Long-Term Phosphorus Application Impacts on Aggregate-Associated Carbon and Nitrogen Sequestration in a Vertisol in the Mediterranean Turkey

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Abstract: Fertilizers are among major contributors to the production of aboveground and belowground biomass. Soil organic carbon (SOC) concentration is an important factor affecting crop productivity in semiarid clayey soils of low fertility. A long-term experiment was established in 1998 on a Vertisol in the Mediterranean coast of Turkey, to assess the effects of four rates of application of inorganic phosphorus (P) fertilizers (0, 50, 100, and 200 kg P_2O_5 ha^{-1}) on soil bulk density (ρ_b), carbon (C) and nitrogen (N) concentrations, SOC and N pools, C and N sequestration rate, aggregate fractions, water-stable aggregates, and the mean weight diameter (MWD). Thus, disturbed and undisturbed soil samples were collected from the 0- to 15-cm depth after wheat (*Triticum aestivum* L.) harvest in June 2010 to analyze soil properties.

Increase in the rate of application of P fertilizers significantly increased ρ_b and reduced porosity (%). The SOC concentration was significantly more in the treatment receiving 200 kg P_2O_5 ha^{-1} than in the control. The carbon-nitrogen ratio was less than 10 in control and greater than 10 in high P fertilizer treatments. Total amount and rate of C and N sequestration increased with increase in the rate of application of P fertilizers. The mean rate (kg C ha^{-1} y^{-1}) of C sequestration was −110.9 for control and 556.9 for the highest rate of P fertilizer. Increase in fertilizer rate also significantly increased the rate (kg N ha^{-1} y^{-1}) of N sequestration, which was −0.21 for control and 28.9 for the highest rate of P fertilizer treatment. The SOC concentrations differed among aggregate-size fractions, which were greater in 0.5- to 2.0-mm aggregate size than those in the fraction of less than 0.25 mm. Greater SOC concentrations were observed in 1- to 2-mm and 0.5- to 1-mm size fractions than in fractions of less than 0.25 mm. Concentrations of C and N decreased significantly with decrease in aggregate size of less than 0.25 mm. There was a general trend of increase in water-stable aggregates and MWD with increase in application of P fertilizer. The highest MWD (1.81 mm) was observed in the high P rate and lowest (1.36 mm) in the control. Macroaggregates were more enriched in C and N than microaggregates. The mean rate of SOC sequestration with 200 kg P_2O_5 ha^{-1} treatment is ~560 kg C ha^{-1} y^{-1}. The effects of long-term P fertilizer application on C and N sequestration finding(s) of from this study have not been previously observed.

Key words: Soil organic carbon, phosphorus fertilizers, mean weight diameter, water-stable aggregates, long-term experiment.

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Vertisols are a dominant soil type covering 308 million hectare (M ha) globally (Coulombe et al., 1996), of which 1.7 M ha of mainly basaltic Vertisols (General Directorate of Rural Services, 1987) occur in the Mediterranean region of Turkey. Vertisols are structurally unstable and have a high swell-shrink property. Soil mineralogical characteristics include high Fe and Al oxides and smectite-dominated clay mineralogy with high surface area. The SOC in Vertisols forms a major component of soil organic matter (SOM) (Follett et al., 2005) and is a principal determinant of soil biological and physical quality. Organomineral complexes are important for binding soil particles into macroaggregates and microaggregates (Singh et al., 2009).

The SOC pool and its long-term retention have several beneficial effects on soil quality and agronomic yield. These parameters promote and accentuate aggregation, nutrient reserves and supply, soil biotic activity, soil fertility, and biomass productivity (Karlen et al., 1997). The SOC pool is a function of the net input of organic residues by the cropping system (Follett et al., 2005; Gregorich et al., 1996).

Sequestration of SOC and its transfer into aggregates are important for enhancing the mean residence time (Lal, 2002, 2010; Singh et al., 2009; Six et al., 2000a; Ussiri et al., 2006; Wilson et al., 2009). Thus, it is important to enhance soil aggregation for increasing SOC sequestration, especially within stable microaggregates and macroaggregates. Transfer of SOC pool into stable microaggregates is important to long-term soil C sequestration (Huang et al., 2010). Management practices involving increased return of crop residues-C improve aggregate stability and enhance aggregate-associated SOC levels (Lal, 2009). Indeed, soil aggregation is an important process that mediates numerous chemical, physical, and biological properties and improves soil quality and sustainability (Moreno-de las Heras, 2009). Aggregates encapsulate SOC and reduce the rate of its decomposition (Lal, 2008), a process crucially important in the semiarid Mediterranean climate. The SOC associated with microaggregates is persistent and protected from microbial attack and decomposition (Elliott, 1986).

A judicious management of biomass affects many soil properties, including SOC pool through a transfer of the atmospheric CO_2 into terrestrial ecosystems (Lal, 2010). Soil management strongly influences formation and stabilization of aggregates and SOC sequestration. Increasing C sequestration in agricultural ecosystems and making soil a net sink of atmospheric CO_2 can be achieved by adoption of the best management practices such as reduced tillage, fertilizer application, organic amendments, and improved residue management (Jarecki and Lal, 2003).

