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Phosphorus distribution after three decades of different soil management and cover crops in subtropical region

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Keywords No-tillage; conventional tillage; P forms

Taxonomy Winter Cover Crops, Continuous Non-Tilled

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Abstract

The no-tillage system with normally comprise winter cover crops and crop rotation may increase the efficiency use of soil P and phosphate fertilizer. The objective of this study was to evaluate the effect of three decades of different soil management systems and winter cover crops on the fractions of P in a clayey oxisol of Paraná State, Brazil. The bi-factorial experiment with three replicates was installed in 1986. The main plots consisted of seven treatments of winter cover plants and in the subplots, two tillage systems were used: no-tillage and conventional tillage. The soil was sampled in the 0-10 cm soil layer in the post-harvest periods of the corn (harvest 2014-2015), the flowering of the winter cover plants (2015) and soybean flowering (harvest 2015-2016). The different pools of P were evaluated. In a short time of no-tillage adoption (30 years) with phosphate fertilizers addition, the amount of P organic pools and type of P organic compound were likewise to the natural biome. The no-tillage guaranteed higher inorganic and organic P, both rapid and moderately available, in comparison to the conventional tillage system. However, in the conventional tillage during (40 years - 80 plows + 160 harrows), despite the addition of 3 Mg ha⁻¹ of P₂O₅, the available and moderately phosphorus in soil surface were very similar to soil under natural forest. In the natural biome, the amount of P stored in soil microbial biomass was stable in the time showing a homeostatic equilibrium. Nevertheless, in cultivated soil and fertilized with inorganic phosphate, there was a synchronism between plant demand and storage in soil microbial biomass.

Key words: No-tillage; conventional tillage; cover crop; P adsorption/desorption; microbial biomass; natural forest

1. Introduction

World population growth is increasing demand for food (Bijl et al., 2017), which highlights the importance of the current stimulation of more efficient agricultural production systems. For this purpose, the main strategies are the intensification of the existing agricultural land and the expansion of cultivated areas to the detriment of native

vegetation. However, both strategies are detrimental to global biodiversity. Another option possible could be recovering soil of degraded pastureland. In Brazil, the new arable land available for cropland is restricted to the degraded pasture or natural biome, specially Cerrado biome, which predominate very old and weathered soils. Thus, at the beginning of the exploitation of these new agricultural areas, the soils need high P application rates in order to overcome their large P adsorption capacity. Beyond, the intensification of cultivated land suggests additional fertilizer application resulting in amounts of P twice times the plants' demands (Withers et al. 2018). In addition, natural reserves of phosphate fertilizers are limited and they are reducing all over the world (Elser et al., 2014). In this way, soil management in agricultural land needs to be improved by the better use of fertilizers. Soil management can alter the dynamics of P transformations in a beneficial way by better utilization of P in the soil or harmful way through losses of this nutrient and the exacerbated applications (Rheinheimer et al., 2008a). In the no-tillage system (NT) it is recommended to keep the straw on the soil surface, providing the accumulation of organic matter. The presence of straw results in the increase of microbial activity and in greater mineralization of nutrients such as P (Corbeels et al., 2016), making readily available nutrients for plant roots uptake.

Another way to change the dynamics of soil P is growing different plant species in a consortium (mixing) and/or singles (Soltangheisi et al., 2018). This is due to the different strategies of obtaining P by the distinct species and to the lability of P in the forms accumulated in their tissues (Tiecher et al., 2012a, 2012b; Horst et al., 2001). The main strategies used by plants to intercept and absorb P are: (i) the increase in the ratio between root and shoot, (ii) the increase of root bristle in quantity, shape or thickness to ensure greater exploration of soil volume, (iii) the root exudation of the acid phosphatase enzyme or low molecular weight organic compounds which, through binder exchange reactions or occupation of the adsorption site, promote the mobilization of P and also (iv) through association with other microorganisms (Chavarría et al., 2016; Zhang et al., 2014). In addition, cover crops may have a regulatory effect on the enzymatic activity related to mineralization of soil organic P (Po). For example, radish (*Raphanus sativus* Mill.), a crop with large root excretion of malic acid, can significantly increase the availability of P in the soil solution (Pavinato and Rosolem, 2008).

Therefore, the rotations of different cover crops with different strategies to access P from the soil, especially with well-developed root systems, can increase soil P availability, changing the P cycle mainly in the long-term (Soltangheisi et al., 2018, Damon et al., 2014).

This effect can be optimized when the plant residue is maintained in the soil surface, such as in NT system (Rheinheimer et al., 2008a). Accordingly, to the soil management and plants physiological adaptations, the utilization efficiency of P added via fertilizer can be improved (Teles et al., 2017). The consequence of a more efficient use of P that is already in the soil is the reduction of its wide application in agricultural land, resulting in a more sustainable agriculture. Another important factor in crop management and rotation is microbiological activity since the P cycle in the soil is mainly controlled by microbial activity (Yevdokimov et al. 2016). The microorganisms are responsible for the storage of P through the immobilization inside their tissues, avoiding P adsorption in the inorganic colloids of the soil. By the senescence, death and cellular lysis of these organisms, P becomes part of the P_o of the soil, being mineralized and available to the soil solution and then uptake by the plant roots (Bünemann, 2015; Darch et al., 2014; Martinazzo et al., 2007; Conte et al., 2002). Thus, changes in P_o availability are also susceptible to soil management, especially in the long-term.

It is commonly known that continuous addition of cover crops residues in the long-term on the soil surface of no-tilled soils in succession to commercial crops markedly changes the magnitude of soil chemical and biological processes (Pavinato et al., 2017, Damon et al., 2014, Tiecher et al., 2012a 2012b; Wutke et al., 2014). However, long term studies about these changes are still scarce, especially in consolidated crop systems. Recently Haygartha et al. (2018) in their perceptive paper “On the history and future of soil organic phosphorus research: a critique across three generations” affirm that the “Soil organic phosphorus has broad agronomic and ecological significance, but remains a neglected topic of research”.

The objective of this study was to compare the P distribution in subtropical soils cultivated three decades under different soil tillage systems and winter cover crops.

