

Article

Labile and Non-Labile Fractions of Phosphorus and Its Transformations in Soil under Eucalyptus Plantations, Brazil

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Abstract: Phosphorus (P) is the main limiting nutritional factor in the cultivation of *Eucalyptus* in Brazil. Despite this, little is known about the P fractions and dynamics in *Eucalyptus* soils in tropical regions. The aims of this study were (1) to evaluate the distribution of labile and non-labile fractions of P in soil in 10 sites of *Eucalyptus* in the state of São Paulo, Brazil and (2) to determine the interactions among the P fractions and to identify which fractions act as sinks or sources for the available P fraction. The P fractions were separated using the Hedley sequential extraction method, and the P transformation processes were evaluated via structural equation modeling. The labile P (resin-Pi + Pi and Po in NaHCO₃) varied between 6% and 16%, the moderately labile P (Pi + Po in NaOH) varied between 28% and 40%, the occluded P (Pi + Po in Sonicate + NaOH) varied between 6% and 19%, the P associated with Ca (HCl-Pi) varied between 0.4% and 3% and the residual-P varied between 35% and 47%. The hypothetical structural model of soil P cycle was composed of four P pools (latent variables): the organic pool, occluded pool, inorganic pool, and available pool. The structural model was adequate to identify functional pools of P in soil, and to identify transformation processes of P based on the source-sink relationships. The available P pool was highly dependent on inorganic and occluded pools and the organic pool acted predominantly as a sink of P on available and inorganic pools. The results reinforce the high level of recalcitrance of the organic pool and the fact that *Eucalyptus* plants must access pools of limited-availability P to meet their nutritional demands.

Keywords: tropical forest soils; organic P pool; inorganic P pool; occluded P pool; structural equation modeling

1. Introduction

Eucalyptus represents more than 20 million hectares grown over more than 90 countries [1] and has played an increasingly important role in satisfying global demand for wood products. Now, it accounts for 8% of cultivated forest worldwide, with a third of this found in the tropics [2]. Brazil has 7.6 million hectares of cultivated forests, of which 72% are in the *Eucalyptus* genus, and account for 17% of the harvested wood worldwide [3]. Characteristics of *Eucalyptus* genus such as rapid growth, high productivity and adaptability to varying soil and climate conditions, tolerance to acidity, toxicity to Al and low base saturation, explain such a high proportion of total cultivation in forests both in Brazil and elsewhere.

The initial stages of cultivation usually involve mineral fertilization, and responses to P applications are often of a high magnitude [4]. The exploitation of low natural fertility soils, such as those in the Brazilian Cerrado, has increased, thus exposing the soils' nutritional limitations. These limitations, particularly of nutrients P, K and B, are common in *Eucalyptus* cultivation [5]. Nowadays, fertilization is responsible for gains of between 30% and 50% in wood production. P, besides being a minor macronutrient in plants, has a significant impact on wood productivity [3].

Well-known for limiting the primary productivity of natural tropical forests [6], P is also the main limiting nutritional factor in the development of forest cultivation of *Eucalyptus* in Brazil. This problem occurs mainly in highly weathered soils, like most tropical and subtropical areas, and is characterized by a low content of total P and available P, and with high P fixation capacities. Phosphorus is found in soils in inorganic forms (Pi) and organic forms (Po) and according to the degree of stability or solubility, these allow different bioavailability of P. Pi can be found in solution (P-solution) and fixed through the adsorption phenomenon, with oxides of Fe and Al (of clay fraction). This process establishes either weak adsorption (labile P) or strong adsorption (moderately labile P) with these oxides and, precipitated with Al, Fe and Ca, establishes insoluble forms (non-labile P) [7]. Po is formed by phosphate ions, which are bonded to C moieties and its lability is directly related to the decomposition susceptibility of the organic moiety to which the phosphate is bonded [8]. In natural systems or in soils with little or no P addition, the element availability is directly related to the organic forms cycling [9–11]. Thus the soil can be a source of P, while providing stocks of available P to plants or a P sink when part of the added fertilizer is fixed. This leads to competition between plants and soils for the available P.

The complex dynamics of this nutrient in tropical conditions validates other studies about the relationships between fractions, which aim to enhance knowledge of the P transformations that occur in these soils. Hedley *et al.* [12] developed a method of sequential extraction or fractionation of P that characterizes different organic and inorganic fractions of P from soil based on solubility of inorganic and organic P. This method has been widely applied since then and has become almost standard to characterize P forms in soils [13]. It enables us to distinguish labile from non-labile fractions and to rank stable fractions and organic fractions by availability to plants. Based on this extraction, P can be classified as available to plants or microorganisms (labile P), which includes the sum of Pi and Po extracted in resin and bicarbonate. The refractory forms or non-labile P include the sum of remaining fractions (Pi and Po in hydroxide and in sonicate + hydroxide, Pi in HCl and residual P in sulfuric digestion) [14]. In a review on the use of the Hedley method, contrasting agroecosystems of tropical and temperate climates, with different soil use and managements, Negassa and Leinweber [15] reported that inorganic and organic fractions of P could act as a source or sink of available P to plants, as a result of several factors such as climate, soil management and mineral fertilization.

Although some literature supports the potential of the Hedley method in studies that involve the dynamics of P in soil [16–21], there are few studies into tropical soil in *Eucalyptus* plantations. In general, the management of soil fertility for estimating the available P in *Eucalyptus* plantations in Brazil has been based only on P acid extraction (Mehlich-1 P) [22] or resin-extracted [5] and has not considered other P forms in the soil that may be P sources for plants. The aims of this study were (1) to evaluate the distribution of labile and non-labile fractions of P in soil in 10 sites of *Eucalyptus* in the state of São Paulo, Brazil and (2) to build a hypothetical structural model of the P cycle in soil to determine the interactions among the P fractions and to identify which fractions act as a sink or source of the available P fraction.

2. Material and Methods

2.1. Study Sites

In order to conduct this study, 10 sites forested with *Eucalyptus grandis* Hill (ex Maiden) and *Eucalyptus grandis* × *E. urophylla* S.T. Blake clonal hybrid were selected in the state of São Paulo, with

ages varying from 1.4 to 11 years, all of which are managed with minimum cultivation. The sites belong to forestry companies in São Paulo state and to the Department of Forestry Science of ESALQ/USP and are located in different regions (Table 1).