Aggregate stability is directly related to SOC concentration, which depends on the aboveground and belowground plant biomass. Soil amendments are important to improve soil fertility and aggregation and maintain and enhance crop yields in soils of low inherent fertility. Long-term increase in crop yields and

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biomass returns with regular fertilizer applications resulted in a higher SOC concentration and biological activity than control (Haynes and Naidu, 1998; Lal, 2003). Improvements in soil quality with application of fertilizers and amendments are due to more biomass residues returned in fertilized than unfertilized soils (Campbell et al., 2001). A balanced application of fertilizers (N, P, K) and manures can also increase SOC levels in agricultural soils (Rudrappa et al., 2006). Balanced fertilization, using mineral fertilizers with organic amendments, can improve the nutrient status of soil, improve crop yields, and produce high levels of residues that are essential to increasing the SOC concentration (Holeplass et al., 2004). Improvement in soil fertility through nutrient management is also important for SOC sequestration (Lal, 2005). Furthermore, concentrations of SOC and N are key indicators of soil quality and productivity because of their favorable effects on physical, chemical, and biological processes (Bauer and Black, 1994) including nutrient cycling, water retention, root development, plant growth, and environmental quality (Sainju and Good, 1993).

A combination of monocropping and heavy tillage decreases soil organic carbon (SOC), water permeability, soil aggregation, and soil macrofauna and microfauna but exacerbates soil compaction and nutrient imbalance (Lal et al., 1989; Tisdall and Oades, 1980, 1982). It is difficult to increase the SOC pool under these conditions. In general, soils of the semiarid Mediterranean regions have low SOC concentrations, high clay and carbonate contents, weak soil structure, and low water infiltration rates. Supraoptimal temperatures, high $CaCO_3$ and clay contents, and excessive tillage accentuate decomposition of SOM and reduce productivity of soil, especially in the eastern part of the Mediterranean regions. Depletion of SOM and the degradation of soil structure are also exacerbated by continuous cropping on arable land because of the traditional practices of burning crop residues and excessive tillage. Reduced SOM content is one of the major reasons of low crop yields in the Mediterranean region (Khresat et al., 2008; Ryan et al., 2008). Because of their high clay and lime contents, most soils in the Mediterranean region are poor in macronutrient and micronutrient availability, which is also indirectly related to soil aggregation.

Enhanced biomass production increases the SOC concentration and directly affects soil microbial dynamics. Consequently, management of soil physical (i.e., bulk density and aggregation), chemical (accumulated organic matter, optimum pH), and biological (microbial diversity, mycorrhizae, and nitrogen-fixing *Rhizobia*) fertility parameters can promote a high and sustainable crop production. Phosphorus is one of the major fertilizers used in all agricultural systems. However, the majority of P applied is immobilized in the soil and unavailable. Furthermore, a high rate of P application can negatively affect soil organisms such as mycorrhizae development (Ortas, 2003).

Little is known about the effects of long-term P application on soil aggregation and its relationship with C stabilization in Vertisols in the semiarid Mediterranean region. There are few, if any, data on the impact of P application on SOC storage and aggregation under long-term field experiments. Long-term soil management experiments are needed to assess soil C sink capacity through a complete life cycle analysis, including direct and hidden C costs (Lopez-Bellido et al., 2010). Therefore, the objective of this study was to determine the long-term effects of different rates of P application on aggregate-associated carbon and nitrogen sequestration and the related soil properties under wheat (*Triticum aestivum*)–maize (*Zea mays* L.) cropping system in a Vertisol in semiarid Mediterranean conditions. The study was based on the hypothesis that P fertilization enhances C and N concentrations in soil aggregates, increases aggregation, and improves soil structure and tilth.

MATERIALS AND METHODS

Location and Experimental Layout

The study was conducted at the Çukurova University Experimental Station of the Soil Science Research Center in the Mediterranean region of Adana, Turkey (37°00.47.75 N lat. and 35°21′31.92 E altitude long. 33 m.a.s.l.). The experiment was established in 1998 and continued through 2011, with two consecutive crops a year: maize in summer and wheat in winter. Predominant soil series is Arik, which is classified as typic Haploxererts. The regional climate is typical Mediterranean with long-term average annual air temperature of 19.1°C (ranging from 14.2°C in January-February to 25.5°C in July-August) and precipitation of 670.8 mm. About 80% of the annual precipitation is received between November and April, with a mean annual humidity of 66% (Anonymous, 2008).

The experiment was composed of 12 plots laid out in a randomized block design with three replications and plot dimensions of 10 × 20 m. The four treatments were as follows: (i) P0 control, (ii) P1 50 kg P_2O_5 ha^{-1}, (iii) P2 100 kg P_2O_5 ha^{-1}, and (iv) P3 200 kg P_2O_5 ha^{-1} as $3Ca\ (H_2PO_4)_2{\cdot}H_2O$. In addition, 160 kg N ha^{-1} as $(NH_4)_2SO_4$ for wheat and 200 kg N ha^{-1} as $(NH_4)_2NO_3$ for maize were applied along with a uniform application of 50 kg K ha^{-1} as K_2SO_4. Residues of maize and wheat were not removed and were incorporated by moldboard plowing to 15-cm depth after each harvest. Annually, the inorganic fertilizers were uniformly spread on the soil surface just before sowing and incorporated into the surface 0- to 15-cm layer with a disc harrow.

Soil Sampling, Preparation, and Analyses

In July 2010, bulk soil sample was obtained from 0- to 15-cm depth after the harvest of wheat from each replicate of all treatments. After air drying, part of the sample was kept aside as bulk soil fraction, and the other ground and sieved through 2-mm sieve. Soil bulk density (ρ_b) was measured by the core method (Blake and Hartge, 1986). However, the missing initial ρ_b for 1998 was estimated with pedotransfer functions using soil textural data (Calhoun et al., 2001). Total porosity f_t was calculated using the equation: $[1 - (\rho_b / \rho_s)] \times 100$, where ρ_b = bulk density, ρ_s = particle density (assumed to be 2.65 Mg m^{-3} soil) (Singh et al., 2009).