2. Material and methods

2.1. Study site and experimental design

The experimental area is located at the Experimental Station of Paraná Agronomic Institute - IAPAR (52° 41' W, 26° 07' S, 700 m asl), in Paraná state, Brazil. The relief in the area is smooth wavy with slope ranging from 4 to 7%. The soil of the region was formed from basaltic and is classified as very acid Oxisol (clayey Rhodic Hapludox) (70% clay, 15% silt and 15% sand). The soil mineralogy is composed of 130 g kg⁻¹ of 2:1 clay minerals, 680 g kg⁻¹

¹ of 1:1 clay minerals (kaolinite and halloysite), 140 g kg⁻¹ of iron oxides (51% hematite, 36% goethite and 13% magnetite) and 50 g kg⁻¹ of gibbsite (Costa, 1996). According to the classification of Köppen, the climate of the region is Cfb subtropical humid with average annual precipitation ranging from 1,200 to 1,500 mm and minimal and maximal mean annual temperature of 15.6 and 25.7 °C. Variation of temperature and precipitation from October 2014 to March 2016 is presented in Figure 1.

In the summer of 1976, the native forest was cleared to grow corn (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.). Both species were cultivated for 10 years under conventional tillage (CT) with plowing that resulted to intensive soil erosion, soil degradation and decrease of soil quality. In 1986, the experiment was set up with a randomized block design, in a factorial system of subdivided plots (bi-factorial), with three replications. The winter cover species were allocated to the main plots (20 × 12 m) and the subplots (20 × 6 m) with the soil preparation systems of CT and NT. The treatments of the main plots consisted of seven winter cover crops: ryegrass (*Lolium multiflorum* L.), rye (*Secale cereal* L.), common vetch (*Vicia sativa* L.), consortium of hairy vetch (*Vicia villosa* Roth) and black oat (*Avena strigosa* Schreb), radish (*Raphanus sativus* Mill.), wheat (*Triticum aestivum* L.) and fallow. During the summer, the whole area was cultivated with soybean (*Glycine max* L.) or corn (*Zea mays* L.) (Table 1). Soil under CT continued to be prepared with one plowing and two disc-harrowing, before each cultivation.

All treatments received the same amounts of limestone, nitrogen (N), phosphorous (P) and potassium (K), according to the recommendation of IAPAR (2003). The nutrients P and K and one third of N were applied in the line during the sowing, and the remaining N was applied 45 days after sowing. A total of 2,020 kg ha⁻¹ of P₂O₅, 995 kg ha⁻¹ of K₂O and 668 kg ha⁻¹ of N were applied from 1986 to 2016. In addition, between 1976 and 1986, approximately 1,000 kg ha⁻¹ of P₂O₅ was added in the experimental area. The last two lime applications were performed in 2011 and 2015, using a rate of 2 Mg ha⁻¹ (Table 1). The herbicides were used to control weeds in the fallow and, in some cases, to complement the thinning of the aerial part of the winter cover crops. Wheat was harvested for grain until 1995 (7 crops) and after that was managed as a cover crop (Table 1).

During the 30 years of the experiment, the dry matter of the aerial part of the cultivated plants was evaluated during the winter and summer (Table 2). In the fallow treatment, without annual crop cultivation in the winter period, the biomass yield represents weeds and summer crops.

2.2. Soil sampling and physical-chemical characterization

The soil was sampled in three periods: (i) after the corn crop cycle (March 2015); (ii) in the flowering of the winter species (September 2015); (iii) in the flowering of soybean (February 2016). Soil sampling was performed in triplicate in the 0-10 cm soil layer using a cutting blade. In addition, “reference” soil samples were taken in an adjacent area with native forest. After sampling, the soil was packed in polystyrene boxes, stored at 4°C, and transferred to the laboratory, where it was sieved with a 185 µm mesh for analysis.

The total N content was obtained through digestion, according to the methodology proposed by Tedesco et al. (1995). Total organic carbon (TOC) was determined by the Mebius method in the block (Rheinheimer et al., 2008b). The exchangeable K was extracted by Mehlich-1 (0.05 N HCl and 0.025 N H₂SO₄) solution and determined by atomic emission spectrometry. The exchangeable calcium (Ca²⁺), magnesium (Mg²⁺) and aluminum (Al³⁺) cations were extracted with 1 mol L⁻¹ KCl. Aluminum was determined by titration with NaOH 0.0125 mol L⁻¹ and Ca and Mg by atomic absorption spectrometry (Tedesco et al., 1995).

The pH value was determined in water (soil:solution ratio 1:1) and the potential acidity (H + Al) was estimated using the TSM method (37.5 mM triethanolamine; 17.3 mM deimidazole; 30.7 mM 2-(N-morpholino) ethanesulfonic acid; 20.7 mM calcium acetate and 721 mM calcium chloride, with adjusted pH at 7.5) (Santa Maria Buffer - Toledo et al., 2012). The sum of bases (S) was determined by the sum of the K⁺ + Ca²⁺ + Mg²⁺ cations. The effective CEC (CEC_{effective}) was calculated by the sum of S plus the concentration of Al³⁺. The cation exchange capacity at pH 7.0 (CEC_{pH7}) was calculated by the sum of (H⁺ + Al³⁺) + Ca²⁺ + Mg²⁺ + K⁺. The base saturation (V) was calculated using the equation: $V (\%) = ((S / CEC_{pH7}) \times 100)$. The aluminum saturation (m) was calculated by the equation: $m (\%) = ((Al^{3+} / CEC_{effective}) \times 100)$. The specific surface area (SSA) of the soil was determined by the BET isotherm technique using Quantachrome equipment, model NOVA 3200E. All these parameters are presented in Table 3.

