybean rotation				
Si	0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.0147	NS
Fe	<0.0001	0.0012	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
Si × Fe	0.0003	0.0332	0.0016	<0.0001	0.0274	<0.0001	NS
De	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
Si × De	<0.0001	NS	<0.0001	NS	<0.0001	NS	0.0001
Fe × De	NS	NS	<0.0001	0.0265	0.0315	NS	NS
Si × De × Fe	0.0058	NS	0.0051	0.0036	0.0047	0.0378	0.0264

† NS = not significant ($P > 0.05$).

though the difference in TOC between the Fp and Wp treatments was <1 g C kg^{-1} in the 0- to 20-cm depth at the other sites, San Alfredo experienced a difference of 2.5 g C kg^{-1} for the same treatment comparison (Table 4).

All experimental sites resulted in a high concentration of C toward the topsoil depth increment. The vertical TOC distribution increased in the order 10- to 20- < 5- to 10- < 0- to 5-cm soil depths for both Wp and Fp treatments at all locations evaluated (Table 3). In the case of TOC, fertilization effects tended to disappear at the 10- to 20-cm soil layer at two of the four locations (La Hansa and La Marta sites; Table 3).

The POM-C decreased more rapidly with depth than TOC. The POM-C/TOC ratio was, on average for both rotations, slightly greater for the fertilized treatment plots (24.4%) than their unfertilized counterparts (22.6%) at the 0- to 20-cm soil depth (Table 5). Both rotations produced enough crop residue returned to the soil to produce a sustainable increase in both POM-C and TOC fractions. Moreover, the changes in both fractions were more related to the initial soil C content and soil characteristics than the cropping systems evaluated. This was clearly observed in the greater magnitude of mean C changes observed at San Alfredo (high organic C value) than at the other sites (Table 4).

Phosphorous Fractions

Both TP and POM-P showed a significant three-way interaction of site × depth × fertilization for both rotations (Table 2). In the 0- to 20-cm soil depth, the average TP was 469, 503, 533, and 543 mg P kg^{-1} soil for San Alfredo, La Marta, La Hansa, and La Blanca, respectively (Table 3). The POM-P fraction was 10.5, 6.8, 7.0, and 7.4 mg P kg^{-1} soil at San Alfredo, La Marta, La Hansa, and La Blanca, respectively (Table 3).

As expected, P fertilization promoted a significant increase in soil TP (Table 3). The fertilized plots had an average of 52 mg P kg^{-1} more TP, at 0 to 20 cm, than the unfertilized plots, which represents a relative difference between fertilization treatments of 11% (Table 4; Fig. 1).

As occurred with POM-C, POM-P was more sensitive than TP to the addition of P; the relative difference between

Table 3. Effect of continuous P fertilization on the vertical distribution of total organic C (TOC), total P (TP), and C and P in particulate organic matter (POM-C and POM-P, respectively) at three soil depths with (Fp) and without (Wp) continuous P fertilization at different experimental sites under two crop rotations, corn–soybean–double-cropped wheat/soybean (C-S-W/S) and corn–double-cropped wheat/soybean (C-W/S).

Site	Soil depth	TOC		POM-C		TP		POM-P	
		Wp	Fp	Wp	Fp	Wp	Fp	Wp	Fp
	cm	g C kg^{-1}				mg P kg^{-1}			
				C-S-W/S rotation					
La Blanca	0–5	23.6 b†	25.2 a	6.8	7.8	564 d	659 a	6.7 cd	11.9 a
	5–10	21.3 d	22.8 c	4.9	5.7	568 d	596 c	5.8 de	9.6 b
	10–20	15.8 h	16.1 g	3.0	3.4	474 h	502 g	5.4 e	7.2 c
Mean	0–20	19.1	20.1	4.4	5.1	520.0	564.8	5.8	9.0
La Hansa	0–5	21.3 d	22.6 c	6.2	6.8	541 e	624 b	7.1 c	11.3 a
	5–10	16.4 f	16.8 e	4.1	4.4	493 g	629 b	6.3 d	9.4 b
	10–20	15.0 i	15.2 i	3.2	3.4	472 h	517 f	4.2 f	6.6 cd
Mean	0–20	16.9	17.5	4.2	4.5	494.5	571.8	5.5	8.5
				C-W/S rotation					
La Marta	0–5	19.7 g	20.1 g	5.3	5.6	514 d	571 a	6.6 h	10.7 d
	5–10	16.4 i	17.8 h	3.4	4.1	478 e	538 b	5.7 i	8.4 f
	10–20	14.5 j	14.7 j	2.5	2.8	449 h	512 d	4.7 j	6.9 h
Mean	0–20	16.3	16.8	3.4	3.8	472.5	533.3	5.4	8.2
San Alfredo	0–5	28.0 b	30.9 a	7.8	9.0	470 f	522 c	9.7 e	15.4 a
	5–10	27.3 d	29.5 c	6.5	7.4	455 g	474 e	7.5 g	13.5 b
	10–20	22.0 f	24.4 e	4.6	5.9	452 gh	464 f	6.4 h	12.5 c
Mean	0–20	24.8	27.3	5.9	7.1	457.3	481.0	7.5	13.5

† Only for the parameters that presented a significant three-way interaction, different letters indicate significant differences at $P < 0.05$.