Soil Carbon and Nitrogen Analyses

Soil sieved through 2-mm mesh was ball-milled and again passed through a 0.25-mm mesh for determination of the total C and N concentrations by the dry combustion method at 900°C using a C and N elemental analyzer (LECO Corporation, St Joseph, MI). Inorganic C was determined by measuring total $CaCO_3$ content by treating 2 g of finely ground soil with 6 *M* HCl as per the modified pressure calcimeter method (Sherrod et al., 2002). The SOC concentration was obtained by subtracting soil inorganic carbon from total C. Total N concentration was measured by the semi-Kjeldahl method (Jackson, 1973).

Soil Water-Stable Aggregate Analyses

Air-dried bulk soil samples were gently crushed and sieved through 8-mm sieve. Fifty grams of aggregates was used for the wet-sieving technique (Yoder, 1936; Youker and McGuiness, 1957). Wet sieving was done by using a nest of five sieves (4.75, 2, 1, 0.5, 0.25 mm) after prewetting for 30 min. The nest of sieves was oscillated in water for 30 min (Lal and Blanco-Canqui, 2007). Aggregates retained on each sieve were

TABLE 1. Soil Initial Physical, Chemical, and Biological Characteristics of Arik Soil Series

Properties	Unit	Depth 0–20 cm
Clay	g kg^{-1}	535 ± 15
Silt		291 ± 35
Sand		174 ± 44
Soil organic carbon	g kg^{-1} soil	0.89 ± 0.08
Inorganic carbon		3.41 ± 0.43
Total nitrogen		0.09 ± 0.01
CEC	$Cmol^{+}$ kg^{-1}	35 ± 1.00
pH	H_2O	7.57 ± 0.55
Salt	%	0.03 ± 0.03
P	mg kg^{-1}	15.35 ± 1.64
K		1132.3 ± 15.3
No. mycorrhizal spores	10 g^{-1} soil	89.7 ± 24.03

Mean of two replicates ± S.D.

CEC: Cation exchange capacity.

backwashed with deionized water for determining percentage of water-stable aggregate (WSA). Soil aggregates on different sieves were oven dried at 40°C, and the mean weight diameter (MWD) was computed (Nimmo and Perkins, 2002; van Veen and Kuikman, 1990). Each aggregate-size fraction was also ball-milled and passed through a 0.25-mm sieve for determination of C and N concentrations.

Calculations of SOC and Nitrogen Pools

The SOC and total soil N (TSN) pools were calculated for 15-cm soil depth using the following equation (Lal et al., 1998):

$$\text{Mg SOC or TSN ha}^{-1} = \text{\% C or N} \times \text{soil depth (m)} \times \rho_b\,(\text{Mg m}^{-3} \times 10^4\ \text{m}^2\,\text{ha}^{-1} / 100) \qquad (1)$$

The SOC and TSN sequestration rates were calculated with reference to the control treatment as baseline and by dividing the change in the SOC and TSN pools by 12 years of the experiment duration.

Data Analysis

All data were statistically analyzed using analysis of variance in the SAS program (SAS, 2009) to assess the effects of different P treatments on soil properties. Means were compared using the least significant difference test when the analysis of variance showed significant treatment effects ($P \leq 0.05$). The path analysis was done by Lisrel program to search types of direct and indirect relation effects. The effects of P addition on SOC %, N %, ρ_b, SOC and TSN pools, WSA, and MWD were calculated and interpreted.

RESULTS AND DISCUSSION

Soil Properties, Bulk Density, and Total Porosity

Soil initial physical, chemical, and biological characteristics of Arik soil series are presented in Table 1. In general, the soil has high clay content, and the initial SOM is low.

Soil bulk density and porosity are important soil physical fertility parameters that are affected by soil and crop managements. After 12 years of cropping, ρ_b was significantly changed ($P < 0.05$) (Table 2). There was a noticeable trend of higher ρ_b in the plots treated with P3 fertilizers. In 0- to 15-cm depth, ρ_b was 1.31 Mg m^{-3} in the control compared with 1.44 Mg m^{-3} in the P3 treatment. Similar results of increase in ρ_b at high rate of P application were reported by Singh et al. (2009), who observed that the ρ_b was the highest in the chemical fertilizer compared with the organic treatments. It was expected that 12 years of maize and wheat growth and development of roots loosen up the soil and decrease ρ_b. However, these results are contrary to these reported by Thompson et al. (1987), who concluded that root system development, addition of biomass, and little anthropogenic perturbation after 15 to 20 years of reclamation improved soil structure, decreased ρ_b and increased porosity.

In accord with an increase in $\rho_{b,}$ total porosity (f_t) was significantly ($P < 0\ .0002$) reduced by the addition of P (Table 2). Soil porosity, a reverse trend to that of ρ_b, was the highest in the control treatment and the lowest in the P3. The highest f_t of 50.4% was in the control and the lowest of 45.7% for P3-treated soils (Table 2).

SOC and Nitrogen Pools and Carbon-Nitrogen Ratio

The data in Table 3 summarize results on inorganic and organic C and N concentrations and total SOC and N pools for different rates of P fertilizer applications. Increase in P fertilizer application significantly impacted ($P < 0.03$) total C concentrations, which was 4.29% in control compared with 4.63% in P3 treatment. The average soil inorganic carbon concentration, not affected by increase in P fertilization, was 3.42%. The SOC concentration increased significantly with the increase in rate of P application ($P < 0.04$), ranging from 0.84% to 1.20% (average, 1.01%) (Table 4). The most notable increase in SOC concentration occurred in the P3 plots, where it was 142% more than in the control (0.84% vs 1.20%). The TSN concentration (%) did not increase with the increase in P fertilization ($P <$ 0.23), ranging from 0.09% to 0.11%, with an average of 0.10% (Table 3). Su et al. (2006) observed in China that removal of residues and with only input of organic materials through root biomass using chemical fertilizers without animal manure did not maintain the SOC pool in the long term. Nardi et al. (2004) also reported that incorporation of limited crop residues had little effect on the SOC pool.