2.3. Phosphorous distribution

2.3.1. P stored in soil microbial biomass

The P stored in soil microbial biomass (P_{SMB}) was estimated by the fumigation-extraction method (Hedley et al., 1982; Brookes and Powlson, 1982; Morel et al., 1996). For

this purpose, 0.500 g of soil were weighed in four sets of tubes: the first set (PA) received the fumigation treatment on a saturated surface of chloroform in a vacuum oven for 24 hours (25 °C); the second (PB) did not receive any treatment; the third set (PC) received a solution with 50 mg L⁻¹ of P, together with the extractor to determine the adsorption capacity of P by the soil; the latter set was used to determine the soil moisture. The extraction of P from the three sets was done with 0.5 mol L⁻¹ NaHCO₃ at pH 8.5. After extraction, the samples were centrifuged at 2500 × g for 15 minutes and filtered through (pore size) filters to remove particulate matter. An aliquot of 3,0 mL of the filtered extract was taken and 5 mL of 7% solution of ((NH₄)₂S₂O₈) (w/v) and 0.5 mL of 1:1 (v/v) H₂SO₄ solution were added. Afterward, the samples were submitted to digestion in an autoclave for 2 hours, at 1 atm and 121°C, according to USEPA (1971). The determination of the P concentration present in each soil sample set was performed according to Murphy and Riley (1962). The content of P stored in the SMB expressed in relation to the dry soil was estimated by:

$$P \text{ stored in the SMB} = \frac{50 \times (PA - PB)}{0.40 \times (PC - PB)}$$

2.3.2. Available P

For the other analyzes of P fractions, the soil collected was oven dried at 55 °C, ground with a roller and sieved with a 2 mm mesh. The available P was extracted by the method of Mehlich-1 (Mehlich, 1953) and by the anion exchange resin - AER (plates AR 103 QDP 434, Rheinheimer et al., 2003). The P present in the extracts was determined according to Murphy and Riley (1962).

2.3.3. Moderately available P

Moderately available P was estimated by using a 0.5 mol L⁻¹ NaOH extraction by adding 10 mL to 0.5 g of soil followed by agitation for for 16 hours in an over and over agitator at 33 rpm, with subsequent centrifugation at 3000 × g for 15 minutes. Inorganic P (P_{NaOH}) was determined by the Dick and Tabatabai (1977) method. The total P extracted by NaOH (P_{TNaOH}) was estimated after digestion of 3 mL of extract in an autoclave following the procedure presented in section 2.3 using the USEPA method (1971) and quantifying P by Murphy and Riley (1962) method. Subsequently, P_{ONaOH} was obtained by the difference

between $P_{T_{NaOH}}$ and $P_{i_{NaOH}}$. The organic fraction ($P_{O_{NaOH}}$) is represented by monoester phosphates, predominantly (Rheinheimer et al., 2002).

2.3.4. Total organic and inorganic P

The $P_{O_{total}}$ content in the soil was estimated by calculating the difference between the P extracted from a sample ignited at 550 °C for two hours inside muffle furnace and the non-ignited sample. For both treatments, 0.25 mol L⁻¹ H₂SO₄ was used (Olsen and Sommers, 1982). P_{total} was quantified using 0.05 g of soil mineralized with concentrated sulfuric acid (H₂SO_{4(conc.)}) and a solution of saturated MgCl_{2(sat.)} and (1: 7.5 - v / v) at 200 °C for 1.5 hour. After cooling, 3 ml of H₂O₂ was added and again heated at 100 °C for 1 hour. P was quantified by Murphy and Riley (1962) method. $P_{i_{total}}$ was determined by the difference between P_{total} and $P_{O_{total}}$.

2.4. ³¹P-NMR analysis

Liquid-state ³¹P-NMR was used to identify the main form of P..... The analyses were performed for two replicates of natural forest and common vetch winter cover crops (no-tillage and conventional tillage). The phosphorus was extracted with 50 mL centrifuge tubes with 40 mL of 0.25 mol L⁻¹ NaOH + 50 mmol L⁻¹ EDTA (Turner et al., 2005). We used duplicate extraction of 4 g of dried soil samples. The tubes were agitated for 4 h on an end-over-end shaker at 20°C and centrifuged at 3,000 x g for 15 minute. The extract of one subsample was used to estimate total and organic P, Ca, Mg, Fe, and Al concentration and the second was frozen and lyophilized. Subsequently, the lyophilized extract was dissolved into 2.7 mL of 0.25 mol L⁻¹ NaOH + 50 mmol L⁻¹ EDTA and 0.3 mL D₂O was added and the mixture vortex-stirred for 5 min. After 120 minutes of contact, the supernatant was separated by centrifugation (3,000 × g for 15 min), filtered (<0.45 μm) and transferred to 10 mm NMR tubes. The P spectra were obtained in a Bruker Advance DPX 400 spectrometer at a frequency of 162 MHz with proton decoupling. A pulse angle of 90° was used at 20°C an acquisition time of 0.5 and 3 s relaxation time. The number of scans was of 23,568 to 31,600. The peak area was determined by electronic integration at a baseline of 1 Hz.

The MestRe-C v 2.3a software was used for peak area integration. The chemical shifts of 7.49 ppm for orthophosphate, 6.33 to 5.31 ppm for monoester, -0.81 to -0.69 ppm for diester, -2.55 to 3.59 ppm for pyrophosphate, and 9.35 ppm for adenosine diphosphate

(Turner et al., 2003; Cade-Menun, 2015). Total inorganic P was obtained from the sum of NaOH EDTA extractable inorganic orthophosphate and pyrophosphate, while total organic P was the sum of NaOH EDTA extractable orthophosphate monoesters, diesters and adenosine diphosphate.

2.8. Statistical analysis

The statistical model adopted was a tri-factorial, where the winter crops were the main plots, the soil tillage systems the subplots, and the sample time, the sub-subplots. Statistical analysis was carried out using ANOVA. Mean of treatments were tested for level of significance at 5% probability of error. When the effects of the treatments were significant, the differences between winter crop media, soil preparation systems, and soil collection times were compared by the Tukey test (Table 4). The values for microbial P and Mehlich P did not follow a normal distribution and were presented in scale.

3. Results and discussion

3.1. Consequence of the conversion of a natural forest to an agroecosystem

The content of P stored in soil microbial biomass (Table 5) in the natural forest was approximately eight times higher than the amount of available P extracted by Mehlich 1 or resin extractors (Table 6). This is a normal behavior in the subtropical natural biome (Martinazzo et al., 2007). Although P_{SMB} is considered one of the soil insoluble P forms, it can be considered as a source of P potentially available for plant nutrition (Blackwell et al., 2010; Darch et al., 2014). After thirty years of NT system, the P_{SMB} content (mean = 47.9 mg kg⁻¹) was higher than in the natural forest (mean = 40.3 mg kg⁻¹). The absence of soil disturbance and sufficient P input rate promote high crop grain yield, adequate environment for soil microbiota development and slow and constant organic matter increase. Biotic processes such as microbial immobilization, remineralization of immobilized P and non-microbial Po mineralization by soil microorganisms have great relevance for P cycling in soil (Bünemann, 2015). In contrast, in CT system, with the same rate of P inorganic fertilization, the P_{SMB} content was only 24.7 mg kg⁻¹ (mean). This represent only 52% and 61% of the amount of P_{SMB} accumulated in soil under NT and under natural forest, respectively.