The sites are located at altitudes from 570 m to 889 m. According to the Köppen classification, climates correspond to types: Aw (Agudos and Altinópolis) and Cwa (Angatuba, Botucatu, Capão Bonito, Itatinga, São Miguel Arcanjo, Paraíbuna and Votorantim). In accordance with EMBRAPA (1999) [23], the soils were classified as Dystrophic Red Yellow Latosol (Oxisol) (Itatinga, São Miguel Arcanjo and Paraíbuna), Quartzarenic Neosol (Entisol) (Altinópolis, Angatuba and Botucatu), Dystrophic Red Latosol (Oxisol) (Agudos and Capão Bonito 2), Dystrophic Yellow Latosol (Oxisol) (Capão Bonito 1), Dystrophic Red Yellow Argisol (Ultisol) (Votorantim) and Dystrophic Haplic Cambisol (Inceptisol) (Capão Bonito 3) (Table 1). These are the main soils used in afforestation in São Paulo state [4].

In all sites, the forest plantations were established using a minimum cultivation system with the maintenance of plant residues (litter and harvest residues) on soil, followed by soil preparation in planting rows. At each site, three fixed plots were installed. The plots consisted of different plant densities on each site (Table 1). For the purposes of this study, sample plots were not fertilized with P and soil sampling was conducted 1.5 m away from the row planting.

2.2. Physical-Chemical Characterization of Soil

On each site in 2010, ten single soil samples were collected in order to form a composite sample at depths of 0–20 cm in each plot (three plots per site). The samples were collected in a diagonal transect to a representative area of plots between the row planting. It was assumed that with this sampling procedure, there was no influence from fertilization in planting holes conducted at the sites. The soil samples were air-dried, homogenized, prepared and passed through 2 mm sieves. These samples were used for physical and chemical characterizations and determination of P fractions. Table 2 displays physical and chemical characterizations and demonstrates that the sites presented great variability in such properties [23]. The soils were characterized as very acidic, the levels of P Mehlich-1, exchangeable K and Ca are considered low in most of the sites, and levels of exchangeable Mg were satisfactory in all sites, based both on an average annual increment of wood equal to $50\text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ and on nutritional levels considered critical in *Eucalyptus* plantations [22]. The low level of exchangeable bases and high potential acidity resulted in levels of base saturation (V) bellow the ideal (30%), in most of the sites and the saturation by aluminum (m) within the maximum acceptable for *Eucalyptus* (45%), in most sites [24].

2.3. Soil P Fractions

The P fractions of soil were sequentially extracted according to the Hedley method [12], which separates P of soil into five inorganic fractions, three organic and one residual. The fractionation was done with resin membrane (available Pi), NaHCO_3 (labile Pi and Po adsorbed on the soil surface), NaOH (moderately labile Pi and Po held more strongly by chemisorption to surfaces of Al and Fe oxides), Sonicate + NaOH (Pi and Po adsorbed within surfaces of Al and Fe oxides of soil aggregates—occluded P), HCl (P associated to Ca, derived from primary mineral—apatite) and a digestion with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ (residual P—non-labile; stable Po forms and relatively insoluble Pi forms).

Table 1. Sites, species, plant density, climate types, soil orders, soil texture and age in Brazilian *Eucalyptus* plantations.

Site *	Species	Density (m)	Plot (m ²)	Plants per Plot	Climate ⁽¹⁾	T (°C)	Precipitation (mm)	Soil ⁽²⁾	Soil Texture	Age (Years)
AG	<i>Eucalyptus grandis</i> (G-232)	3.0 × 2.0	144	24	Aw	21.6	1170	LVd	loam	3.0
ANG	<i>E. grandis</i> × <i>E. urophylla</i>	3.0 × 2.0	216	36	Cwa	20.6	1262	RQ	sandy	2.0
BOT	<i>E. grandis</i> (G-232)	3.0 × 2.0	144	24	Cwa	20.2	1302	RQ	sandy	3.0
ITA	<i>E. grandis</i> (Suz.)	3.0 × 2.0	144	24	Cwa	19.9	1308	LVAd	loam	4.0
ALT ⁽³⁾	<i>E. grandis</i> × <i>E. urophylla</i>	3.0 × 2.5	180	24	Aw	20.7	1517	RQ	sandy	7.0
CB1 ⁽⁴⁾	<i>E. grandis</i> × <i>E. urophylla</i>	3.0 × 3.0	360	40	Cwa	20.1	1210	LAd	clayey	9.0
CB2 ⁽⁵⁾	<i>E. grandis</i> × <i>E. urophylla</i>	3.0 × 2.0	144	24	Cwa	20.1	1210	LVd	very clayey	1.4
CB3 ⁽⁶⁾	<i>E. grandis</i> × <i>E. urophylla</i>	3.0 × 2.0	144	24	Cwa	20.1	1210	CXBd	loam	1.6
PA ⁽⁷⁾	<i>E. grandis</i> (Parb.)	3.0 × 2.5	180	24	Cwa	20.8	1249	LVAd	clayey	11.0
VOT ⁽⁸⁾	<i>E. grandis</i> × <i>E. urophylla</i>	3.0 × 2.0	96	16	Cwa	21.3	1287	PVAd	very clayey	1.8

* AG—Agudos, ANG—Angatuba, BOT—Botucatu, ITA—Itatinga, ALT—Altinópolis, CB1—Capão Bonito 1, CB2—Capão Bonito 2, CB3—Capão Bonito 3, PA—Paraibuna, VOT—Votorantim. ⁽¹⁾ Aw means tropical climate with dry winter season and temperature in the coldest month of the year exceeding 18 °C and Cwa means humid temperate climate with dry winter and hot summer. ⁽²⁾ According to Brazilian System of Soil Classification (EMBRAPA 1999): LVd = Latossolo Vermelho distrófico (Oxisol), RQ = Neossolo Quartzarénico (Entisol), LAd = Latossolo Amarelo distrófico (Oxisol), CXBd = Cambissolo Háplico distrófico (Inceptisol), LVAd = Latossolo Vermelho-Amarelo distrófico (Oxisol), PVAd = Argissolo Vermelho-Amarelo distrófico (Ultisol). Six sites received NPK fertilization at planting: ⁽³⁾ 123 kg·ha⁻¹, ⁽⁴⁾ 290 kg·ha⁻¹, ⁽⁵⁾ 183 kg·ha⁻¹, ⁽⁶⁾ 183 kg·ha⁻¹, ⁽⁷⁾ 120 kg·ha⁻¹, and ⁽⁸⁾ 183 kg·ha⁻¹ of the formula 04-28-06. The sites AG and BOT received fertilization applying 15 kg·ha⁻¹ of N and K₂O, 18 kg·ha⁻¹ of N and K₂O, respectively, besides 13% of S and 10% of B, at each site. Supplementary fertilization was also held at planting with other elements to the sites CB1 (0.3% of B), CB2 (10% of Ca, 4% of S, 0.3% of Cu and 0.7% of Zn), CB3 (10% of Ca, 4% of S, 0.3% of Cu and 0.7% of Zn), PA (2.4% of Mg) and VOT (10% of Ca, 4% of S, 0.3% of Cu and 0.7% of Zn). Still was held cover fertilization on the sites CB1 at 3 and 9 months (8 kg·ha⁻¹ of B); CB2 at 3 months (~41 kg of K₂O and 1.5% of B), at 6 months (~36 kg of K₂O, 0.5% of B and 1% of Zn) and at 12 months (~35 kg of K₂O, 0.7% of B and 1% of Zn); CB3 at 3 months (~40 kg of K₂O and 1.5% of B), at 6 months (~75 kg of K₂O, 0.5% of B and 1% of Zn) and at 12 months (~60 kg of K₂O, 0.7% of B and 1% of Zn); and VOT at 3 months (~41 kg of K₂O and 0.3% of B and 1% of Zn) and at 9 months (~126 kg of K₂O and 1.5% of B).