Soil carbon-nitrogen (C:N) ratio affects soil fertility and is impacted by the rate of P application. The data show a trend of increase in C:N ratio with increasing P fertilization. These results are in agreement with those of Moore et al. (2011). The most notable increase in C:N ratio occurred in the P3 plots,

TABLE 2. Effect of Different Levels of Phosphorus Fertilizers on Bulk Density and Total Porosity

Fertilizer Treatments	Bulk Density Mg m^{-3}	Porosity %
P0	1.31 ± 0.02c	50.44 ± 0.87a
P1	1.39 ± 0.01b	47.42 ± 0.44b
P2	1.42 ± 0.01a	46.29 ± 0.44c
P3	1.44 ± 0.01a	45.66 ± 0.38c
Pr>F	0.0002	0.0002
LSD	0.0312	1.172

Mean of three replicates ± S.D. Means in the same row followed by the same letter represent significant differences ($P \leq 0.05$) among treatments at the same depth.

P0: Control, P1: 50 kg P_2O_5 ha^{-1}, P2: 100 kg P_2O_5 ha^{-1}, P3: 200 kg P_2O_5 ha^{-1}, LSD: least significant difference.

TABLE 3. Initial Soil Organic and Inorganic Carbon, Nitrogen Concentrations, and Bulk Density, and the Effects of Organic and Inorganic Fertilizers Application on Total Soil Organic Carbon and Total Nitrogen Pools

Fertilizer Treatments	Total Soil Carbon (%)	Inorganic Carbon (%)	SOC (%)	n (%)	C:N Ratio	SOC Pool (Mg ha^{-1})	TSN Pool (Mg ha^{-1})
P0	4.29 ± 0.09b	3.45 ± 0.03	0.84 ± 0.08b	0.09 ± 0.02	9.84 ± 1.82	16.50 ± 1.77b	1.71 ± 0.32b
P1	4.39 ± 0.10b	3.40 ± 0.03	0.98 ± 0.13ab	0.10 ± 0.02	9.61 ± 1.33	20.56 ± 2.73ab	2.18 ± 0.47ab
P2	4.44 ± 0.11b	3.40 ± 0.03	1.04 ± 0.09ab	0.11 ± 0.00	9.47 ± 0.93	22.19 ± 1.86ab	2.35 ± 0.10a
P3	4.63 ± 0.11a	3.43 ± 0.01	1.20 ± 0.11a	0.11 ± 0.00	11.03 ± 0.68	25.85 ± 2.45a	2.33 ± 0.13a
Pr>F	0.0297	0.2002	0.0455	0.2340	0.5155	0.0181	0.089
LSD	0.2019	0.057	0.231	0.026	2.673	4.864	0.548

Mean of three replicates ± S.D. Means in the same row followed by the same letter represent significant differences ($P \leq 0.05$) among treatments at the same depth.

P0: Control, P1: 50 kg P_2O_5 ha^{-1}, P2: 100 kg P_2O_5 ha^{-1}, P3: 200 kg P_2O_5 ha^{-1}, LSD: least significant difference.

where it was higher than in the control by 112%. The C:N ratio ranged between 9.84 and 11.03, with an average of 10.0 (Table 3). Banger et al. (2009) observed that the C:N ratio was the lowest in the clay fraction and increased with increase in particle size and is about 10 in loam and clay soils (Hassink, 1994). Since the soil has high clay content (Table 1), the SOC is absorbed by the clay surface (Chan et al., 2008). The positive effect of P fertilization on C mineralization may be due to differences in litter quality. Ghani et al. (2003) also observed a positive impact of long-term P fertilization of pastures on labile organic C pools. In Ohio, United States, Jacinthe and Lal (2007) observed a significant effect of P fertilization on the pool of mineralizable C in the surface layer of a reclaimed mine soil.

Total SOC and Nitrogen Pools

The SOC pools in the bulk soils were significantly affected by P fertilization ($P < 0.02$), which produced high crop residues and root biomass, and consequently contributed to the high SOC and TSN pools. The data in Table 3 indicate that the increased P fertilizer application increased the SOC pool in the 0- to 15-cm depth compared with control. Soil receiving the highest level of P had the highest SOC pool (25.9 Mg C ha^{-1}) relative to the control (16.5 Mg C ha^{-1}). The trend of somewhat higher SOC concentration in P3 compared with control may be due to incorporation of more residues and root biomass produced by P fertilization.

Despite the lack of significant differences, there is a trend of increase in TSN pool with increase in P application ($P < 0.08$) (Table 3). The TSN pool was 1.71 Mg ha^{-1} for control and 2.33 Mg ha^{-1} for the P3 fertilizer treatment.

In India, Hati et al. (2008) observed that the use of NPK with manure and lime significantly increased the SOC pool in plots receiving N alone compared with an unfertilized control treatment. Ghani et al. (2003) observed a positive impact of long-term P fertilization of pastures on labile organic C pools. Agbede (2010) reported that application of inorganic NPK fertilizer increased TSN. On the contrary, however, Huang et al. (2010) indicated that the use of inorganic fertilizers (N and NPK) did not affect the concentrations of either total SOC or any C fraction compared with unfertilized control. Gregorich et al. (1996) also indicated that although adequate fertilization increases crop yields and soil C storage, it may not significantly alter the rate of turnover of the native SOM pool. Nardi et al. (2004) reported that incorporation of limited crop residues had little effect on the SOC pool.