The study of P distribution highlights that three decades of agricultural practice can significantly modify its distribution. Thus, under natural forest, 33.6 % of P_{total} is in organic form (436 mg kg^{-1}) and under cultivated soil the organic P account for 30.2 % (371 mg kg^{-1}) and 30.3% (463 mg kg^{-1}) of total P in CT and NT, respectively (Table 6). The same tendency was observed for the moderately labile organic P (389 , 364 and 277 mg kg^{-1} , for natural forest, NT and CT, respectively). The soil mobilization by plow may decrease slowly the soil organic carbon and, consequently, promote the mineralization of organic P. After soil degradation, it is more difficult to recover the original amount of soil organic carbon observed in the natural biome. In this case, a very clayed soil, ten years of inadequate soil use and management (deforestation followed by CT system from 1976 to 1986) need 30 years (1986 – 2016) of adequate and high productions system for recovery the similar soil quality. The intensive soil mobilization reduced the soil organic carbon and nitrogen stocked in soil biomass microbial, the polysaccharide content and phosphatase activity, in relation to NT system (Balota et al., 2014).

Only 4.6 mg kg^{-1} of the $1,298 \text{ mg kg}^{-1}$ of total P was available in natural forest due to the adsorption on the minerals with 680 mg kg^{-1} of 1:1 clay mineral and 190 mg kg^{-1} of iron and aluminum oxides. These results highlight that soil P solubility is essentially controlled by sorption/desorption and mineralization/immobilization process. The organic carbon accumulated in soil surface (3.6 %) (Table 2) and constant low-molecular organic acids (Hou et al., 2018) production by microbial activity can be uphold bioavailable pool of “biological P” (biomass microbial and plant residues) and the plant demand is more biological than chemical dependent. Secondary and not negligible are the root and microbial phosphatase enzymes production and mycorrhizal symbiosis (Wang et al., 2017) and trees high P-resorption efficiency (Tsuji et al., 2017). After Walker and Syers (1976) study, it is admitted that in soils with very advanced stage of weathering, soil organic carbon cycling generally control the biological availability of P. Recently, Huang et al. (2017) showed that all soil chronosequence under natural aerobic conditions, soil organic P was dominated by phosphate monoesters (inositol phosphates), followed by phosphate diesters and phosphonates. However, the monoesters are very strongly adsorbed and their bioavailability is very limited (Turner et al., 2005).

The liquid-state ^{31}P -NMR analyses showed four P compound groups in natural forest and no-tillage system (orthophosphate, monoester, diester and adenosine diphosphate). In the CT, adenosine diphosphate was not detectable (Table 7). The ratios monoester/diester were 5.9, 8.5 and 7.4 for forest, NT and CT, respectively. The organic P compounds groups were

responsible to 46.4%, 33.7% and 40.9% of total P present in NMR extract for forest, NT and CT, respectively. Despite the higher absolute value, the lower percentage of organic P in NT in relation of CT is due higher available and moderately available inorganic P (Table 6 and 7) from inorganic fertilizer added in soil surface and no soil mobilization. The adequate soil management and P addition in subtropical conditions guarantee high plant production, high microbial activity and diversity of organic P similar to the natural forest.

However, the representatively of total organic P in liquid-sate ^{31}P -NMR extract is doubtful. Only --.% of total P was extracted by NaOH-EDTA. This value is much closed to observed for tropical soils (37% - Turner, 2008) and forest soil (34% - Cade-Menun et al., 2002). In the natural forest soil approximately --.% of total organic P was extracted; in contrast more than --.% in cultivated soils. Therefore, the NaOH-EDTA extractor has a selective capacity of organic P extraction and can compromised the liquid-sate ^{31}P -NMR results. Aleixo (2018) showed that organic P extracted by NaOH-EDTA was less than 10% of total P in tropical soil in natural forest and cocoa agroforests production. The amount of total P extracted depend of anthropic P addition, and is recovery in inorganic pools (18, 23 and 44% for natural forest, cacao without P fertilizer and cacao with P fertilizer, respectively). Doolette et al. (2011) observed similarly results in Australian soils.

3.2. Effect of a soil tillage practice

After 30 years of adequate soil management adoption for subtropical conditions (NT, cover crops and regular fertilization) the P stored in soil microbial biomass was twice as the CT (Table 5). In the NT system, 240.7 Mg ha^{-1} of shoot plant residues was deposited in soil surface during the last three decades. Considering that root/shoot biomass is of 46% for corn and 55% for cover crop (Austin et al., 2017), the biomass input was nearly twice. The minimum soil disturbance promoted more stable and diverse microbial activity and high C, N, S and P turnover in relation to the CT system. The high organic carbon (Table 2) in soil surface make possible the incorporation of P in organic pools, especially in moderately bioavailable form extracted by NaOH (Rheinheimer and Anghinoni, 2006) and in P_{SMB} (Conte et al., 2002; Rheinheimer et al., 2008a; Damon et al., 2014; Mackay et al., 2017). The P immobilization by the SMB delays the specific adsorption with high binding energy of P by the soil clay minerals (Martinazzo et al., 2007; Rodrigues et al., 2016; Zamuner et al., 2008). Then, after lysis of the microbial cells and P mineralization, the P inorganic is released into the soil solution and can be uptake by plants and microorganisms.

The amount of moderately bioavailable organic P in NT was 31.4% higher than in the CT (364 and 277 mg kg⁻¹, respectively - Table 5). This organic P pool is constituted mainly by orthophosphate monoester (Figure 2). They are strongly adsorbed by ligand exchange at surface functional groups of iron and aluminum oxides and kaolinite (Turner et al., 2005) forming inner sphere complexes, which hinders its mineralization by microorganisms (Pavinato and Rosolem, 2008) and then accumulate in soils in the long-term (Jian et al., 2017). These authors showed that steady state for P organic monoesters were 113 and 91 years in paddy and no-paddy soils in China, respectively. In our case, we can consider that the steady state for moderately bioavailable organic phosphorus (389 mg kg⁻¹) in natural forest occur in the long-term because the natural forest was established around 20 thousand years ago. In a short-time of NT adoption with phosphate fertilizers addition (only 30 years), the amount of this P organic pools is likewise to the natural biome.

