Soil organic phosphorus in Eucalyptus plantations, Brazil: extraction methods

Lais Chierici Bernardes Rinaldi Alvarenga, Marlon Gomes da Costa, Antonio Carlos Gama-Rodrigues, Seldon Aleixo, Emanuela Forestieri Gama-Rodrigues, José Leonardo de Moraes Gonçalves

ABSTRACT: In-depth knowledge of total soil organic phosphorus (TPo) as a potential P source for plants allows for a comprehensive understanding of the adoption of an efficient management system of phosphorus fertilization in forest plantations. Thus, we aimed to compare three TPo extraction methods (Hedley; Bowman; Bowman and Moir) in different Eucalyptus plantations on strongly weathered soils. The TPo concentrations obtained by the “Hedley” (mean of 130 mg kg⁻¹), and “Bowman and Moir” methods (mean of 131 mg kg⁻¹) were similar. The “Bowman” method extracted less than 50 % of the TPo content extracted by the other methods. Both “Hedley” and “Bowman and Moir” methods showed similar TPo extraction efficiency compared to TP determined by acid digestion. However, the “Hedley” method is the most expensive and time-consuming analytical technique. In view of this, the NaOH+Na₂EDTA extractor (Bowman and Moir) would be the most suitable since, in addition to showing high extraction efficiency, it offers fast analysis execution, low analytical error, and lower cost.

Keywords: basic EDTA extraction, forest soils, phosphorus fractionation, sequential acid-base extraction

Introduction

Soil organic phosphorus (PO) is of fundamental importance to the maintenance of production systems. The planted forest areas in Brazil are largely located on soils with high phosphorus (P) deficiency, mainly those under Eucalyptus grandis Hill (ex Maiden) and Eucalyptus grandis × E. urophylla S.T. Blake clonal hybrid (Eucalyptus spp.) which represents 76 % of the planted forest area in Brazil [SNIF, 2019]. Thus, the quantification of the PO pool and its potential use as a source of this nutrient makes it possible to reduce the use of phosphate fertilizers, a resource that is limited, since P reserves in the soil are finite and non-renewable in the short term [Laclau et al., 2010]. Soil PO is an important reserve pool in forest systems in temperate [Prietzel et al., 2022] and tropical climates [Spohn, 2020]. Given this, estimating soil PO concentrations is relevant to P studies as PO has high potential for supplying inorganic P (Pi) to forest cover through mineralization processes [Aleixo et al., 2019; Spohn and Stendahl, 2021]. However, few studies deal with PO extraction methods and their efficiency in estimating the real soil contents. Thus, it is necessary to improve the knowledge about the different extraction methods used to determine the total PO (TPo) to identify the methodology best suited to soils with high P buffering capacity.

Three methods are commonly used for TPo determination. Hedley et al. [1992] proposed the sequential P fractionation, widely used in the most diverse studies on P transformations in different soil classes and land uses, both in temperate and tropical ecosystems [Condron and Newman, 2011; McDowell and Burkitt, 2022]. Sequential extraction allows for characterizing different P and PO fractions on a scale of solubility variation based on changes in different pH levels and extracting strength, in decreasing order of soil lability, in which TPo is the sum of fractions extracted in sodium bicarbonate [NaHCO₃], sodium hydroxide [NaOH] and NaOH + Sonification [Aleixo et al., 2017; Viana et al., 2018]. Another method is Bowman’s sequential extraction [Bowman, 1989], considered suitable for comparing P levels in different tropical soil classes [Condron et al., 1990], and especially the soil TPo dynamics in pastures and forest systems [Cunha et al., 2007; Zaia et al., 2008, 2012; Rita et al., 2013]. The method proposed by Bowman and Moir [1993] was developed to obtain an easy and reproducible methodology in a single step using an alkaline source such as sodium hydroxide [NaOH] associated with Na₂EDTA. Under this methodology, PO associated with soil organic matter is solubilized by the extractor alkaline matrix, and Na₂EDTA helps in the formation of paramagnetic ion complexes present in the soil extract [e.g., aluminium [Al], iron [Fe] and manganese [Mn]] [Turner, 2008], which can be precipitated after quick centrifugation, increasing the P recovery percentage during soil extraction.
Soil \( P_O \) is not directly determined by colorimetry, being, therefore, obtained by the difference between total phosphorus (TP) and \( P_I \) contents. This implies that different methods present different results (Turner et al., 2005). Given this backdrop, this scientific note aims to compare three analytical methods of TP\( _O \) extraction to identify which method is best suited to planted Eucalyptus forests.