Table 2. Physical and chemical attributes of soils (0–20 cm) under Brazilian *Eucalyptus* plantations.

Site *	Clay	C	P ¹	pH	K	Ca	Mg	CEC	Al sat.	BS
	g·kg ⁻¹	g·dm ⁻³	mg·kg ⁻¹	CaCl ₂		mmol _c ·dm ⁻³			%	%
AG	167	12.2	4.0	3.9	4.8	4.8	2.4	70.7	52.2	17
ANG	100	10.4	8.5	4.0	0.6	5.0	2.5	47.0	44.5	17
BOT	100	8.7	8.3	4.0	0.4	4.0	5.0	49.5	36.9	25
ITA	193	11.0	2.4	2.6	0.9	2.1	2.4	65.6	67.8	8
ALT	67	11.6	6.2	4.3	0.3	11.0	4.0	58.0	20.7	28
CB1	478	16.2	1.4	3.9	1.2	1.7	2.3	94.9	78.2	5
CB2	653	20.3	1.4	4.4	2.7	15.2	9.3	107.3	31.8	25
CB3	272	12.8	3.5	4.1	1.4	9.9	9.5	102.5	43.9	21
PA	365	12.2	2.1	4.1	1.9	6.8	4.2	58.0	33.8	22
VOT	670	29.0	2.2	4.0	5.0	5.0	2.1	125.6	58.4	9

* AG—Agudos, ALT—Altinópolis, CB1—Capão Bonito 1, CB2—Capão Bonito 2, CB3—Capão Bonito 3, ITA—Itatinga, PA—Paraibuna, VOT—Votorantim, ANG—Angatuba, BOT—Botucatu; ¹ P Mehlich-1; CEC—cation exchange capacity at pH 7.0; Al sat.—aluminum saturation = (exchange Al/CEC)100; BS—base saturation.

The sequential separation of fractions of inorganic P (Pi) followed the order: (1) resin-Pi—extracted with 10 mL of deionized water and a resin membrane (1 × 5 cm, ANION 204UZRA, Renics, São Paulo, Brazil) in a horizontal agitator (Tecnal, São Paulo, Brazil) at 120 rpm per 16 h. Afterwards, the resin was removed and placed in a falcon tube of 15 mL, 10 mL of HCl 0.5 mol·L⁻¹ was added and then, after agitation and repose, the extract was used to determine P. (2) NaHCO₃-Pi—soil from the previous stage was centrifuged at 6000 rpm for 20 mins and the supernatant was discharged, then 10 mL of extractor NaHCO₃ 0.5 mol·L⁻¹ at pH 8.5 was added, followed by agitation for 16 h in a horizontal agitator at 120 rpm. Shortly after, the extract was centrifuged at 6000 rpm for 20 mins, the supernatant was put aside (extract), and later more 5 mL of NaCl 0.5 mol·L⁻¹ was added to the extract, derived from agitation at 6000 rpm for 5 mins, which produced the final volume. (3) NaOH-Pi was extracted with 10 mL of NaOH 0.1 mol·L⁻¹ and followed the same subsequent stages used in extraction with bicarbonate; (4) Sonic-Pi (Sonicate + NaOH)—extracted with 10 mL of NaOH 0.1 mol·L⁻¹ following the same subsequent stages used in the previous extraction. However the samples in falcon tubes of 15 mL were subjected to ultrasonification for 2 mins (model: USC-1800A, frequency US: 40 KHz, power US: 153 W; Tecnal, São Paulo, Brazil), before agitation of 16 h. (5) Pi-HCl—extracted with addition of 10 mL of HCl 1.0 mol·L⁻¹ following the same stages with agitation and centrifugation applied for bicarbonate; (6) In the remaining soil, after all extractions, digestion was conducted H₂SO₄ + H₂O₂, at 200 °C, to determine the residual P fraction. In order to determine total P (Pt) in extracts of NaHCO₃ 0.5 mol·L⁻¹, NaOH 0.1 mol·L⁻¹ and sonicate + NaOH 0.1 mol·L⁻¹, an aliquot was taken out and 1 mL of H₂SO₄ 24 mol·L⁻¹ was added and 10 mL of (NH₄)₂S₂O₈ at 7.5% that was autoclaved at 121 °C and 103 kPa for 3 h. After cooling, the volume was increased to 20 mL with distilled water. In extracts derived from different extractions, fractions of Pi and residual P were determined by the colorimetric method of Murphy and Riley [25] after adjusting the pH, using *p*-nitrophenol as an indicator. Regarding Pt fractions, the determination was carried out using the colorimetric method of Dick and Tabatabai [26]. The organic P was calculated through the difference between the concentration of Pt and Pi in each extract (Po = Pt – Pi). The organic fractions were denominated NaHCO₃-Po, NaOH-Po and Sonic-Po.

2.4. Statistical Analysis

Each *Eucalyptus* site was considered to be a fixed-effect treatment due to several sources of variation, such as soil orders and management practices (Table 1). Thus, the data was subjected to the Kolmogorov-Smirnov and Lilliefors normality test and homoscedasticity and normal distribution residuals, which evaluates the normal distribution of analyzed variables. In sequence, the data regarding the P fractions were then submitted to principal component analysis (PCA), which allowed

clustering of the sites evaluated in diagrams that consider the similarity of the data set of the variables along ordination axes, thus seeking associations of the P fractions among *Eucalyptus* sites [27].