Table 4. Mean change (2000–2006) in total organic C (TOC), total P (TP), C and P in particulate organic matter (POM-C and POM-P, respectively), and their relationships between continuous P fertilization (Fp) and no P applied (Wp) in the 0- to 20-cm soil layer at the four experimental sites.

Site	TOC	POM-C	TP	POM-P	POM-C/TOC	POM-P/TP	POM-C/POM-P
	— g C kg^{-1} —		— mg P kg^{-1} —		———— % ————		kg C kg^{-1} P
La Blanca	0.9	0.7 a†	44.7 b	3.2	2.1 a	0.4	−194
La Hansa	0.5	0.3 b	77.4 a	3.0	1.1 b	0.4	−237
La Marta	0.6 b	0.4 b	61.2 a	2.8 b	1.7	0.4 b	−160 a
San Alfredo	2.5 a	1.2 a	24.1 b	6.0 a	2.3	1.2 a	−258 b

† Only for the parameters that presented a significant site effect within each rotation, different letters indicate significant differences at $P < 0.05$.

fertilization treatments was 47% (Table 3). The mean change between fertilization treatments (Fp − Wp) in POM-P averaged 3.1 mg P kg^{-1} in the 0- to 20 cm soil depth for the C-S-W/S rotation and 4.4 mg P kg^{-1} for the C-W/S rotation. As was observed for C, the greatest mean change in POM-P was observed at the San Alfredo site (6.0 mg kg^{-1} at 0–20 cm; Table 4). In general terms, the vertical distribution of P fractions increased in the order 10- to 20- < 5- to 10- < 0- to 5-cm soil depths for both Wp and Fp treatments at all locations evaluated (Table 3).

The greatest differences in POM-P between fertilization treatments were observed in the top layers of the soil (Table 3); however, significant effects of the fertilization treatments on TP and POM-P were observed in all three soil layers evaluated (Table 3).

The POM-P/TP ratio in the 0- to 20-cm soil depth was fairly similar among all the experimental sites. On average, the POM-P/TP ratio was greater under the Fp (1.8%) than the Wp treatments (1.2%). Pooling both fertilization treatments, this ratio averaged around 1.3%. San Alfredo again showed a unique behavior, with a ratio of 2.2% (Table 4).

Table 5. Effect of continuous P fertilization (Fp) or no P fertilizer (Wp) on the vertical distribution of C in particulate organic matter (POM-C) and total organic C (TOC), the relationship between P in particulate organic matter (POM-P) and total P (TP), and the ratio between POM-C and POM-P at different experimental sites under two crop rotations, corn–soybean–double-cropped wheat/soybean (C-S-W/S) and corn–double-cropped wheat/soybean (C-W/S), at three soil depths.

Site	Soil depth	POM-C/TOC		POM-P/TP		POM-C/POM-P	
		Wp	Fp	Wp	Fp	Wp	Fp
	cm	———— % ————				g C kg^{-1}/mg P kg^{-1}	
		C-S-W/S rotation					
	0–5	28.8	31.0	1.2 f†	1.8 a	1015	655
La Blanca	5–10	23.0	25.0	1.0 h	1.6 b	845	594
	10–20	19.0	21.1	1.1g	1.4 d	556	472
Mean	0–20	22.5	24.6	1.1	1.6	743.0	548.3
	0–5	29.1	30.1	1.3 e	1.8 a	873	602
La Hansa	5–10	25.0	26.2	1.3 e	1.5 c	651	468
	10–20	21.3	22.4	0.9 i	1.3 e	762	515
Mean	0–20	24.2	25.3	1.1	1.5	762.0	525.0
		C-W/S rotation					
	0–5	26.9 c	27.9 b	1.3 h	1.9 e	803 b	523 f
La Marta	5–10	20.7 g	23.0 f	1.2 i	1.6 f	596 d	488 g
	10–20	17.2 i	19.0 h	1.1 j	1.4 g	532 f	406 i
Mean	0–20	20.5	22.2	1.2	1.6	615.8	455.8
	0–5	27.9 b	29.1 a	2.1d	3.0 a	804 b	584 d
San Alfredo	5–10	23.8 ef	25.1 d	1.6 f	2.8 b	867 a	548 e
	10–20	20.9 g	24.2 e	1.4 g	2.7 c	719 c	472 h
Mean	0–20	23.4	25.7	1.6	2.8	777.3	519.0

† Only for the parameters that presented a significant three-way interaction, different letters indicate significant differences at $P < 0.05$.