Materials and Methods

Sites description

The present study was carried out on Eucalyptus plantations \([E. \textit{grandis}, E. \textit{urophylla}, E. \textit{grandis} \, \text{[Parb.], E. \textit{grandis} \,[G-232], E. \textit{grandis} \,[Suz.]}}\) in nine sites with different soil orders, climatic conditions and management, distributed in seven municipalities in the state of São Paulo, Brazil. The sites are located at altitudes from 570 m to 889 m. Soils were classified according to the Brazilian Soil Classification System [EMBRAPA, 2013]: Latossolo Vermelho-Amarelo distrófico [equivalent to Oxisol in USDA Soil Taxonomy [Soil Survey Staff, 2014] – Itatinga [ITA], Neossolo Quartzarênico [Entisol – Angatuba (ANG) and Botucatu (BOT)], Latossolo Vermelho distrófico [Oxisol – Agudos (AG) and Capão Bonito 2 (CB2)], Latossolo Amarelo distrófico [Oxisol – Capão Bonito 1 (CB1)], Argissolo Vermelho-Amarelo distrófico [Ultisol – Votorantim (VOT)] and Cambissolo Háplico distrófico [Inceptisol – Capão Bonito 3(CB3)]. The municipality’s climate where the soils were collected was considered Cwa, according to the Köppen classification [Costa et al., 2016], except for the AG site, which was characterized as Awa. The site’s ages ranged from 1.4 to 11 years with a spacing of 3.0 m \( \times \) 2.0 m, except for the CB1 (3.0 m \( \times \) 3.0 m spacing), and the PA (3.0 m \( \times \) 2.5 m spacing) sites. In all sites, ten simple soil samples were collected in each experimental plot (three plots per site) at 11 years with a spacing of 3.0 m \( \times \) 2.0 m, except for the AG site, which was considered as Aw, according to the Köppen classification [Costa et al., 2016].

Table 1 – Physical and chemical properties of soils in stands of different eucalyptus sites.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Clay</th>
<th>C</th>
<th>P( ^{I} )</th>
<th>pH</th>
<th>K</th>
<th>Mg</th>
<th>T</th>
<th>m</th>
<th>V</th>
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<td>4.0</td>
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<td>102.5</td>
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<td>5.2</td>
<td>107.3</td>
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<td>2.4</td>
<td>70.7</td>
<td>52.2</td>
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</table>

Bowman’s sequential acid-base extraction [Bowman, 1989]

Total \( P_O \) was determined in a sequential extraction with concentrated sulfuric acid and dilute base. For the determination of \( P_O \), 3.0 mL of 18 mol L\(^{-1}\) \( \text{H}_2\text{SO}_4 \) were added to falcon tubes containing 2.0 g of soil samples (in triplicate). The tube was manually shaken, then subjected to the addition of 4.0 mL of distilled water in 1.0 mL aliquots at a time, followed by manual shaking again, a further addition of 43 mL of distilled water and the tube was shaken again. Subsequently, the tubes were centrifuged at 83.3 Hz for 10 min. The supernatant was filtered with \( \text{N}^\circ \) 42 Whatman filter paper and transferred to a clean vial. The filter paper used to filter the acid extracts was removed and returned to the same centrifuge tube containing the remaining soil and 40 mL of 0.5 mol L\(^{-1}\) \( \text{NaOH} \) was added. After adding \( \text{NaOH} \), the suspension was taken to the end-over-end shaker at 2 Hz for 30 min. The centrifuge tube was placed in a water bath at 80 °C for 2.0 h. After this period, the centrifuge tube was cooled in running water, then centrifuged and filtered as in the acid extraction procedure.