The data were also analyzed using one-way analysis as well as the Scott-Knott test to group the means among the *Eucalyptus* sites, at 5% probability. In each *Eucalyptus* site, the three plots were treated as pseudo-replication and were separated by at least several hundred meters from each other. Pseudo-replication is a limitation of the present study, as in many other *Eucalyptus* studies in paired sites and in chronosequences [28,29]. The relationships between all of the P fractions and the physical and chemical attributes of the soil were measured using the Pearson correlation coefficient. The program SAEG 9.1 (SAEG Inst. Inc., Viçosa, Brazil) was employed to conduct all statistical analysis.

Structural equation modeling (SEM) was applied to build a model of the P cycle, to identify interactions among P fractions and to identify which fractions acted as a sink or source of available P. For general fitting, the Chi-square test (χ^2) was employed. Due to the high sensitivity of this test in terms of sample size, the indexes of goodness of fit (GFI), comparative fit (CFI) and root mean squared error of approximation (RMSEA) were also considered as measurement alternatives. The Maximum Likelihood method was used for estimation of parameters. The analyses of SEM were conducted with the aid of the program AMOS, version 22 (IBM—SPSS Inc., Chicago, IL, US).

3. Results

3.1. Distribution of Organic and Inorganic P Fractions

High dissimilarity was observed among the forest sites based on PCA (Figure 1). In the PCA, the direction and length of the arrows show the degree of correlation between fractions and the principal components (X and Y axes). The arrows that correspond to the P fractions with low angles are strongly correlated, whereas the arrows that are perpendicular indicate absence of correlation. Three significant components were obtained (auto values > 1 ; factorial loading ≥ 0.70 in the first component). Only the two first components were used in order to facilitate interpretation of the bi-dimensional graph because component 3 constituted less than 7% of the total data variance. Thus, seven distinct groups were established. The sites ALT (Altinópolis) and BOT (Botucatu), AG (Agudos) and ITA (Itatinga), CB3 (Capão Bonito 3) and PA (Paraibuna) constituted the different groups. The sites ANG (Angatuba), VOT (Votorantim), CB1 (Capão Bonito 1) and CB2 (Capão Bonito 2) did not present similarity with any other site. The components 1 (45%) and 2 (26%) explained 71% of total variation in the data set. The variables most associated with component 1, in descending order of factorial loading, were residual-P (0.90), sonic-Po (0.88), NaHCO₃-Po (0.86), NaOH-Po (0.81) and sonic-Pi (0.74). The fractions of resin-Pi and NaHCO₃-Pi were closely associated to component 2, with factorial loading of 0.94 and 0.92, respectively. They contributed little to dissimilarity between sites, such as fractions of NaOH-Pi and HCl-Pi, which had weak association (<0.70) with the two first components (Figure 1).

There was wide variation in content for all P fractions across the sites (Table 3), as well as in the relative contribution of P fractions to total extracted P (Figure 2). The labile P (resin-Pi + Pi and Po in NaHCO₃) varied between 6% and 16% (average of 10%), the moderately labile P (Pi + Po in NaOH), varied between 28% and 40% (average of 35%), the occluded P (Pi + Po in Sonicate+NaOH) varied between 6% and 19% (average of 12%), the P associated to Ca (HCl-Pi) varied between 0.4% and 3% (average of 1.4%) and the residual-P varied between 35% and 47% (average of 42%). The Po corresponded in average to 70% of total labile P, 51% of moderately labile P and 53% of occluded P (Table 3). Although the soil samples present variations across the organic and inorganic fractions, in general the relative proportions of total Pi and Po, in relation to total extracted P, were similar. Values varied between 18% and 37% for total Pi (average of 28%) and between 16% and 37% for total Po (average of 30%) (Figure 3). Thus, the geochemical P (total Pi + residual-P) constituted around 70% of total extracted P.

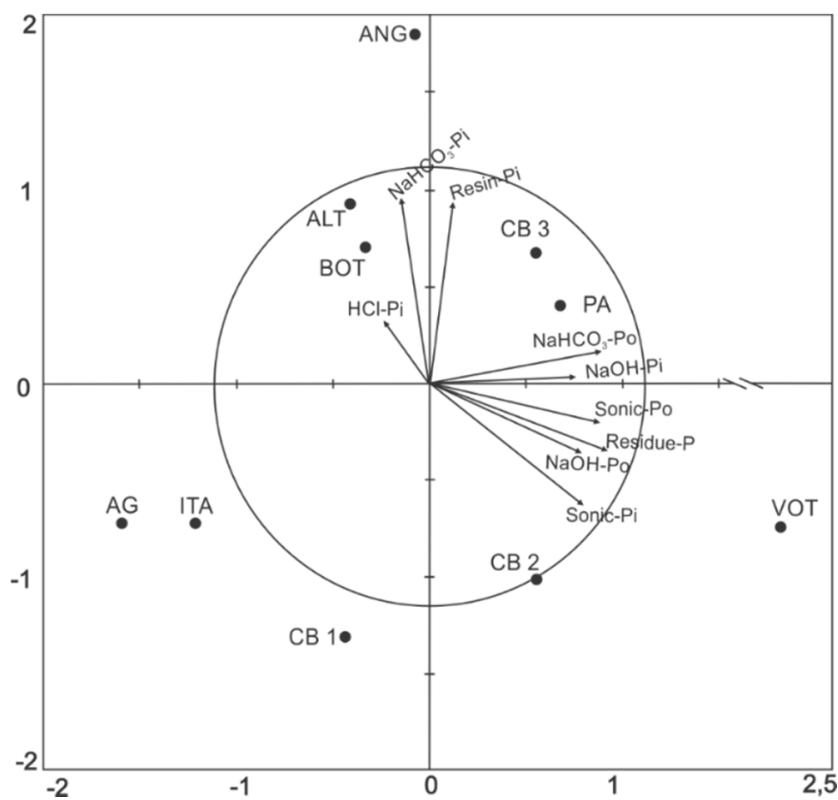


Figure 1. Ordination diagram produced by principal component analysis of soil P fractions (0–20 cm) in Brazilian *Eucalyptus* plantations. Sites: AG—Agudos, ANG—Angatuba, BOT—Botucatu, ITA—Itatinga, ALT—Altinópolis, CB1—Capão Bonito 1, CB2—Capão Bonito 2, CB3—Capão Bonito 3, PA—Paraibuna, VOT—Votorantim.

Table 3. P fractions of soils (0–20 cm) under Brazilian *Eucalyptus* plantations.