The Pi moderately bioavailable was also higher in NT than CT (211 and 127 mg kg⁻¹, respectively). In NT system, the inorganic P added by fertilizers was partially adsorbed with moderate energy contrasting with intensive soil mobilization (CT). In this case, CT, the Pi moderately bioavailable was lower than accumulated in the natural forest. The soil plowing exposes the P in the soil solution to new active adsorption sites present in the inorganic colloid surface, especially in hydroxyl groups coordinated by one Fe atoms or in the Al-OH functional groups at the edges of kaolinite. In the goethite surface, the P can be formed binuclear inner sphere complexes in monocoordinated hydroxyl groups (Barrón and Torrent, 1996; Parfitt, 1989). However, in NT system the dissolution of fertilizer is restricted to a small volume of soil around fertilizer granule and the functional groups are quickly saturated and the binding energy of P with the soil decrease (Parfitt et al., 1975). The new phosphate added will then be less adsorbed because the soil is already saturated (Barrow, 1998). Additionally, the higher organic carbon content recovers the surface of clay minerals and oxides (Kleber and Johnson, 2010) decreasing their reactivity.

In more sustainable soil management for tropical and subtropical conditions regions, like no-tillage system, the protection of soil surface by plant residues decreases the fluctuation of water content and the average soil temperature (Blanco-Canqui and Ruis, 2018). The biologic activity is more stable in the time and the rate of organic carbon mineralization is lower than in conventional tillage system (Calegari et al., 2008). Similarly, to a natural biome, there are constant and dynamic low-molecular organic acids production that can compete with active sites of the soil inorganic colloids, reducing the adsorption of P on their surface (Fink et al., 2016).

The available inorganic P (P_M and P_R) contents were higher in the soil under NT in relation to the CT soil (Table 6). The value of available P in CT is lower than the critical level for grain production (9 mg kg^{-1} - SBCS/NEPAR, 2017). Even after the addition of almost 3 Mg ha^{-1} of P_2O_5 in the last 40 years of cultivation (80 plows + 160 harrows), the available P in soil surface (0-10 cm) of CT was very similar to the soil under natural forest (Table 6). The P added via inorganic fertilizer is redistributed and diluted in the plowed layer (0-20 cm - Tiecher et al., 2017; 2012a) and it is adsorbed in inorganic colloid surface by inner-sphere complex. The predominance of inorganic forms and unavailable pools are related to high concentrations of hematite, goethite and 1:1 clay-minerals in the soil (Table 2).

Using the same P fertilizer rates, the soybean and corn production with CT is 6% and 11% lower than in NT system (Calegari et al., 2008; 2012). So, the different soil management systems affect the dynamics of P pools and their bioavailability to crops. Changes in P pools after 30 years of fieldwork indicate that the results can be interpreted as "laws" (general trend) rather than just as punctual results. These changes may not be detected in the short-term as they affect the redistribution of P in the soil gradually.

3.3. Effect of winter cover crops on soil phosphorus forms

Soils continuously under fallow with spontaneous vegetation in the winter presented the lowest levels of P stored in soil microbial biomass (mean = 28 mg kg^{-1}) for the three sampling time compared to soils with cultivated species (mean = 39 mg kg^{-1}), except for wheat (Table 5). Total aboveground biomass yield during 30 years in fallow was 58.0 and 44.7 Mg ha^{-1} in NT and CT system, respectively. When winter cover crops were cultivated the values were increased up to 119.5 and 86.1 Mg ha^{-1} , in NT and CT system, respectively. Furthermore, the lower soil surface covering in fallow treatment expose the soil to erosion and fluctuation of soil water content and temperature, as well as lower effects of winter crops (shoot and roots), contributed to decreases the microorganism activity.

For the others P pools, regardless its nature (organic or inorganic) or degree of bioavailability, the winter cover crops effect was despicable or absent, compared to winter fallow effects (Table 6). The pools of organic and inorganic moderately available P, and, organic total P in soil cultivated with radish were lower than others winter cover plants. Radish is a non-mycorrhizal plant, with higher activity of the acid and alkaline phosphatase enzymes in the soil, promoting a greater mineralization of organic P (Dalla Costa and Lovato, 2004). Due to its main and aggressive central root system and/or due to the high

decomposition rate of its dry biomass (Loss et al., 2015), radish may have contributed to the distribution and recycling of P in depth and decreasing its concentration in the less labile pool in the superficial layer. The soil cultivated with ryegrass has less total inorganic P and total P in relation to others winter plants.

It should be noted that only 19 of the 30 winter's seasons were grown with the different winter cover crops. In the other eleven years, all treatment, except fallow, was cultivated with the same species, especially, black oat (Table 1). Moreover, in spring/summer all treatments were cultivated with the same culture (corn or soybean). Approximately 54 and 57% of total residues was originating from maize + soybean (Table 2). The plant type effect in soil P dynamic is small or unclear comparatively to soil management system. Much contradiction about the effects of cover crops on microbial community and activity in bulk soil and rhizosphere is reported in the literature (Ridder-Duine et al., 2005). Marschner et al. (2001) supported that bacterial community arrangement is the result of a complex plant and soil interaction.

3.4. Effect of soil sampling time on soil phosphorus forms

In the natural biome, the P stored by SMB oscillated to 38 from 45 mg kg⁻¹ between the sampling times (Table 5). The “mature” subtropical natural forest is a system in homeostatic equilibrium. The P in plant biomass, litter and soil microbial biomass are connected by microbial activity (Solomon et al., 2002), but the stock of P_{SMB} is very stable over time. In contrast, in perturbed natural biome, the fluctuation of P_{SMB} content is very large and linked to root / shoot alteration (Rheinheimer et al., 2008a). In very clayey, highly weathered acidic soils, which severe P scarcity, the plant–microorganism interaction and trees high P-resorption efficiency are very developed. Richardson and Simpson (2011) stated that extension or stimulation of root growth and/or direct physiological and metabolic were the mechanism of microbial-mediated plant growth promotion. The nutrients stored in SMB are an intermediate pool between litter decomposition and plant absorption. The mineralization and immobilization process of P due to microbial activity occurs rapidly and close to the roots (rhizosphere), allowing only a small fraction of P to be adsorbed by Fe and Al oxides (Vincent et al., 2010).