A 5.0 mL aliquot of each of the acid and alkaline extracts was removed for TP determination with the addition of 1.0 mL of \( \text{MgCl}_2 \) (at saturation point) plus 1.0 mL of 69 % \( \text{HClO}_4 \) [mL 100 mL\(^{-1}\)] in acid extracts and only 1.0 mL of \( \text{HClO}_4 \) at 69 % [mol 100 mL\(^{-1}\)] in basic extracts. Digestion tubes were manually shaken and then placed in the digester block at 80 °C, with a slow temperature increase to 180 °C. Digestion was terminated when a colorless gel formed at the bottom of the tube. After tube cooling, 5.0 mL of distilled water was added and an aliquot was removed for the quantification of TP. \( P_O \) was determined using 20 mL of acid and alkaline extracts after clarification by filtration in 0.5 cm\(^2\) of activated carbon (Guerra et al., 1996). Due to the occurrence of \( P_I \) in the filter element [activated carbon], the purification was carried out with
successive washings using solutions of HCl 6.0 mol L⁻¹ [acid], NaHCO₃ 0.5 mol L⁻¹ and NaOH 0.5 mol L⁻¹ [alkaline]. P concentrations in clarified acid extracts were determined at 880 nm following the method of Murphy and Riley (1962). P concentrations in clarified alkaline extracts were determined at 700 nm following the Dick and Tabatabai method (1977). All extracts from the steps were analyzed by colorimetry for P reactive to ammonium molybdate in SPECORD 210 PLUS. Total P₀ (TP₀) concentrations were calculated by summing the differences in concentrations between TP and P₁ of all acid and alkaline extracts as follows:

\[
[P₀ \text{ acid extract} = TP \text{ acid (digested)} - P₁ \text{ acid (extracted)}]
\]

\[
[P₀ \text{ basic extract} = TP \text{ alkaline (digested)} - P₁ \text{ alkaline (extracted)}]
\]

\[
[TP₀ = P₀ \text{ acid extract} + P₀ \text{ alkaline}]
\]

**Extraction with NaOH+Na₂EDTA by Bowman and Moir (Bowman and Moir, 1993)**

Total P₀ (TP₀) was determined by extraction with modified NaOH+Na₂EDTA. For this, 1.0 g of soil was used to which 10 mL of solution with 0.25 mol L⁻¹ NaOH plus 0.05 mol L⁻¹ Na₂EDTA was added, followed by end-over-end stirring at 2 Hz for 10 h (25 °C) in 15 mL falcon tubes. Next, the tubes were centrifuged at 83.3 Hz for 15 min, and the supernatant was filtered through 45 μm cellulose membranes [75-80 % nitrate and acetate] and P₁ was determined. An aliquot (3.0 mL) of each NaOH+Na₂EDTA soil extract for total P digestion was autoclaved at 103 kPa and 121 °C for 3.0 h with a solution containing 10 mL of [(NH₄)₂S₂O₈ at 7.5 % (g 100 mL⁻¹)] plus 1.0 mL H₂SO₄ at 24 mol L⁻¹. TP in the extracts was determined at 880 nm by the method of Murphy and Riley (1962). The P₁ in extracts was determined at 700 nm by the method of Dick and Tabatabai (1977). All extracts from the steps were analyzed by colorimetry for P reactive to ammonium molybdate in SPECORD 210 PLUS. The difference between TP and P₁ concentrations in each NaOH+Na₂EDTA extract calculated organic P (PO).

**Determination of total soil P by sulfuric and nitric-perchloric digestions**

Total soil P by sulfuric digestion [Hedley et al., 1982] was determined using 0.1 g of soil macerated in a digestion tube and 1.0 mL of MgCl₂ (at saturation point) plus 2.0 mL of H₂SO₄ at 49 % (mL 100 mL⁻¹) were added. The tubes were taken to the digester block by heating them at 200 °C for 1.0 h, and after cooling 2.0 mL of H₂O₂ at 30 % (mL 100 mL⁻¹) was added and heated in the digester block at 100 °C for another 1 h. After cooling, the volume was adjusted to 50 mL with deionized water. TP by nitric-perchloric digestion, described by Guerra et al. [1996], was determined using 0.2 g of soil macerated in a digestion tube and 3.0 mL of HNO₃ and HClO₄ in a 2:1 ratio (mixture containing 120 mL of HNO₃ and 60 mL of HClO₄) was added. The tubes were taken to the digester block by slowly heating them to 160 °C, remaining for ~40 min (volume reduced to half). After this period, the temperature was raised to 210 °C to obtain a clear solution, which gave off dense vapors of HClO₄ (~20 min). After cooling, 50 mL of distilled water was added and the sample was transferred to a 250 mL volumetric flask. The extracts were filtered through n° 42 Whatman filter paper and stored for TP determination. Total P (TP) was determined by the colorimetric method after pH adjustment, using p-nitrophenol as an indicator. All extracts were analyzed by colorimetry for P reactive to ammonium molybdate by spectrophotometry at 880 nm [Murphy and Riley, 1962] in SPECORD 210 PLUS. The TP concentrations from both methods were used to estimate the TP₀ recovery capacity following the Hedley, Bowman, and Bowman and Moir extraction methods [(TP₀ extracted × 100) / TP by digestion].