Site *	Resin Pi	NaHCO ₃ Pi	NaHCO ₃ Po	NaOH Pi	NaOH Po	Sonic Pi	Sonic Po	HCl Pi	Residue P
	$\text{mg} \cdot \text{kg}^{-1}$								
AG	3.4 C	4.4 C	19.1 B	45.0 B	33.8 E	7.7 B	5.6 D	3.4 C	81.9 F
ANG	8.5 A	12.9 A	23.6 B	53.5 B	59.1 D	7.3 B	16.0 C	3.1 C	98.3 F
BOT	6.9 A	7.0 B	26.7 B	52.2 B	59.2 D	7.9 B	17.6 C	3.6 C	108.7 F
ITA	2.5 C	6.4 B	17.9 B	47.2 B	62.0 D	12.3 B	13.3 C	6.2 B	142.3 E
ALT	5.2 B	8.2 B	22.6 B	83.1 A	19.9 F	10.9 B	8.5 D	10.5 A	150.9 E
CB1	2.8 C	2.8 C	21.0 B	73.2 A	78.5 C	43.3 A	18.0 C	3.7 C	169.9 D
CB2	4.3 B	2.1 C	25.8 B	76.1 A	98.0 B	52.0 A	46.5 B	3.2 C	214.6 B
CB3	5.0 B	7.2 B	35.0 A	79.3 A	79.3 C	18.1 B	17.0 C	6.6 B	165.9 D
PA	5.2 B	7.4 B	25.8 B	84.5 A	38.3 E	26.3 B	58.4 A	4.0 C	188.6 C
VOT	3.5 C	4.4 C	38.2 A	73.9 A	166.8 A	49.6 A	67.7 A	2.6 C	332.3 A
Mean	4.7	6.3	25.6	66.8	69.5	23.5	26.9	4.7	165.3

Groups of means with different letters in the columns differ between itself by Scott & Knott test at 5% probability.

AG—Agudos, ANG—Angatuba, BOT—Botucatu, ITA—Itatinga, ALT—Altinópolis, CB1—Capão Bonito 1, CB2—Capão Bonito 2, CB3—Capão Bonito 3, PA—Paraibuna, VOT—Votorantim.

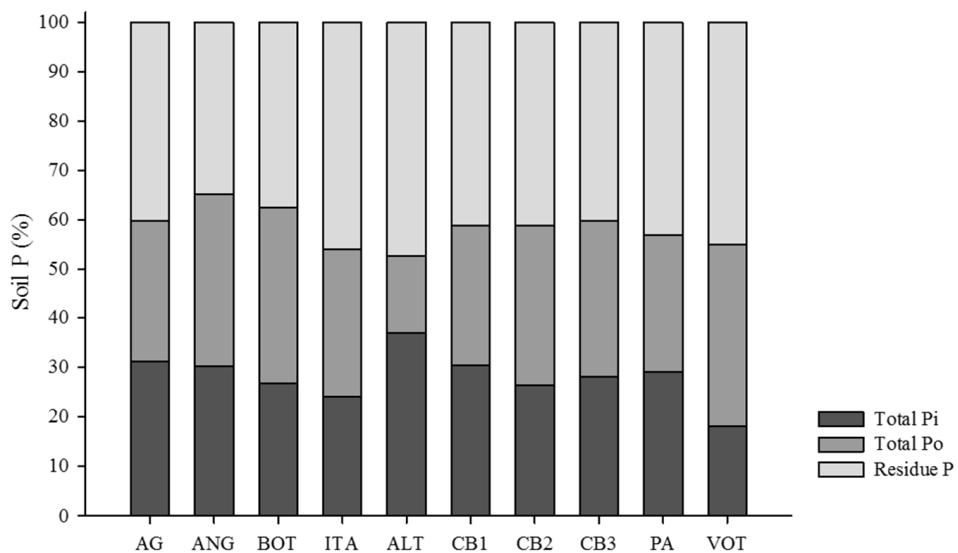


Figure 2. Relative distribution of total organic P, total inorganic P and residue P fractions of total P in soils under Brazilian *Eucalyptus* plantations. Sites: AG—Agudos, ANG—Angatuba, BOT—Botucatu, ITA—Itatinga, ALT—Altinópolis, CB1—Capão Bonito 1, CB2—Capão Bonito 2, CB3—Capão Bonito 3, PA—Paraibuna, VOT—Votorantim.

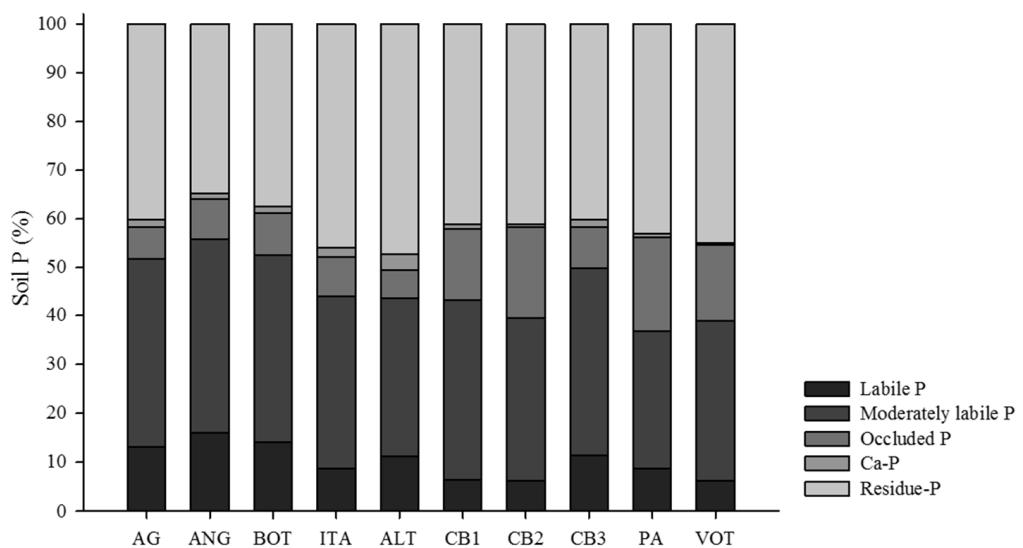


Figure 3. Soil P fractions for each *Eucalyptus* site as a percentage of total P. Sites: AG—Agudos, ANG—Angatuba, BOT—Botucatu, ITA—Itatinga, ALT—Altinópolis, CB1—Capão Bonito 1, CB2—Capão Bonito 2, CB3—Capão Bonito 3, PA—Paraibuna, VOT—Votorantim. Labile P (Resin-Pi + Pi and Po in NaHCO_3), moderately labile P (Pi and Po in NaOH), occluded P (Pi and Po in Sonicate+NaOH), Ca-P (HCl -Pi) and residue-P.