The changes in both fractions were apparently related with the initial soil Bray-1 P for TP and with the initial soil TOC for the POM-P fraction (Tables 1 and 3). Our observation was that the greatest difference in the mean TP change occurred at the La Hansa site (which had the highest Bray-1 P), and the highest mean POM-P change occurred at the San Alfredo site (with the highest initial TOC) between fertilization treatments (Wp − Fp) at the 0- to 20-cm soil depth (Table 4).

Relationships between Carbon and Phosphorus Pools

The POM-C/POM-P ratio declined with depth due to a greater decline in POM-C than POM-P. This indicates a greater stratification of POM-C than POM-P (Table 5). From the mean changes between fertilization treatments (Table 4), it can be observed that negative values resulted from greater levels of the ratio in Wp treatments than in Fp plots. On average, the treatment with continuous P applied showed a lower POM-C/POM-P ratio (512 kg C kg^{-1} P) than the Wp treatment (724 kg C kg^{-1} P) (Table 5). In relative terms, the POM-C changed less than the POM-P between fertilization treatments.

Overall, TOC, POM-C, TP, and POM-P mass contents were greater in the Fp than in the Wp treatments in the 0- to 20-cm soil depth (Fig. 1). For the C-S-W/S rotation, the changes between fertilization treatments (Fp − Wp) were 188 g TOC m^{-2}, 124 g POM-C m^{-2}, 16 g TP m^{-2}, and 0.8 g POM-P m^{-2}. For the C-S-W/S crop rotation, differences between treatments were 423 g TOC m^{-2}, 212 g POM-C m^{-2}, 12 g TP m^{-2}, and 1.3 g POM-P m^{-2} (Fig. 1).

Relationship between Phosphorus in Particulate Organic Matter and Corn Plants

The P accumulation by corn plants at silking time was significantly and positively related to soil POM-P (0–20 cm) following a polynomic exponential function (Fig. 2). Increased POM-P values led to a greater P accumulation in corn up to a critical point of 13 mg POM-P kg^{-1}. Beyond this threshold, a slight decline in P accumulation was observed. The Fp treatment increased soil POM-P, which could be related to a higher P accumulation in these corn plants than in the unfertilized plots.