**Statistical analysis**

Each Eucalyptus site was considered a pseudo-replication. The data were submitted to the Lilliefors and Kolmogorov-Smirnov normality test, showing a normal distribution of the analyzed variables. Subsequently, the data were submitted to descriptive analysis and test of means (Tukey) at 5 % probability. The relationships between the three extraction methods of TP₀ and TP; between extracted TP and digested TP; between TP₀ and clay content; and, TP₀ recovery values and clay contents were analyzed using Pearson’s correlation coefficient. Data were analyzed using the R 3.2.1 program (R Core Team, 2016) with the ISwR package [Dalgaard, 2008] and ExpDes.pt (Ferreira et al., 2014). For the construction of the graphs, the Sigmaplot 12.0 program was used.

**Results**

The TP₀ levels by the three extraction methods evaluated are shown in Table 2. The TP₀ contents extracted by the “Hedley” fractionation and by the “Bowman and Moir” extraction [NaOH+Na₂EDTA] were much higher (~63 %) than the contents determined by the “Bowman” method. The mean values of TP₀ contents between the “Hedley” and “Bowman and Moir” methods were similar, ranging between 58 and 273 mg kg⁻¹ [Hedley] and 80 and 214 mg kg⁻¹ [Bowman and Moir]. While the TP₀ extracted by “Bowman” quantified, on average, less than 50 mg kg⁻¹ for almost all forest sites (Table 2; Figure 1). On the other hand, there were no significant differences between the three extraction methods for the inorganic P fraction (Table 2; Figure 1). The highest levels of TP extracted were obtained by the Hedley method, followed by sulfuric digestion (H₂SO₄+H₂O₂+MgCl₂), “Bowman”, “Bowman and Moir” methods (NaOH+Na₂EDTA) and nitric-perchloric digestion [HNO₃+HClO₄] (Table
The difference between the TP and the sum of TP\textsubscript{O} and TP\textsubscript{I} extracted by the "Hedley" method corresponds to the residual P content, a P fraction considered recalcitrant.

Significant positive correlations existed between the three TP\textsubscript{O} extraction methods (Figure 2). The average TP\textsubscript{O} recovery rate was 34 % for both the "Hedley" method, and the "Bowman and Moir" method (NaOH+Na\textsubscript{2}EDTA), and 11 % for the "Bowman" method compared to the PT by sulfuric digestion (Figure 3). On the other hand, when compared to TP by nitric-perchloric digestion, the mean recovery rate of TP\textsubscript{O} was 56 % for the "Hedley" method, 56 % for the "Bowman and Moir" method (NaOH+Na\textsubscript{2}EDTA), and 18 % for the "Bowman" method (Figure 3). The three extraction methods evaluated were significantly correlated with each other for TP (r = 0.97, p < 0.001, n = 9) for "Hedley vs. Bowman and Moir"; r = 0.91, p < 0.001, n = 9 for "Hedley vs. Bowman"; and r = 0.90, p < 0.001, n = 9 for "Bowman vs. Bowman and Moir". These extracted TP contents also correlated significantly only with the TP contents determined by nitric-perchloric digestion (r = 0.98, p < 0.001, n = 9 for "Hedley"; r = 0.97, p < 0.001, n = 9 for "Bowman and Moir" and r = 0.96, p < 0.001, n = 9 for "Bowman"). There was no significant correlation between the two methods of TP determination by acid digestion. For TP\textsubscript{I}, the significant correlations between the "Bowman and Moir" and "Bowman" methods (r = 0.87, p < 0.01, n = 9), and between the "Bowman" and "Hedley" methods (r = 0.83, p < 0.01, n = 9).