3.2. Relations between P Fractions and Soil Attributes

Significant correlations were observed across all P fractions, except for NaOH-Pi and HCl-Pi (Table 4), which corroborates the results of PCA (Figure 1). The resin-Pi demonstrated a positive relationship with only NaHCO_3 -Pi, which correlated negatively to sonic-Pi. All organic fractions and the sonic-Pi showed positive correlation with residual-P. Regarding soil attributes, there were negative correlations between NaHCO_3 -Pi and clay content and cation exchange capacity (CEC at pH 7.0). The fractions of NaOH-Po, sonic-Pi and residual-P demonstrated a positive correlation with the contents of

clay and organic C and with CEC, and Sonic-Po fraction with both clay and organic C. No significant correlations between the other P fractions and soil attributes were found (Table 5).

Table 4. Pearson correlation coefficients for soil P fractions (0–20 cm) in Brazilian *Eucalyptus* plantations.

Fractions	Resin Pi	NaHCO ₃ Pi	NaHCO ₃ Po	NaOH Pi	NaOH Po	Sonic Pi	Sonic Po	HCl Pi	Residue P
Resin Pi	1	0.78 **	0.13	-0.05	-0.27	-0.45	-0.11	-0.05	-0.37
NaHCO ₃ Pi	-	1	-0.04	-0.16	-0.40	-0.69 *	-0.28	0.23	-0.43
NaHCO ₃ Po	-	-	1	0.48	0.71 *	0.41	0.60	-0.17	0.70 *
NaOH Pi	-	-	-	1	0.17	0.54	0.50	0.31	0.58
NaOH Po	-	-	-	-	1	0.74 *	0.65 *	-0.49	0.83 **
Sonic Pi	-	-	-	-	-	1	0.73 *	-0.39	0.82 **
Sonic Po	-	-	-	-	-	-	1	-0.45	0.85 **
HCl Pi	-	-	-	-	-	-	-	1	-0.18
Residue P	-	-	-	-	-	-	-	-	1

Significant at ** $p < 0.01$; * $p < 0.05$.

Table 5. Pearson correlation coefficients between P fractions and soil attributes (0–20 cm) in Brazilian *Eucalyptus* plantations.

Fractions	Clay	C	pH	SB	Al sat.	CEC
Resin Pi	-0.47	-0.43	0.42	0.04	-0.58	-0.56
NaHCO ₃ Pi	-0.69 *	-0.55	-0.03	-0.27	-0.34	-0.68 *
NaHCO ₃ Po	0.47	0.59	0.41	0.41	-0.15	0.61
NaOH Pi	0.46	0.37	0.59	0.52	-0.37	0.41
NaOH Po	0.79 **	0.88 ***	0.04	0.15	0.37	0.82 **
Sonic Pi	0.98 ***	0.86 **	0.28	0.35	0.23	0.82 **
Sonic Po	0.78**	0.73 *	0.27	0.29	-0.08	0.51
HCl Pi	-0.48	-0.37	-0.06	0.12	-0.37	-0.24
Residue P	0.84 **	0.91 ***	0.18	0.29	0.10	0.78 **

Significant at *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; SB—sum of bases; Al sat.—aluminum saturation; CEC—cation exchange capacity.

3.3. Soil P Transformations

The hypothetical structural model of soil P cycle was composed of four P pools (latent variables): (i) the organic fractions in NaHCO₃ and NaOH were grouped in the organic pool; (ii) the recalcitrant fraction residual-P and sonic-P (Pi and Po), in the occluded pool; (iii) the NaHCO₃-Pi fraction, in the inorganic pool, and (iv) the resin-Pi only in the available pool (Figure 4). The general fitting of the model was highly satisfactory ($X^2 = 14.03$, $df = 9$, $P = 0.121$; GFI = 0.891; CFI = 0.957; RMSEA = 0.139). The available P pool (endogenous latent variable) was directly dependent on pools of inorganic P (Pi), organic P (Po) (endogenous latent variables) and occluded P (exogenous latent variable). However, only the Pi pool had significant direct effects on the available P pool. In turn, the exclusion of direct relationships between the pools of Po and occluded P with the available P pool decreases the model's fit, thereby justifying the relevance of these two pools in the model. Thus, these three pools together explained 50% of total variance in the available P pool. The Po pool had also an indirect negative effect via Pi pool on the available P pool, causing a general negative effect ($\beta = -0.27 + \{(-0.22) \times 0.66\} = -0.415$). The occluded P pool had an indirect negative effect on the pools of Po + Pi and positive effect via Pi pool, but its general effect was positive ($\beta = 0.30 + \{(0.90 \times (-0.27)) + (0.63 \times 0.66) + (0.90 \times (-0.22) \times 0.66)\} = 0.342$). Therefore, the overall effect of these three pools on the available P pool was positive ($\beta = (-0.415) + 0.342 + 0.66 = 0.587$).

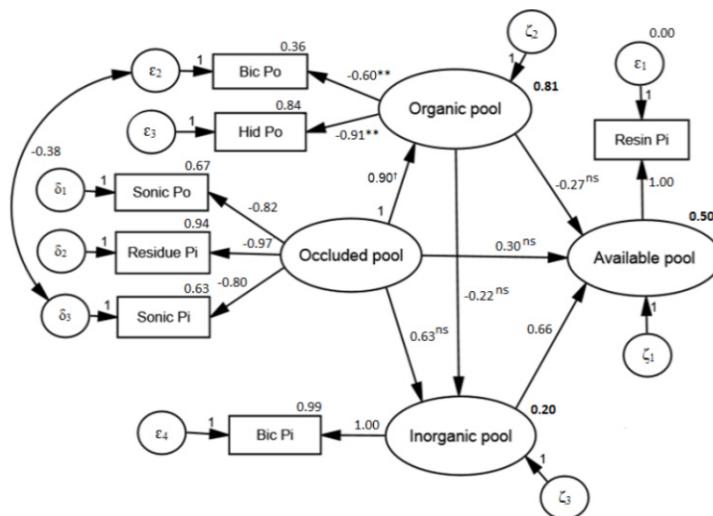


Figure 4. Structural equation modeling for the soil P cycle in Brazilian plantations. All measured variables (in boxes) are represented as effect indicators associated with latent variables (in circles). The numbers correspond to the standardized parameters estimated ($p < 0.001$) and the R^2 values (numbers in bold). [†] Significant at $p < 0.10$, * at $p < 0.05$, ** at $p < 0.01$. Error variables ($\epsilon_1-\epsilon_5, \delta_1-\delta_3$) are standardized values. Model $\chi^2 = 14.03, df = 9, p = 0.121$.