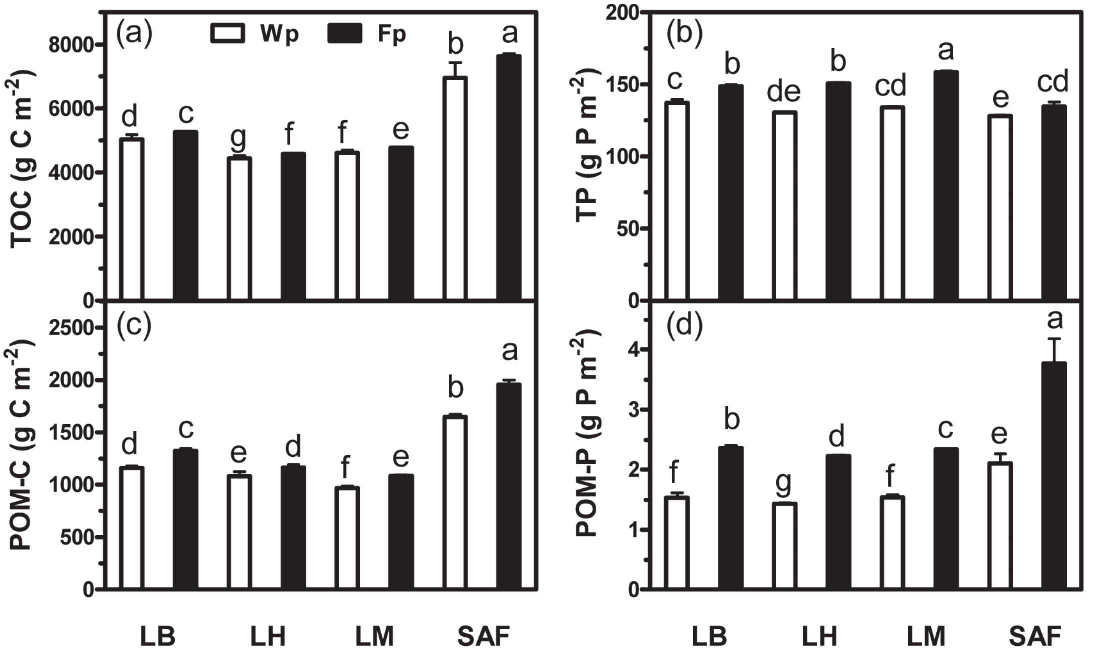

Fig. 1. Effect of no P fertilizer additions (Wp) and continuous P fertilization (Fp) on the (a) total organic C (TOC), (b) total P (TP), (c) C associated with particulate organic matter (POM-C), and (d) P associated with particulate organic matter (POM-P) for the 0- to 20-cm soil depth at La Blanca (LB) and La Hansa (LH) under a corn–soybean–double-cropped wheat/soybean rotation and La Marta (LM) and San Alfredo (SAF) under a corn–double-cropped wheat/soybean rotation. Different letters represent significant differences at $P < 0.05$.

DISCUSSION

Carbon Pools

The effects of P fertilization on soil C and P fractions depends greatly on the fertilization rates, the initial available soil P levels, and the amount of P removed in harvested products. In that sense, our results were constrained by our experimental conditions, which comprised a single annual P fertilization rate of 34 kg P yr^{-1}, a range of initial soil available P from 11.2 to 45.5 mg P kg^{-1}, and a total amount of P removed by crops ranging from of 148.1 to 194.5 kg P ha^{-1} (Ciampitti et al., 2011). Overall in our study, P fertilization management led to either a negative (Wp treatment) or a positive (Fp treatment) net P budget in the soil system. Particulate organic matter C was more sensitive than TOC to both soil depth and P fertilization treatments. This indicated that POM-C was the fraction more responsive to positive balances of P fertilization in the cropping system. The POM fraction has been found to be very sensitive to changes in residue inputs, tillage system, and other management practices (Schwenke et al., 2002; Chan et al., 2002; Fabrizzi et al., 2003).

A concentration gradient of TOC and POM-C toward the top layer of the soil (0–5 cm) was observed (Table 3). This stratification was probably related to the deposition of residues on the soil surface under the no-till system used by the farmers. Furthermore, POM-C vertical stratification was greater than that of TOC, in agreement with Wander et al. (1998), Needelman et al. (1999), and Fabrizzi et al. (2003). In our research, the average POM-C/TOC ratio in the first 20 cm of the soil was 23.5% and was slightly greater in the Fp than the Wp treatments. The POM-C/TOC ratio at this depth varied from 20 to 25% in four Brazilian soils (Amado et al., 2006) and from 22 to 38% in 35 Argentinean soils from the Pampas region (Gutiérrez Boem et al., 2008).

In terms of C sequestration, our results indicate that the C sequestration capability of no-till systems may depend on the initial SOM status and is positively influenced by soil nutrient enrichment associated with higher crop yields and residue deposition (Barber, 1979; Fabrizzi et al., 2003).

The initial C values of our experimental plots reflect the equilibrium of the SOM with the common agricultural practices of the farms at the beginning of this decade. These practices

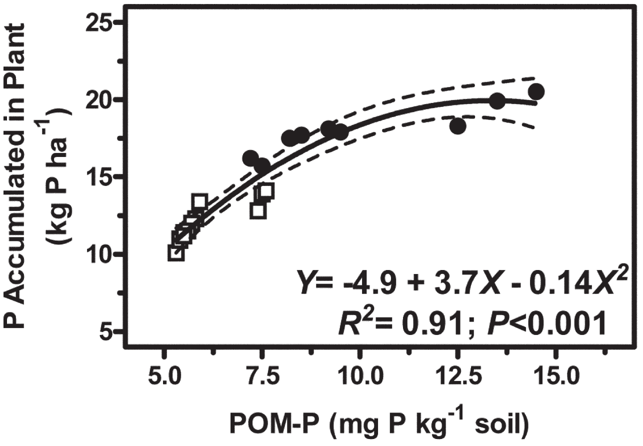

Fig. 2. Relationship between P associated with particulate organic matter (POM-P) in the 0- to 20-cm depth and P accumulated in corn plants at silking time. The dashed lines represent the 95% confidence interval for the data set. Filled circles represent the treatment with continuous P fertilization and empty squares refer to the control plots with no P added to the soil.

generally involved continuous cropping with fertilization rates equal to or below the actual nutrient requirements for maximum yields. In this context, our results strongly indicate that P fertilization levels at rates that raise soil-test P beyond the critical values will promote increased TOC values. Promotion of C sequestration by P applications should not necessarily be expected in P-rich soils or in low-P soils that have been artificially enriched in SOM, for example by the application of manures. Thus, the prediction of the responses of soil C to P fertilization practices should take into account the initial conditions (Liang and MacKenzie, 1992; Darmody and Peck, 1997).