Carbon and Nitrogen Sequestration

Total soil N is the key parameter for SOC pool in ecosystem. Increasing TSN can enhance SOC sequestration. The data in Table 4 show that the total magnitude of C sequestration (Change) ranged from −1.6 to 7.8 Mg ha^{-1} for 0- to 15-cm depth. There was a linear increase in the amount of C sequestered with increase in the P application (Fig. 1). The difference in C sequestration between control and high P fertilizer application was 9.4 Mg C ha^{-1}, with an average increase across all treatments of 3.2 Mg ha^{-1}. The mean annual rate of C sequestration over the 12-year period ranged from −110.9 to 556.9 kg C ha^{-1} y^{-1} ($\bar{X}$ = 237.7) (Table 4).

The amount of N sequestration ranged from −0.21 (control) to 0.4 Mg N ha^{-1} (P3 fertilizer) with an average of 0.22 Mg

TABLE 4. Mean Rate of Carbon and Nitrogen Sequestration 1998 and 2010

		Carbon Sequestration						Nitrogen Sequestration			
						SOC		TSN			
Fertilizer	ρ_b	SOC	N	1998	SOC Pool	2010	Rate of C Sequestration	1998	2010	ΔTSN	Rate of N Sequestration
Treatments	Mg m^{-3}	%		Mg ha^{-1} C			kg C ha^{-1} y^{-1}	Mg ha^{-1} N			kg N ha^{-1} y^{-1}
P0	1.32	0.91	0.09	18.1	16.5	−1.6	−110.9	1.92	1.7	−0.21	−15.3
P1					20.6	2.5	179.3		2.2	0.25	17.9
P2					22.2	4.1	295.5		2.4	0.42	30.1
P3					25.9	7.8	556.9		2.3	0.40	28.9
Mean					21.3	3.2	230.2		2.1	0.22	15.4

P0: control, P1: 50 kg P_2O_5 ha^{-1}, P2: 100 kg P_2O_5 ha^{-1}, P3: 200 kg P_2O_5 ha^{-1}.

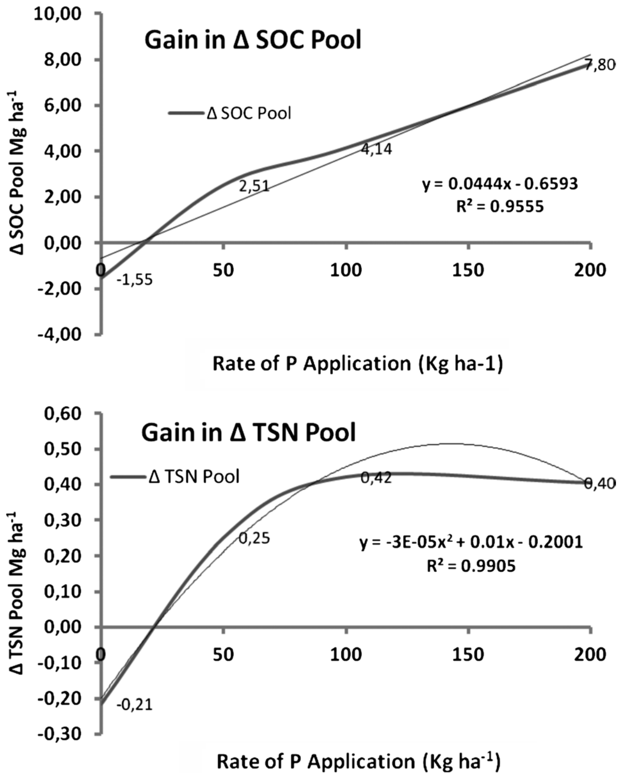

FIG. 1. Relationship between SOC and TSN pool gain and phosphorus application over 12 years' time.

N ha^{-1} (Table 4). The mean annual rate of N sequestration ranged from −15.3 to 28.9 kg N ha^{-1} y^{-1}, with an average of 15.4 kg N ha^{-1} y^{-1}. The relationship between the rate of P fertilizer and the N sequestration is polynomial. Increase in P fertilizer increased the total N pool up to 100 kg P_2O_5 ha^{-1} (Fig. 1).

With reference to the control, the highest rate of P application increased soil organic carbon pool by 3.2 Mg ha^{-1}. However, the rates of SOC sequestration in this study are higher than those reported by Lal (2005) for the semiarid climate, probably because of the clayey texture of the soil. Follett et al. (2005) observed that SOC sequestration was 0.2 and 0.6 Mg y^{-1} in 0- to 15- and 15- to 30-cm depths, respectively, in the conventional wheat-maize experiment in Vertisols in Central Mexico. Follett et al. (2005) hypothesized that there was a significant correlation between the aboveground crop residues produced and the amount of SOC sequestered. The SOC sequestration in irrigated Vertisols from several experimental sites in New South Wales, Australia, indicated that the highest rates were observed where minimum tillage and rotation with wheat had been practiced for more than 10 years (Hulugalle, 2000).

Increase in SOC pool with fertilizer application depends on two factors: (i) increase in crop yield and thus more residues returned and (ii) application of balanced fertilizers over a long period (Dumanski et al., 1998; Haynes and Naidu, 1998). In some cases, application of chemical fertilizers in conjunction with FYM (5–10 Mg ha^{-1} y^{-1}) can increase soil organic carbon pool (Derpsch and Bohm, 2001). In contrast, application of inorganic fertilizers alone (without FYM) may have no significant effect on C sequestration compared with unfertilized control (Su et al., 2006). The results support the hypothesis that long-term application of P fertilization increases C sequestration in cropland soils.