4. Discussion

4.1. Distribution of Organic and Inorganic P Fractions

The high level of dissimilarity among P fractions suggests that each *Eucalyptus* site consisted of a specific environment and any change in production system, management practices and soil conditions could therefore affect the dynamics of P. The sites presenting the greatest clay content also showed the greatest organic C content and CEC (at pH 7) (Table 2). Variables highly correlated with each other ($r = 0.87, p < 0.001$ between clay and C; $r = 0.85, p < 0.01$ between clay and CEC; $r = 0.87, p < 0.01$ between C and CEC). In these sites, the greatest contents of total Po were found, evidencing the strong correlation between Po and soil organic matter [6,9]. Thus, forest management practices that increase soil organic matter tend to lead to an increase of P stocks in organic forms.

An average content of $6.6 \text{ mg} \cdot \text{kg}^{-1}$ of resin-Pi is common in tropical soils of low natural fertility and unfertilized soils [18,21,30,31]. The predominance of Po in NaHCO_3 and NaOH fractions, in most of the sites, had significant linkages to the availability of P to plants. In tropical soils of low fertility, the organic forms of P are considered the main sources of P to plants [32–34], if the maintenance of Po stocks occurs through the process of organic matter recycling.

The organic fractions remained similar to the inorganic fractions, with approximately 30% of total P in each (excluding the residual-P). This proportion of Po is within the range found in tropical regions for several soil orders (from low to medium content of organic C) and land-use systems, with average values of total Po from 26% [9,35,36] to 31% [11,19,37]. Oliveira *et al.* [38] found proportions of total Po ranging from 36% to 46% for soils with high contents of organic matter, a conclusion that supports these findings. The results of the current study suggest that the minimum cultivation system could be a management strategy in the maintenance of high soil Po contents in *Eucalyptus* plantations in Brazil. Residue retention on the soil surface increased organic matter and nutrient status in *Eucalyptus* sites [4].

The content of fractions that compose labile P (resin-Pi + Pi and Po in NaHCO_3) are within the range found by other authors in several soil orders and land-use systems [14,35,39]. The average content of $37 \text{ mg} \cdot \text{kg}^{-1}$ of labile P, which corresponds to an average quantity of $73 \text{ kg} \cdot \text{ha}^{-1}$ of labile P (bulk density = $1 \text{ g} \cdot \text{dm}^{-3}$), could satisfy demand for immobilized P in the aboveground biomass of *Eucalyptus* (leaves, branches, wood and bark), in a cultivation cycle of seven years, assuming

the predominance of the P mineralization process of immobilization. *Eucalyptus* production with a cultivation cycle of 7 years accumulates between 23 and 38 kg·ha⁻¹ of P [40–43]. Thus, in all sites there is potential supply for a high demand of P (50 kg·ha⁻¹) in *Eucalyptus* plantations [44,45]. This indicates the need to improve routine analysis methods for the quantification of available P in soil.

The maintenance of forest residues, such as bark and canopy, has a great effect on P availability in forest sites; together they represent between 57% and 70% of P accumulated in the aboveground biomass of *Eucalyptus* [3,42,43,46]. These sources partially replace the element quantity in the labile fraction, decreasing demand for phosphorus fertilization.

The contents of moderately labile P and occluded P were higher than the average values reported by Gama-Rodrigues *et al.* [21] for different tropical soil orders. The HCl-Pi contents were within the range expected for highly weathered tropical soils [14], which were found in relative proportions of less than 1%. The residual-P contents were also within the expected range for most tropical soils [14,21,31,39], although in reality this P fraction is only used in the long term by the *Eucalyptus*, as it is not affected in the short term by soil management systems and fertilizer application [47].

4.2. Structural Model

In spite of demonstrating association between most P fractions, the simple correlation analysis did not provide enough information to distinguish the direct and indirect effects of all P fractions on resin-Pi. Thus, by applying the SEM method, it was possible to quantify levels of interdependence among P pools and consequently the cause–effect relationship in the transformation of P in soil could be established.

The structural model of P transformations in soil showed that pools of Pi and occluded P act as sources and Po pools act as a sink of P for the available P pool. The main source of P was the Pi pool ($\beta = 0.66$) once the source function of the occluded P pool ($\beta = 0.342$) was counterbalanced by the sink function of the Po pool ($\beta = -0.415$). The adjusted model demonstrated the complexity of the inter-relation of geochemical and biological processes on the available P pool, validating the theoretical concept that indicators of P pools are fractions of P determined by the sequential extraction method, as reported by Sales *et al.* [34]. In addition, the HCl-Pi fraction, as an indicator of the primary mineral P pool, was not part of the adjusted model likely because all soil orders used for modeling are strongly weathered (Table 1). This fraction represented on average only 1.4% of the total P extracted (Figure 3).

The direct and indirect effects (via Pi pool) of the occluded pool as a source of P on the available P pool can be attributed to the dissolution of stable forms of Pi and/or to the mineralization of stable forms of Po found in residual-P fraction, besides the Sonic-Po fraction itself, as indicator variables of this pool. The presence of stable forms of Po in the occluded pool would partially explain its positive relationship with the Po pool [34].

However, the transformations of recalcitrant forms of Po from the occluded pool to labile and moderately labile forms of the Po pool did not result in indirect positive effects of occluded pool on the available P pool. These results suggest that in the Po pool there is a deficit of several forms of Po, which are characteristic of cultivation conditions with no or low phosphorus fertilizer supply in several *Eucalyptus* sites that have soils lacking in P (Table 1).

This deficit reveals the sink function of the Po pool, evidencing the predominance of the immobilization process in relation to mineralization, once the enrichment of available P and Pi pools has caused a reduction of P in the organic pool. Under these conditions, the input of Pi and Po in soil solution via mineralization/decomposition of plant litter/roots can increase the Po pool. Pi can be immobilized by several species of organic anions through complexion reactions and chelation [48,49]. Several forms of labile and moderately labile Po can be adsorbed on soil minerals in varying levels of stability [50], thus decreasing the concentration of these organic compounds in soil solution and, consequently, their availability for mineralization.

The adjusted model therefore shows that P bioavailability is regulated by the interaction between pools of Po, Pi and occluded P (Figure 4). The lability of P progresses over time for all fractions in

such a way that, in the long term, all soil P can become labile [17]. Thus in soils poor in P, plants can acquire P in more recalcitrant forms through several acquisition strategies—for instance, through root exudation of organic anions and phosphatases that increases the desorption, solubilization or mineralization of P from sparingly-availability sources in soil [51]. The organic anions can also reduce the soil's capacity for P-fixation as they occupy adsorption sites on mineral phase [52]. Additionally, the extension of root systems and association with mycorrhizal fungi are other strategies to improve P acquisition [53,54].