For TP\textsubscript{I}, the recovery rates of the three extraction methods were similar to each other for both sulfuric digestion (on average 25 %) and nitric-perchloric digestion (on average 44 %) (Figure 4). The recovery rates of TP extracted by the methods of "Hedley", "Bowman and Moir", and "Bowman" were 105 %, 59 % and 39 %, respectively, compared to sulfuric digestion. In turn, the values of recovery rates of TP extracted by the three methods evaluated compared to nitric-perchloric digestion were higher than by sulfuric digestion (Figure 5). The meaning and values of the correlations between TP\textsubscript{O} recovery rates and clay contents differed between sulfuric and nitric-perchloric digestion methods (Table 3). The nitric-perchloric digestion method had significant negative correlations for the "Hedley" and "Bowman and Moir" methods. On the other hand, in the sulfuric digestion method, there was only significant positive correlation for the "Bowman" method. The values and direction of correlations for TP\textsubscript{I} and TP also varied between extraction and digestion methods. Total organic P (TP\textsubscript{O}) contents varied positively and significantly with clay contents for the three extraction methods (Figure 6). Similar results occurred for extracted TP, ["Hedley" (r = 0.88, p < 0.01, n = 9), "Bowman" (r = 0.96, p < 0.001, n = 9), "Bowman and Moir" (r = 0.78, p < 0.05, n = 9)]
and TP ["Hedley" (r = 0.89, p < 0.01, n = 9), "Bowman" (r = 0.95, p < 0.001, n = 9), "Bowman and Moir" (r = 0.83, p < 0.01, n = 9)]. Significant correlations between the clay contents and sulfuric digestion (r = 0.69, p < 0.05, n = 9), as well as the nitric-perchloric digestion (r = 0.93, p < 0.001, n = 9) also varied positively.

**Discussion**

Residual-P values [Hedley’s method], which were not added to P₀ and P₁ totals, presented an overall average of 41.5 % of the soil total phosphorus. Studies carried out in tropical soils by Araújo et al. (2004); Gama-Rodrigues et al. (2014); Viana et al. (2018); Soltangheisi et al. (2021) found similar values. As they do not have clear limits between the P compartments in the soil, the exact quantification of TP₀ does not always have the desired precision. Successive extractions may overestimate TP levels compared to single-step extractions. Viana et al. (2018) showed that concentrations of TP using...
the “Hedley” fractionation technique can be higher than those found with a single-step extraction such as sulfuric digestion. Among those responsible for this high extraction capacity is the use of an anion exchange resin (RTA) in its initial phase that simulates the extraction of readily available P (solution-P) by the plant root system in situ (Hedley et al., 1982), and combined with the fact of obtaining P₀ extracted by NaHCO₃, NaOH and the P₀ resulting from the Sonification (Costa et al., 2016), where strong acids and bases can induce P₀ hydrolysis (Turner et al., 2005).

Thus, during the sequential extraction, the different extractors levels act at different P adsorption sites. For example, in the step of the Pᵢ and P₀ fractions at NaOH extracts plus sonication, the rupture of aggregates occurs, making new available P sites, followed by the Residual-P fraction determination on H₂O₄+H₂O₂+MgCl₂ digestion, where there would thus be a greater amount of TP at the end of the analytical run compared to that measured in the incomplete sulfuric digestion in a single step of the entire volume of soil sampled (Aleixo et al., 2017).

Table 3 – Pearson correlation between total soil phosphorus forms recovery (%) from different extraction methods and clay contents in both sulfuric and nitric-perchloric digestion methods.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Clay</th>
<th>Sulfuric</th>
<th>Nitric-perchloric</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP₀</td>
<td>Hedley</td>
<td>-0.66*</td>
<td>0.6405</td>
</tr>
<tr>
<td></td>
<td>Bowman</td>
<td>-0.55</td>
<td>0.74*</td>
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<td>Bowman</td>
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<td></td>
<td>Bowman and Moir</td>
<td>-0.88**</td>
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</table>

Bold values significant at *p < 0.05; **p < 0.01; ***p < 0.001; TP₀ = total organic phosphorus; TPᵢ = total inorganic phosphorus; TP = total soil phosphorus.