TABLE 5. Effect of Different Organic and Inorganic Fertilizers on C, N Concentration, and C:N Ratio in Aggregate Size Fractions

Fertilizer Treatments	8–4.75 mm	4.75–2 mm	2–1 mm	1–0.5		0.5–0.25	<25
Carbon (%)							
P0	0.74 ± 0.07b	0.76 ± 0.06a	0.83 ± 0.06b	0.80 ± 0.04b	0.77 ± 0.02b	0.46 ± 0.09b	
P1	0.87 ± 0.08ab	0.83 ± 0.09ab	0.97 ± 0.17ab	0.99 ± 0.06a	0.89 ± 0.11ab	0.62 ± 0.19ab	
P2	0.95 ± 0.07a	0.95 ± 0.08a	1.11 ± 0.10a	1.07 ± 0.05a	0.93 ± 0.04a	0.76 ± 0.10a	
P3	0.93 ± 0.15a	0.95 ± 0.04a	1.11 ± 0.08a	1.03 ± 0.09a	0.97 ± 0.10a	0.67 ± 0.10ab	
Pr>F	0.1091	0.188	0.0401	0.003	0.057	0.882	
LSD	0.1847	0.1232	0.2074	0.1203	0.1489	0.2374	
Nitrogen (%)							
P0	0.08 ± 0.01	0.08 ± 0.00	0.09 ± 0.01	0.09 ± 0.00	0.08 ± 0.01ab	0.06 ± 0.01	
P1	0.08 ± 0.01	0.09 ± 0.00	0.08 ± 0.01	0.10 ± 0.02	0.08 ± 0.00a	0.06 ± 0.00	
P2	0.08 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01c	0.06 ± 0.01	
P3	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01bc	0.06 ± 0.01	
Pr>F	0.882	0.258	0.5296	0.2672	0.055	0.6847	
LSD	0.2209	0.011	0.0178	0.0227	0.0119	0.0145	
C:N ratio							
P0	9.06 ± 0.28	9.28 ± 0.47b	9.76 ± 2.00b	9.15 ± 0.78b	9.49 ± 0.92c	7.61 ± 0.61c	
P1	10.24 ± 0.73	9.65 ± 0.49b	11.59 ± 2.55ab	10.21 ± 2.24ab	10.94 ± 1.37bc	9.61 ± 2.81bc	
P2	12.56 ± 3.21	13.00 ± 2.28a	14.76 ± 0.34a	13.24 ± 1.12a	13.42 ± 0.54ab	13.40 ± 1.93a	
P3	10.87 ± 1.74	11.53 ± 1.12ab	13.28 ± 1.84ab	12.53 ± 1.97a	14.02 ± 2.35a	11.37 ± 1.42ba	
Pr>F	0.220	0.0282	0.0522	0.0484	0.016	0.027	
LSD	3.516	2.481	3.519	3.089	2.752	3.524	

Mean of three replicates ± S.D. Means in the same row followed by the same letter represent significant differences ($P \leq 0.05$) among treatments at the same depth.

P0: control, P1: 50 kg P_2O_5 ha^{-1}, P2: 100 kg P_2O_5 ha^{-1}, P3: 200 kg P_2O_5 ha^{-1}, LSD: least significant difference.

TABLE 6. Effect of Different Organic and Inorganic Fertilizers on Water-Stable Aggregates

Fertilizer Treatments	WSA (%)						Total WSA (%)	Total MWD (mm)
	8–4.75 mm	4.75–2 mm	2–1 mm	1–0.5	0.5–0.25	<25		
P0	4.03 ± 2.40	17.89 ± 4.55	20.54 ± 5.26	18.87 ± 1.65	12.72 ± 2.78	17.36 ± 4.80	74.04 ± 8.11	1.36 ± 0.28
P1	7.35 ± 6.94	22.16 ± 7.15	20.32 ± 2.98	18.35 ± 2.56	12.59 ± 4.55	15.12 ± 3.50	80.77 ± 2.48	1.71 ± 0.39
P2	7.88 ± 4.04	23.75 ± 13.01	20.13 ± 5.07	19.46 ± 3.75	12.66 ± 5.50	14.06 ± 7.55	83.86 ± 7.30	1.80 ± 0.24
P3	7.41 ± 3.75	25.14 ± 10.51	20.76 ± 3.66	16.95 ± 2.35	14.44 ± 4.28	12.71 ± 2.81	84.69 ± 5.30	1.81 ± 0.31
Pr>F	0.837	0.825	0.981	0.786	0.956	0.759	0.169	0.421
LSD	7.13	19.2	3.98	6.19	9.97	10.75	10.87	0.696

Mean of three replicates ± S.D.

P0: control, P1: 50 kg P_2O_5 ha^{-1}, P2: 100 kg P_2O_5 ha^{-1}, P3: 200 kg P_2O_5 ha^{-1}, LSD: least significant difference.

Carbon and Nitrogen in Aggregate-Size Fractions

The SOC concentration increased with decrease in aggregate size from 4.75 mm to 0.25 mm. The data support the conclusion that higher SOC concentrations were measured in the aggregates between 0.50 and 2.0 mm than in fractions of less than 0.25 mm (Table 5). Furthermore, concentration of SOC differed significantly among 1- to 2-mm ($P < 0.04$), 0.5- to 1-mm ($P < 0.003$), and 0.25- to 0.50-mm ($P < 0.057$) size fractions (Table 5). The SOC concentration in the unfertilized control ranged from 0.77% for 4.77 mm to 0.46% for aggregates of less than 0.25 mm (Table 5). In comparison, the SOC concentration in the P3 treatment ranged from 0.93% for 4.77 mm to 0.67% for aggregates of less than 0.25 mm. There was a trend of increase in SOC concentration with increase in aggregate size from 0.5 to 2 mm. However, the SOC concentration tended to decrease in aggregates of 0.2- to 0.5 mm. The highest SOC concentration in aggregates was observed in P2 and P3 treatments. Such an increase in concentration of SOC in stable aggregates is crucial to enhancing SOC sequestration. The TSN concentration (%) in different aggregate sizes did not differ among P fertilizer treatments. The lowest TSN (%) was measured in aggregates of less than 0.25 mm. In contrast to N, the C:N ratio was significantly affected by the aggregate size. A high C:N ratio was observed in 1- to 2-mm aggregates (Table 5).