In the agroecosystem, the sampling time has high impact in the P stored in soil microbial biomass, but lower effect in the other P pools (Table 5 and 6). This pool is a highly dynamic and significant changes occur in response to the environment, especially when

inorganic soluble P fertilizers are applied. During flowering stage of winter cover plants and soybean, the P_{SMB} was lower than detected in soil after corn harvest (Table 5). Thus, in cultivated soil and fertilized with inorganic phosphate, there is a synchronism between plant demand and soil microbial biomass storing (Martinazzo et al., 2007). When the P plant demand is high, the root system and its association (mycorrhiza, e.g.) increase the P absorption from soil solution. The P depletion in rhizosphere amendment many chemical and biological process to guarantee the plants requirement. Consequently, there is an increased acid phosphatase activity (Allison et al, 2007; Dalla Costa and Lovato, 2004), promoting the hydrolysis of organic phosphates, especially the diester (Klose and Tabatabai, 1999), and the P_{SMB} is partially mineralized.

After the reproductive stage is reached, plants go into the senescence and death. They drastically reduce P absorption until it completely ceases. Concomitantly, there is release of large amount of exudates in the rhizosphere. Finally, the whole the corn straw fragmentation that is deposited on soil surface, stimulate microbiological activity and thus immobilize large amounts of P into microbial biomass (Table 5). Both biological and geochemical processes of phosphate immobilization / adsorption were rapid (Olander and Vitousek, 2004). Therefore, the immobilized P in SMB is a labile pool that is protected from reactions with inorganic soil colloid, but temporarily unavailable to plants. When the winter cover crops, cultivated in succession to corn, have a demand for P, the rate of release of microbial biomass will be higher and the turnover time is very short. These results are in agreement with Olander and Vitousek (2004), who concluded that in a tropical forest “microbes may play an important role in controlling P partitioning into biological versus geochemical sinks even in soils that have high sorption capacity”.

4. Conclusion

The use of no-tillage system and adequate rate of phosphorus inorganic fertilizer for 30 years allowed the increase phosphorus stored in soil microbial biomass, recovering the amount stored in natural forest. In a short time of no-tillage adoption with phosphate fertilizers addition, the amount of phosphorus organic pools is likewise to the natural biome. The no-tillage guaranteed higher inorganic and organic phosphorus, both rapid and moderately availability, in comparison to conventional system. The phosphorus not exported by the grain harvested, it was accumulated in the moderately labile and non-labile pools. However, in the conventional tillage during 40 years with 80 plows + 160 harrows, despite

the addition of 3 Mg ha⁻¹ of P₂O₅, the available and moderately phosphorus in soil surface was very similar to soil under natural forest.

In the natural biome, the amount of phosphorus stored in soil microbial biomass was stable in the time showing a homeostatic equilibrium. Nevertheless, in cultivated soil and fertilized with inorganic phosphate, there was a synchronism between plant demand and stocking in soil microbial biomass.

Soils continuously with spontaneous vegetation in the winter (fallow) presented the lowest levels of phosphorus stored in soil microbial biomass compared to soils cultivated with winter cover crops, except for wheat. For the others phosphorus pools, regardless its nature (organic or inorganic) or degree of bioavailability, the winter cover plants effect was despicable or absent, compared of winter fallow.

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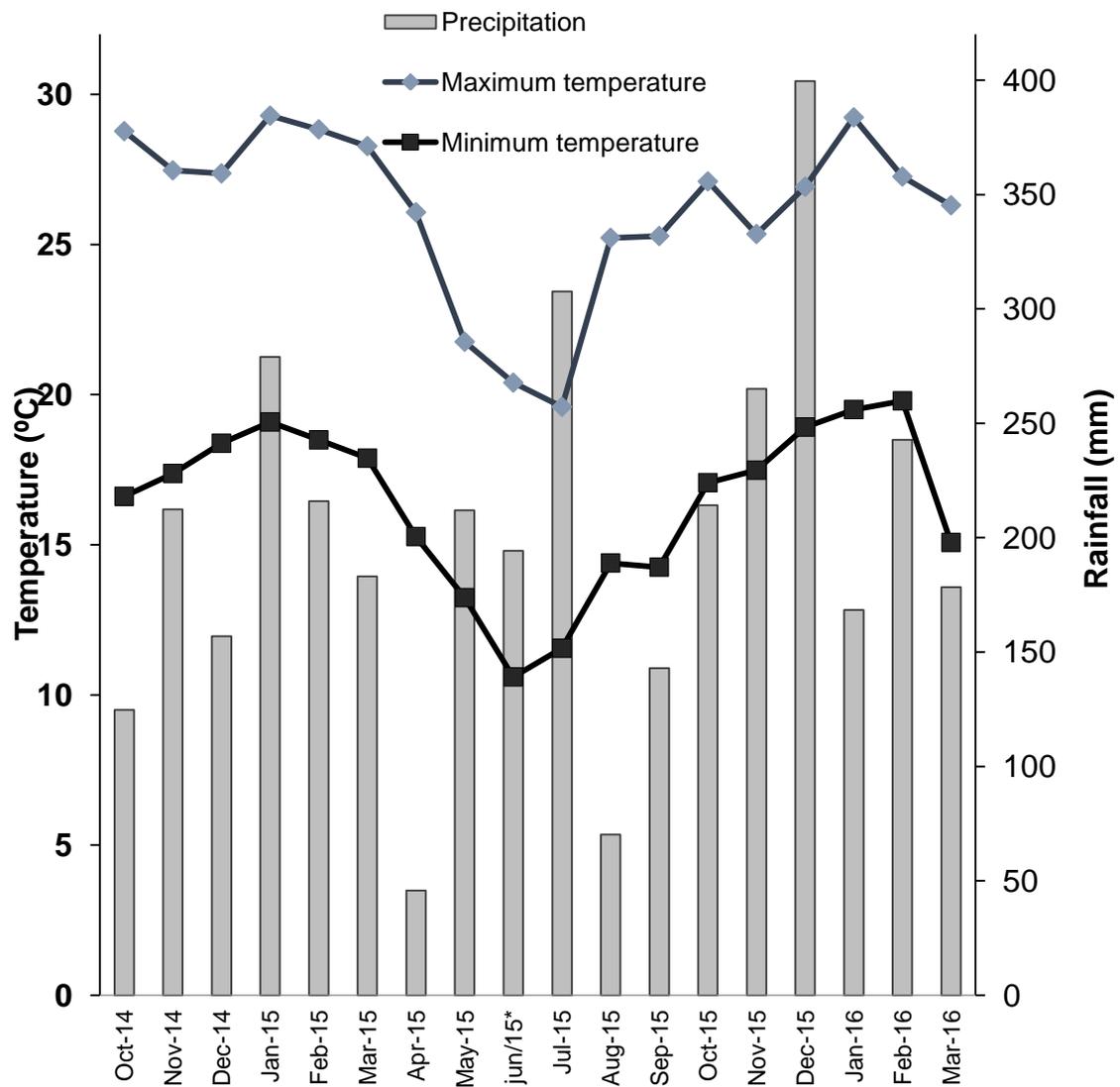
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* Average of the last 30 years. Source: IAPAR Weather Station