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Thus, during the sequential extraction, the different extractors levels act at different P adsorption sites. For example, in the step of the Pᵢ and P₀ fractions at NaOH extracts plus sonication, the rupture of aggregates occurs, making new available P sites, followed by the Residual-P fraction determination on H₂O₄+H₂O₂+MgCl₂ digestion, where there would thus be a greater amount of TP at the end of the analytical run compared to that measured in the incomplete sulfuric digestion in a single step of the entire volume of soil sampled (Aleixo et al., 2017).
The highest PT values extracted by the “Bowman and Moir” method, comparing directly with the “Bowman” methodology are also shown in Table 2. Among the few studies available comparing TP₃ methods in tropical soils, Condron et al. (1990) analyzed 23 soils, 20 of which were Brazilian. They obtained differences in TP₃ determinations between the tested methods, concluding that using an alkaline substance, such as NaOH, allows the TP₃ fraction to be quickly accessed in strongly weathered tropical soils. The superior extraction efficiency by adding EDTA, mainly in soils with higher organic matter contents, was reported by Bowman and Moir (1993). This differs from the method initially proposed by “Bowman”, where only the inorganic orthophosphate was measured by colorimetry.

The alkaline solutions (e.g. those containing NaOH preferentially) are highly efficient in obtaining TP₃ from the soil [Turner et al., 2005]. As observed, the “Bowman” and “Bowman and Moir” methods are positively correlated in all evaluated methodologies [Figure 2]. This is explained by the fact that extraction with the addition of Na₂EDTA was a proposal initiated to facilitate and expand the extraction of TP₃ since certain methods proposed to evaluate soil TP₃ compartments are poorly reproducible and require multiple steps, which increases the possibility of analytical errors in the determinations. Thus, the inclusion of NaOH+Na₂EDTA helps in the complexing of the metals of the colloid functional group through the complexing of cations (i.e., Fe and Al paramagnetic ions), and NaOH promotes the cations hydrolysis with P being released from the soil compound (i.e., due to the addition of the hydroxyl anion); thus TP₃ associated with soil organic matter (SOM) is available for detection in these extracts [Bowman and Moir, 1993; Turner et al., 2005]. Furthermore, the “Bowman and Moir” method presented values (extraction efficiency and soil TP₃ recovery) statistically similar to the “Hedley” fractionation.

These analyzed soils present distinct physical and chemical properties with textures varying from medium to very clayey, being characterized as very acidic and low fertility in all sites [Table 1]. In this study, it was observed that sulfuric digestion presented a significant positive correlation with the evaluated methods, in contrast to nitric-perchloric digestion, which presented negative correlation with the methods compared to their clay contents. It is believed that this fact was due to incomplete and/or inefficient digestion using nitric acid, not dissolving the total P content in the soil. A significant limitation associated with acid extractions is the presence of chemically functional P₃ species that are not detected by colorimetric methods (e.g., P complexes can form with ammonium molybdate), which can affect the actual concentration of TP₃ form present in soils [Turner et al., 2005]. On the other hand, the option of sulfuric digestion to extract TP from the soil can promote an acidic dissolution, where the extracting solution containing H₂SO₄ partially breaks the soil inorganic colloids, which allows access to the PO and P₃ forms that were protected [Aleixo et al., 2017]. Levels of TP obtained by sulfuric digestion can be very similar to total phosphorus in different soil classes (i.e., Inceptisol and Ultisol), where the extractions step with NaOH plus Sonication was omitted [Szott and Melendez, 2001]. Thus, due to its superiority in the P content digesting, it is expected that its correlation with the other methods will be positive.

In all evaluated sites, soil P recovery rates compared to sulfuric digestion showed a ~40 % of the recovery using the “Bowman” method, which is within the values observed in the literature, which ranges from 30 to 169 % (Condron et al., 1990; Guerra et al., 1996; Cunha et al., 2007; Zaia et al., 2008; Oliveira et al., 2014), levels predominates in studies with strongly weathered tropical soils compared to PO forms using the “Bowman” method (Guerra et al., 1996; Rita et al., 2013; Oliveira et al., 2014). Thus, P₃ values with this method showed relatively low responsive values (on average 27 %) compared to TP. Similar results were observed by Rita et al. (2013), who found P₃ levels between 11 % and 32.5 %, and Oliveira et al. (2014), with contents ranging from 36 to 46 %.