Effects of inorganic and organic fertilizers on SOC and TSN pools have been widely studied under long-term experiments (Banger et al., 2009; Follett et al., 2005; Hati et al., 2008; Hernanz et al., 2002; Huang et al., 2010; Nardi et al., 2004). However, few, if any, studies have been conducted to determine the distribution of C among the aggregate-size fractions related to long-term application of P fertilizers.

Higher SOC concentrations were measured in the aggregates between 0.50 and 2.0 mm than in fractions of less than 0.25 mm (Table 5). A general trend of increase in SOC concentration with increase in aggregate size may be related to increase in stability of aggregates in relation to P management. Guo et al. (2010) also observed that the number of soil aggregates in the 1- to 2-mm and 0.5- to 1-mm categories and the stability of aggregates significantly increased with increase in rate of P application. Similarly, Saroa and Lal (2003) observed the highest SOC (%) in the 0.25-mm aggregate-size fraction.

It is widely recognized that macroaggregates play an important role in stabilizing SOC and TSN concentrations (Tisdall and Oades, 1982). In the present experiment, however, there were no effects of aggregate size on TSN concentration. The concentrations of SOC and TSN in bulk soil were more than those in aggregates after sieving in water. This trend may imply that wet sieving is not a suitable technique for measuring TSN in aggregate-size fractions. Being highly soluble, N is washed out of the aggregates during the wet-sieving procedure.

Water Stable Aggregation and the Mean Weight Diameter

Size distribution of WSA increased with increase in P fertilizer, albeit with no significant differences among treatments. In general, a higher WSA (%) was observed in P3 compared with other treatments. There also existed a trend of higher WSA (%) in the 0.5- to 2.0-mm aggregates. The lowest WSA (%) was observed in the 4.75-mm aggregate-size fraction. Soil receiving P3 fertilizer treatment had a greater proportion of WSA in the 0.25- to 4.75-mm aggregate and a lower proportion of this size fraction in other treatments (Table 6).

Total WSA ranged from 74.0% in control to 847% in P3. An increase of 115% in WSA occurred between P0 and P1 treatments. In addition to more retention of plant residues and root biomass, increase in hyphae and other soil biological factors may also have enhanced aggregate stabilization. Despite the strong trend, there were no significant differences in WSA among the P1, Ps and P3 treatments, probably because of the masking effect of the high clay content.

The MWD ranged from 1.36 mm in control to 1.81 mm in P3 treatments (Table 6). Despite lack of statistically significant differences in MWD (and WSA), there was a trend of a notable increase (133%) in MWD in the P3 compared with the control (Table 6).

In general, increasing SOC increases aggregation. In the present study, however, there were no significant differences in WSA among P1, P2, and P3 treatments, probably because of the masking effect of high clay content. Indeed, a higher proportion of total P (>50%) is associated with the clay fraction (Saroa and Lal, 2004). In China, Huang et al. (2010) also reported no significant differences in aggregate-size distribution among NPK treatments. In addition to greater retention of plant residues and root biomass, increases in hyphae and other soil biological factors may also have enhanced aggregate stabilization. Because WSA and MWD were not measured in 1998, it is not possible to assess evolution of WSA and MWD over time under different treatments. Yet, development of soil structure and the dynamics of WSA and MWD in many soils are often closely related to the cycling of SOM and the magnitude of root and mycorrhizae hyphae (Hallett et al., 2009; Rillig et al., 2010). In India, Hati et al. (2008) observed that application of balanced fertilizer along with manure (NPK+M) or lime (NPK+L) improved soil aggregation, soil-water retention, microporosity, and available water capacity and reduced bulk density of the soil in 0- to 30-cm depth than control.

Correlation Between Soil Aggregates and Carbon and Nitrogen Concentrations

The data in Table 7 show a significant correlation between soil ρ_b and total C and SOC concentrations ($R^2 = 0.6921$, $P < 0.0126$) and also a positive correlation between ρ_b and SOC pool ($R^2 = 0.7658$, $P < 0.0038$). However, these exists a weak correlation between ρ_b and the MWD and WSA ($R^2 = 0.549$, $P < 0.069$) (Table 7). The correlation between SOC concentration and f_t was significantly negative ($R^2 = -0.692$, $P < 0.012$). Yet concentration of TSN was positively correlated with the MWD ($R^2 = 0.765$, $P < 0.0037$). Also, MWD and TSN were significantly correlated with each other ($R^2 = 0.657$, $P < 0.02$).

Thus, there exists a strong correlation between SOC concentration and aggregation (Golchin et al., 1995). This hypothesis is also supported by the hierarchy theory of aggregation (Jastrow et al., 1998; Six et al., 2000b). However, increase in aggregation with increase in SOC concentration may not happen in all soils (Abiven et al., 2007), depending on the SOC concentration, clay mineralogy, texture, and other soil-related factors.

The effects of increasing P addition and other soil parameters on SOC, MWD, and other direct and indirect soil quality parameters were also assessed by the path analysis. This technique comprises the process of constituting the path diagrams that display the relations between the variables related with each other. Direct effects, indirect effects, and joint path coefficients are shown in Table 8. The P additions have higher effects on SOC, TSN, ρ_b, WSA, and MWD in standardized solution paths. However, this effect was not significant with t values analyzed in Table 8. The rate of P application had a direct effect on TSN and SOC concentrations, $\rho_{b,}$ SOC, and TSN pools. However, there was no direct effect of P application on WSA and MWD, and the indirect effect of P on SOC and WSA is higher than that on other parameters.