Phosphorous Pools

The continuous application of P fertilizers led to an increase in TP at the 0- to 20-cm soil layer, as also observed by Guo et al. (2000) and Zheng et al. (2002). Zheng et al. (2002) observed that 8 yr of P fertilization produced an increase in TP as a consequence of changes in the quantity and quality of the crop residues. In our work, the POM-P fraction was more sensitive to P fertilization than TP. This fraction is highly responsive to microbial activity (Chauhan et al., 1979) and experiences a rapid turnover rate (Tiessen et al., 1983; Haynes and Swift, 1988). The greater effects of P fertilization on this parameter observed at San Alfredo may be related to the higher initial TOC (Table 1), as observed by Salas et al. (2003). They suggested that there is an association between TOC and POM-P, in that changes in the SOM pool determine the labile organic P release rate from plant residues and the microbiological transformations of the substrate during decomposition. At the La Hansa and La Blanca sites, the changes in POM-P can be attributed to the direct effect of continuous P additions and in some extent to the effect of the initial soil P content (Table 1). Overall, the changes in the organic P fractions could be attributed to surface residue decomposition, as changes in the POM-C fraction have been (Sanchez et al., 1998; Wander et al., 1998; Sá et al., 2001).

Relationships between Pools of Carbon and Phosphorous

The POM-C/POM-P ratio reflected a substantial difference in the C and P dynamics within the POM fraction. Accordingly, several researchers have reported that POM-P exhibits rapid changes during the residue decomposition period and that this dynamic differs from POM-C and POM-N dynamics (Maroko et al., 1999; Phiri et al., 2001; Salas et al., 2003; Hao et al., 2008). Our P fertilization regimen created a higher concentration of P in the POM, resulting in lower POM-C/POM-P values in the fertilized plots.

Relationship between Phosphorus in Particulate Organic Matter and Corn Plants

The observed higher proportion of P in the POM of fertilized soils could be an indicator of higher soil P availability (Phiri et al., 2001). The POM fraction is comprised of the native POM and the POM derived from recent residues added to the soil. Ha et al. (2008) observed that the proportion of POM derived from native POM was only 10 to 20%. Therefore, the quantity and concentration of P in the POM fraction would be more affected by the P contained in the fresh residues than by the P from the native POM. Not all P released from POM will be available to plants because some portion of POM will remain in residue particles <53 μm, taken up by soil microbes, or fixed by the soil matrix. The quality of the residues also affects the release of P (Ha et al., 2007). Ha et al. (2007) observed that soil P availability in the immediate vicinity of decomposing pea (*Pisum sativum* L.) residues incorporated at flowering time was increased up to eightfold compared with the control (soil without residues). Interestingly, when pea residues were incorporated at the mature stage of the crop, they observed a slight reduction in soil P availability.

The polynomic function obtained to describe the association between POM-P and P accumulation in corn plants at silking time, clearly shows two phases. The first one is characterized by a more than proportional increase of P accumulation following POM-P increases. The second phase is characterized by a plateau, where P accumulation is not affected by POM-P. These results suggest that POM-P appears to be a useful tool in P diagnosis although it is understood that POM-P has a biologically limited capacity as a long term nutrient storage pool.

CONCLUSIONS

The results from this study showed that 6 yr of continuous P fertilization increased the TOC and TP at 0 to 20 cm in Mollisols for both crop sequences used by the farmers (C-S-W/S or C-W/S). Both POM-C and POM-P were the most sensitive variables to P fertilization, indicating that they constitute a useful tool to detect the effects of management practices. This is particularly valid for short- and medium-term evaluations, by which time the TOC and TP might not be capable of detecting the relevant effects. In both crop rotations analyzed, the increase in the crop residue input caused by P fertilization was enough to generate gains in TOC and TP and, therefore, to contribute to C sequestration and soil fertility improvement. Phosphorous fertilization also modified the vertical distribution of TOC, POM-C, TP, and POM-P, leading to an accumulation near the soil surface. This may be related to the residue placement in our no-till system and to the increased quantity of crop residues.

Finally, we demonstrated that POM-P could be a good predictor of the P nutritional status of the crop. More research is needed, however, to calibrate the observed association between POM-P and plant P accumulation with different situations, crops, and phenological stages of crop development.

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