Figure 1. Total rainfall (mm) and averages of the maximum and minimum temperature (°C) monthly from October 2015 to March 2016 in Pato Branco – PR - Brazil.

Table 1. Winter cover species, summer species and rate of limestone application in the last 30 years in a very clayey Rhodic Hapludox located in Southwest of Paraná - Brazil.

Year	Winter cover species	Summer species	Limestone ¹
1986	All species	Corn	-
1987	All species	Corn	-
1988	All species	Corn	-
1989	All species	Soybean	1.0 Mg ha ⁻¹
1990	All species	Soybean	-
1991	Black oat in all plots (except fallow)	Soybean	-
1992	All species	Corn	2.0 Mg ha ⁻¹
1993	Fallow in all plots	Soybean	-
1994	All species	Corn	-
1995	Black oat in all plots (except fallow)	Soybean	3.0 Mg ha ⁻¹
1996	Black oat in all plots (except fallow)	Corn	-
1997	Black oat + radish in all plots (except fallow)	Soybean	-
1998	Black oat in all plots (except fallow)	Soybean	-
1999	All species	Corn	1.5 Mg ha ⁻¹
2000	All species	Soybean	-
2001	All species	Soybean	2.0 Mg ha ⁻¹
2002	Black oat + radish in all plots (except fallow)	Soybean	-
2003	Black oat + radish in all plots (except fallow)	Corn	-
2004	Black oat + radish in all plots (except fallow)	Soybean	-
2005	All species	Soybean	-
2006	Black oat in all plots (except fallow)	Fallow	2.0 Mg ha ⁻¹
2007	All species	Soybean	-
2008	All species	Corn	-
2009	Black oat + radish in all plots (except fallow)	Soybean	2.0 Mg ha ⁻¹
2010	Black oat + vetch in all plots (except fallow)	Corn	-
2011	All winter species	Corn	2.0 Mg ha ⁻¹
2012	All winter species	Corn	-
2013	All winter species	Soybean	-
2014	All winter species	Corn	-
2015	All winter species	Soybean	2.0 Mg ha ⁻¹
2016	All winter species	-	-

¹ Superficial in no-tillage system and incorporated into the conventional tillage

Table 2. Aboveground biomass yield of the plants grown during 30 years, under no-tillage system (NT) and conventional tillage (CT) under clayey Rhodic Hapludox in the experimental area located in the Southwest region of Paraná

Winter species	winter residues		summer residues		cumulative		annual mean	
	NT	CT	NT	CT	NT	CT	NT	CT
	----- Mg ha ⁻¹ -----							
Vetch	106.0	92.6	136.4	122.5	242.3	215.1	8.1	7.2
Wheat	112.8	103.0	124.8	118.5	237.6	221.5	7.9	7.4
Ryegrass	114.1	110.0	126.8	121.6	240.9	231.5	8.0	7.7
Rye	124.6	103.8	133.1	123.9	257.6	227.7	8.6	7.6
Radish	119.5	96.4	136.2	131.2	255.6	227.6	8.5	7.6
Hairy vetch + Black Oat	140.2	113.7	128.1	127.4	268.3	241.1	8.9	8.0
Fallow ¹	58.0	44.7	124.8	122.5	182.7	167.2	6.1	5.6
Average	110.7	94.9	130.0	123.9	240.7	218.8	8.0	7.3

¹ Considering the dry material of the weed overwinter in winter

Table 3. Physical and chemical properties of a clayey Rhodic Hapludox in the 0-10 cm layer under no-tillage system (NT) and conventional tillage (CT) during 30 years.

Parameter	Unit	CT	NT	Forest ¹
pH		5.0 b	5.2 a	4.5
N	%	0.2 b	0.3 a	0.3
TOC		2.7 b	3.5 a	3.6
K		0.23 a	0.28 a	0.13
Ca		3.3 b	5.0 a	1.9
Mg		1.5 b	2.2 a	1.1
Al	cmol _c dm ⁻³	0.5 b	0.3 a	2.7
H+Al		9.4 a	9.7 a	14.6
S		5.0 b	7.5 a	3.1
CTC _{effective}		5.5 b	7.8 a	5.8
CTC _{pH7}		14.4 b	17.2 a	17.7
V	%	34.8 b	45.3 a	17.6
m		9.0 b	3.9 a	46.3
SSA	m ² g ⁻¹	44 a	42 a	44

¹The native forest values were not used in the statistics. Equivalent capital letters do not differ statistically by the Tukey test at 5% for management treatment. TOC: Total Organic Carbon; SSA: Specific Surface Area

Table 4. Variance analysis for soil phosphorus forms in a clayey Rhodic Hapludox (0-10 cm) in a 30 years experiment with different soil management, winter cover crops and sampling time.