Application of P strongly impacted SOC and TSN concentrations and pools but weakly altered aggregation and related standard attributes.

CONCLUSIONS

Long-term application of phosphorus fertilizers significantly affected SOC, ρ_b, and f_t relative to the control. The data are also in accord with the proposed hypothesis and support the following conclusions:

(i) Application of long-term inorganic P fertilizers increased ρ_b and decreased f_t because of increase in soil compaction.
(ii) Concentration of SOC was significantly higher in soils receiving high levels of P fertilizers than control. Pools of SOC and TSN were also higher in soils receiving high P fertilizers than unfertilized control.
(iii) The annual rate of C sequestration was greater in soils treated with high P application than in unfertilized plots. However, the rate of C sequestration was much higher in soils receiving 200 kg ha^{-1} of P_2O_5 than in lower rates of P application.
(iv) Concentrations of SOC in aggregate-size fractions were significantly higher in soils receiving P2 and P3 fertilizer application than control. Soils treated with high P fertilizer contained more C and N concentrations in macroaggregates compared with microaggregates.
(v) Both WSA and MWD, although increased with increase in rate of P application, did not significantly differ among P treatments.
(vi) Concentration of SOC was positively correlated with soil ρb.

TABLE 7. The Correlation Matrix

	x1	x2	x3	x4	x5	x6	x7	x8	x9	x10	x11
x1	1										
x2	−0.17746	1									
	0.5811										
x3	0.98497	−0.34480	1								
	<.0001*	0.2724									
x4	0.59352	−0.74292	0.69651	1							
	0.0419*	0.0056*	0.0118*								
x5	0.54654	0.44429	0.44331	−0.33159	1						
	0.0660	0.1479	0.1489	0.2924							
x6	0.65497	−0.38402	0.69213	0.56946	0.21015	1					
	0.0208*	0.2178	0.0126*	0.0533*	0.5121						
x7	−0.65497	0.38402	−0.69213	−0.56946	−0.21015	−1.00000	1				
	0.0208*	0.2178	0.0126*	0.0533*	0.5121	<.0001*					
x8	0.97581	−0.36052	0.99402	0.70254	0.42878	0.76580	−0.76580	1			
	<.0001*	0.2496	<.0001*	0.0108*	0.1643	0.0037*	0.0037*				
x9	0.65684	−0.72540	0.75383	0.98582	−0.23662	0.69772	−0.69772	0.77327	1		
	0.0203*	0.0076*	0.0046*	<.0001*	0.4590	0.0116*	0.0116*	0.0032*			
x10	0.16132	−0.43373	0.23000	0.38770	−0.20379	0.54097	−0.54097	0.29307	0.44979	1	
	0.6165	0.1589	0.4721	0.2130	0.5252	0.0693	0.0693	0.3552	0.1423		
x11	0.39646	−0.60811	0.48490	0.74916	−0.32271	0.53999	−0.53999	0.51656	0.76531	0.65799	1
	0.2020	0.0359*	0.1101	0.0050*	0.3063	0.0699	0.0699	0.0855	0.0037*	0.0200*	

*Significant at $P < 0.05$.

X1: Total soil carbon %, X2: Inorganic Soil Carbon %, X3: organic C %, X4: nitrogen %, X5: C:N ratio, X6: bulk density, X7: porosity, X8: SOC Mg ha^{-1}, X9: TSN Mg ha^{-1}, X10: WSA %, x111: MWD mm.

TABLE 8. ML Path Analize Ethod (Unstandardized [Unst], Standardized (Std) Solutions, and *t* Values)

	γ			β																	
	P			**% N**			**% C**			**ρb**			**SOC**			**TSN**			**WSA**		
	Unst	**Std**	***t***	**Unst**	**Std**	***t***	**Unst**	**Std**	***t***	**Unst**	**Std**	***t***	**Unst**	**Std**	***t***	**Unst**	**Std**	***t***	**Unst**	**Std**	***t***
% N	1.02	0.62	2.19																		
% Organic C	1.33	0.80	3.86	0.23	0.23	1.17															
ρ_b	3.44	1.47	1.96	−0.18	−0.13	−0.45	−0.66	−0.47	−0.55												
SOC	1.56	0.95	5.40																		
TSN	1.22	0.74	2.97																		
WSA	3.44	2.09	1.58				−1.67	−1.68	−1.26												
MWD	0.53	0.33	0.04	0.29	0.30	0.54	−0.34	−0.35	−0.10	−0.11	−0.16	−0.04	−0.29	−0.30	−0.26	0.66	0.67	1.76	0.53	0.54	0.95

chi-square = 21.00, *df* = 11, *P* = 0.03334, RMSEA (root mean square error of approximation) = 0.302.

Application of phosphorus fertilizers increased soil aggregation and enhanced the rate and total amount of SOC sequestration.

Accurate measurement of changes in SOC concentration, in relationship to the amount of biomass C retained annually, remains to be a major challenge. Generally, measurement of SOC concentration is also affected by the presence of inorganic carbonates. There is a strong need for the development of standard methods for credible assessment of SOC in calcareous soils. Use of fertilizers and amendments must be done to alleviate nutrient imbalance, enhance SOC and TSN pools, and improve soil quality especially with regard to soil structure and aggregation. Also, fertilizer application should be related to sustainable management of soils in the context of projected climate change. More SOC needs to be sequestrated in the soil through biomass by using balanced fertilizers.

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