Although the different methods display significant efficiency in the P extraction, the soil nature must be considered as it can influence the percentage of recovery in the P compartments [Turner et al., 2005]. Therefore, the choice between the methods of “Hedley”, “Bowman”, “Bowman and Moir”, sulfuric and/or nitric-perchloric digestion must be made based on the observation of two soil characteristics under study: (i) chemical solubility of the P forms present in SOM, which may have different quality depending on the vegetation cover inputs. The strong soil acidity present in tropical ecosystems could restrict the SOM decomposition [Aleixo et al., 2017], and counterbalance the conditions of high humidity and temperature that favor this process, or even that a high continuous input of senescent plant material can strongly contribute to the maintenance of P₃ stocks in the soil [Fontes et al., 2014; Lima et al., 2023]; (ii) and characteristics of different clay fraction contents in different soil orders [Turner et al., 2005; Costa et al., 2016; Viana et al., 2018].

All soil P extraction methods, including its P₃ and P₄ forms, obtained significant correlations with clay contents in the present study. The methodology proposed by Hedley stands out in relation to the correlations found, even with a small variation in its analytical course. In this sense, the search to evaluate the sensitivity between the TP₃ extraction methods (as well as TP₃ and TP) showed that the different sites with different soil classes and clay contents have a strong relationship associated with the efficiency of the extractors studied since the soil clay contents can interfere with the P solubilization in the soil and its extraction [Corrêa et al., 2005]. Studies considering P molybdate reactive showed that extractors present susceptible differences compared to...
and leguminous trees (Aleixo et al., 2020). However, soils under plantations (Rinaldi et al., 2021) Eucalyptus pools of the organic and inorganic phosphorus cycle in collaborated to elucidate the transformations in discrete Brazilian planted forests. possible to evaluate its effects in tropical soils under the this fraction through its soil compounds, and make it an improved understanding of the contribution of the composition of the P organic forms will allow for the methods of analyzing total P from the soil. The importance of further studies to verify the methods of extracting soil PO (i.e., organic phosphorus Al oxyhydroxides (Novais and Smyth, 1999). Positive correlations between labile PO (i.e., organic phosphorus forms retained with lower energy in the soil) and the soil clay fraction suggest that soil texture may be closely linked to stabilization and increased PO contents in the soil minerals (Oliveira et al., 2014).

Given this background, modern techniques for analyzing the composition of chemically functional soil PO forms are developed and applied together with certain extraction methods evaluated in the present study. The extraction with NaOH + Na,EDTA (Bowman and Moir, 1993), in order to compose the 31P nuclear magnetic resonance spectroscopy solution (31P NMR), is the most widely used for understanding the soil P cycle (Cade-Menun and Liu, 2014). Furthermore, according to Turner et al. (2005), a joint use of quantification methods, such as sequential "Hedley" extraction, with 31P NMR can collaborate to elucidate the transformations in discrete pools of the organic and inorganic phosphorus cycle in soils under Eucalyptus plantations (Rinaldi et al., 2021) and leguminous trees (Aleixo et al., 2020). However, 31P NMR may have limited use due to its relatively high cost, and it is currently impossible to recommend it as a routine technique used in Brazilian accredited soil analysis laboratories.

The different analytical methods of extracting soil total PO forms showed significant differences compared to the methods of analyzing total P from the soil. The "Bowman and Moir" method appeared as a simple and quick technique, which covered a smaller number of manipulations, which reduced the possibility of interference in the results due to errors in the execution of the analytical sequence. Furthermore, fractionation techniques can be costly regarding time and financial resources. Another advantage is that, through extraction with NaOH + Na,EDTA, there is the future possibility of associating the results with those obtained with the 31P NMR analysis, in order to understand and maximize the sustainability of systems, and establish the potential of the labile PO fractions to provide P to the Eucalyptus plantations. The importance of further studies to verify the composition of the P organic forms will allow for an improved understanding of the contribution of this fraction through its soil compounds, and make it possible to evaluate its effects in tropical soils under the Brazilian Eucalyptus planted forests.

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Authors’ Contributions


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