Factors	P _{SMB}	P _R	P _M	P _{O_{NaOH}}	P _{i_{NaOH}}	P _{O_{total}}	P _{i_{total}}	P _{total}
Management ¹ (M)	17.20 ^{**}	432.48 ^{**}	1032.27 ^{**}	246.67 ^{**}	221.34 ^{**}	340.87 ^{**}	74.82 ^{**}	na [*]
Sampling time ² (E)	92.89 ^{**}	1.05 ^{ns}	2.48 ^{ns}	3.74 [*]	3.74 [*]	17.480 ^{**}	5.45 ^{**}	11.45 [*]
Cover crop ³ (C)	2.72 [*]	3.57 ^{**}	3.23 ^{**}	4.29 ^{**}	2.14 ^{ns}	8.97 ^{**}	7.71 ^{**}	9.49 [*]
<i>Interaction</i>								
M x E	0.66 ^{ns}	9.01 ^{**}	2.67 ^{ns}	3.23 [*]	0.37 ^{ns}	0.48 ^{ns}	0.46 ^{ns}	0.034
M x C	0.40 ^{ns}	2.89 [*]	9.42 ^{**}	2.5772 [*]	3.01 [*]	1.67 ^{ns}	0.72 ^{ns}	0.66
E x C	1.24 ^{ns}	7.02 ^{**}	3.34 ^{**}	7.3662 ^{**}	3.10 ^{**}	4.0590 ^{**}	2.72 ^{**}	1.91
M x E x C	0.79 ^{ns}	6.39 ^{**}	4.54 ^{ns}	1.5516 ^{ns}	0.62 ^{ns}	2.70 ^{**}	1.38 ^{ns}	1.59

¹Management: conventional tillage (CT) no-tillage (NT). ²Sampling time: after corn harvest, winter species flowering and soybean flowering. ³Rotation: fallow, ryegrass, rye, vetch, radish, wheat and consortium hairy vetch and black oat.

na not available value. * significant at the 5% probability level (p<0.05). ** significant at the 1% probability level (p<0.01). ns not significant (p>0.05)

Table 5. Phosphorus stored in soil microbial biomass in clayey Rhodic Hapludox (0-10 cm) in a 30 years experiment with different soil management, winter cover crops and sampling time.

	Fallow	Ryegrass	Rye	Wheat	Vetch	Radish	Consortium ³	Mean	Forest ⁴
	----- mg kg ⁻¹ -----								
Sampling Time ¹									
Corn	46	65	59	47	55	61	74	58 a	38
Winter	17	23	28	26	21	39	22	25 b	38
Soybean	20	30	23	20	39	18	27	25 b	45
Mean	28 B	39 A	37 A	31 B	38 A	39 A	41 A		40
Soil Management ²									
CT	18	28	25	19	26	26	31	25 b	
NT	40	50	48	42	51	52	52	48 a	

¹After corn harvest; winter species flowering; soybean flowering. ²CT=conventional tillage system; NT=no-tillage system. ³Hairy vetch + Black oats. ⁴Values obtained in the forest soil were not included in the statistical analysis.

Means followed by the same lowercase letter comparing the sampling time or soil management system.

Means followed by the same capital letter comparing the winter cover crops.

Table 6. Phosphorus polls in clayey Rhodic Hapludox (0-10 cm) in a 30 years experiment with different soil management, winter cover crops and sampling time.

	P_M	P_{RTA}	P_{NaOH}	P_{iNaOH} mg kg ⁻¹	$P_{O_{total}}$	$P_{i_{total}}$	P_{total}^4
Soil Management¹							
CT	5.0B	7.9B	277B	127B	371B	856B	1227 B
NT	13.9A	18.0A	364A	211A	463A	1047A	1526 A
Sampling Time²							
Corn	9.8a	12.8a	319ab	179a	437a	964ab	1426 a
Winter	9.4a	12.6a	330a	162b	409b	902b	1311b
Soybean	9.0a	13.4a	312b	165ab	404b	989a	1393 ab
Winter crops							
Fallow	10.8a	13.1a	304ab	172ab	397cd	970abc	1367 bc
Ryegrass	9.7a	13.1a	329a	159ab	424abc	858c	1282c
Rye	11.8a	13.0a	328a	168ab	408bcd	893c	1301 bc
Vetch	8.1a	10.3B	333a	169ab	435ab	919bc	1354bc
HairyVetch + Black Oat	9.7a	13.7a	325a	184a	411bcd	911bc	1321bc
Radish	8.9a	14.0a	293b	151b	394d	1021ab	1416 b
Wheat	7.9a	13.1a	329a	177ab	448a	1091a	1595 a
Forest ³	4.6	5.7	389	147	436	832	1298

¹CT = conventional tillage system; NT= no-tillage system. ²After corn harvest; winter species lowering; soybean flowering. ³Native forest values were not used in the statistics. ⁴Log (x) transformation for all data from the extractor column.

Equivalent capital letters, lowercase letters and lower case letters in italics do not differ statistically by the Tukey test at 5% for management treatment, sampling time and winter crops, respectively.

Table 7. Total and partial inorganic and organic phosphorus pools and phosphorus form analyzed by ^{31}P -NMR in clayey Rhodic Hapludox (0-10 cm) in a 30 years experiment cultivated with common vetch winter cover crops (no-tillage and conventional tillage) and natural forest

Parameter	Forest	No-tillage	Conventional
<i>Available P</i>			
Anion exchange resin (mg kg^{-1})	5.0	15.4	7.6
Microbial biomass (mg kg^{-1})	33.4	55.1	23.4
<i>Total Digestion</i>			
Total P (mg kg^{-1})	1,267	1,439	1,296
Pi (mg kg^{-1})	814	1,018	929
Po (mg kg^{-1})	453	421	367
Po / Total P (%)	35.7	29.3	28.3
<i>Extracted by 0.5 mol L⁻¹ NaOH</i>			
Total P (mg kg^{-1})	442	619	424
Pi (mg kg^{-1})	159	245	158
Po (mg kg^{-1})	283	384	266
Po / Total P (%)	64.0	62.1	62.8
<i>Extracted by 0.25 mol L⁻¹ NaOH + 50 mmol L⁻¹ EDTA (^{31}P-NMR Extract)</i>			
Total P (mg kg^{-1})			
Pi (mg kg^{-1})			
Po (mg kg^{-1})			
Po / Total P (%)			
<i>P forms analyzed by ^{31}P-NMR</i>			
Orthophosphate (%)	51.0	62.0	56.8
Monoester (%)	39.3	29.7	35.8
Pyrophosphate (%)	2.6	4.3	2.3
Diester (%)	6.6	3.1	5.1
Adenosine diphosphate (%)	0.6	0.9	0.0
Monoester/Diester ratio	5.9	8.5	7.4
Po / Total P (%)	46.4	33.7	40.9