In this context, *Eucalyptus* trees, which require relatively long cultivation cycles and present high fine roots and root hairs production [4], elevated root activity of acid phosphatase [55] and intense mycorrhizal associations [56], have access to all P pools to satisfy their demand for P. All these strategies for nutrient acquisition can increase the capacity of plants of acquiring soil P and accelerating the recycling rate, although not the quantity, of P within the ecosystems [57]. Nevertheless, in the evaluated *Eucalyptus* sites, the reversion of Po pool from sink function to source on the available pool P was not warranted (Figure 4) creating a need to enhance management practices to ensure this outcome. Mixed-species plantations of *Eucalyptus* with nitrogen-fixing trees have the potential to increase both levels of soil labile Po and soil phosphatase activity [58]. Therefore, hypothetically, leguminous trees can increase the quality of soil organic matter, which could lead to reversion of the sink function of the Po pool (Figure 4), thus increasing P bioavailability for *Eucalyptus* trees. To verify this model, the improvement of regression coefficient adjustments to structural relations is still required, as well as verification using independent data from *Eucalyptus* sites. This is because the sink function of Po pool on the available P pool differed from the structural models proposed by Gama-Rodrigues *et al.* [21] and Sales *et al.* [34], in which the Po pool acted as a source of P in unfertilized tropical soils in different land uses. Additionally, 50% of unexplained variance in available P pool in our model suggesting that there are additional unmeasured factors that could also influence P availability (Figure 4). Thus, the SEM method cannot be used to rule out the possibility that other untested models could provide an even better fit [59].

5. Conclusions

1. There is great dissimilarity across forestry sites regarding P fractions in soil, caused by differences in the physical and chemical attributes of soil and in forest management practices.
2. The fractionation procedure of P establishes the potential for labile fractions to supply *Eucalyptus* trees, which is not possible in routine methods for analysis of soil fertility.
3. The structural model is adequate to identify functional pools of P in soil and to identify transformation processes of P in soil, based on the source–sink relationships.
4. The available P pool is highly dependent on inorganic and occluded pool and the organic pool acts predominantly as a sink of P on available and inorganic pools. This demonstrates the high level of recalcitrance of the organic pool and the fact that *Eucalyptus* plants must access pools of limited-availability P in order to meet their nutritional demands.

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References

1. Booth, T.H. Eucalypt plantations and climate change. *For. Ecol. Manag.* **2013**, *301*, 28–34. [[CrossRef](#)]
2. Laclau, J.P.; Gonçalves, J.L.D.; Stape, J.L. Perspectives for the management of eucalypt plantations under biotic and abiotic stresses. *For. Ecol. Manag.* **2013**, *301*, 1–5. [[CrossRef](#)]
3. Bazani, J.H.; Gonçalves, J.L.M.; Rocha, J.H.T.; Melo, E.S.A.C.; Prieto, M. Nutrição fosfatada em plantações de eucalipto. *Info. Agron.* **2014**, *148*, 1–11.
4. Gonçalves, J.L.M.; Alvares, C.A.; Higa, A.R.; Silva, L.D.; Alfenas, A.C.; Stahl, J.; Ferraz, S.F.B.; Lima, W.P.; Brancalion, P.H.S.; Hubner, A.; et al. Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. *For. Ecol. Manag.* **2013**, *301*, 6–27. [[CrossRef](#)]
5. Gonçalves, J.L.M. Fertilização de Plantação de Eucalipto. In *Encontro brasileiro de Silvicultura, 2011*; Gonçalves, J.L.M., Pulito, A.P., Arthur Junior, J.C., Silva, L.D., Eds.; Anais Piracicaba: Piracicaba, Brazil, 2011; pp. 85–114.
6. Turner, B.L.; Engelbrecht, B.M.J. Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry*. **2011**, *103*, 297–315. [[CrossRef](#)]
7. Yang, X.; Post, W.M. Phosphorus transformation as a function of pedogenesis: A synthesis of soil phosphorus data using Hedley fractionation method. *Biogeosciences* **2011**, *8*, 2907–2916. [[CrossRef](#)]
8. Condron, L.M.; Turner, B.L.; Cade-Menun, B.J. Chemistry and dynamics of soil organic phosphorus. In *Phosphorus: Agriculture and the Environment, Agronomy Monograph 46*; Sims, J.T., Sharpley, A.N., Eds.; SSSA: Madison, WI, USA, 2005; pp. 87–121.
9. Cunha, G.M.; Gama-Rodrigues, A.C.; Costa, G.S.; Velloso, A.C.X. Fósforo orgânico em solos sob florestas montanas, pastagens e eucalipto no Norte Fluminense. *Rev. Bras. Ciênc. Solo* **2007**, *31*, 667–672. [[CrossRef](#)]
10. Turner, B.L. Resource partitioning for soil phosphorus: A hypothesis. *J. Ecol.* **2008**, *96*, 698–702. [[CrossRef](#)]
11. Zaia, F.C.; Gama-Rodrigues, A.C.; Gama-Rodrigues, E.F. Formas de fósforo no solo sob leguminosas florestais, floresta secundária e pastagem no Norte Fluminense. *Rev. Bras. Ciênc. Solo* **2008**, *32*, 1191–1197. [[CrossRef](#)]
12. Hedley, M.J.; Stewart, J.W.B.; Chauhan, B.S. Changes in inorganic and organic soil-phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci. Soc. Am. J.* **1982**, *46*, 970–976. [[CrossRef](#)]
13. Condron, L.M.; Newman, S. Revisiting the fundamentals of phosphorus fractionation of sediments and soils. *J. Soils Sediment.* **2011**, *11*, 830–840. [[CrossRef](#)]
14. Cross, A.F.; Schlesinger, W.H. A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* **1995**, *64*, 197–214. [[CrossRef](#)]
15. Negassa, W.; Leinweber, P. How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: A review. *J. Plant Nutr. Soil Sci.* **2009**, *172*, 305–325. [[CrossRef](#)]
16. Tiessen, H.; Stewart, J.W.B.; Cole, C.V. Pathways of phosphorus transformations in soils of differing pedogenesis. *Soil Sci. Soc. Am. J.* **1984**, *48*, 853–858. [[CrossRef](#)]
17. Guo, F.; Yost, R.S. Partitioning soil phosphorus into three discrete pools of differing availability. *Soil Sci.* **1998**, *163*, 822–832. [[CrossRef](#)]
18. Cardoso, I.; Janssen, B.; Oenema, O.; Kuyper, T. Phosphorus pools in Oxisols under shaded and unshaded coffee systems on farmers' fields in Brazil. *Agrofor. Syst.* **2003**, *58*, 55–64. [[CrossRef](#)]
19. Frizano, J.; Vann, D.R.; Johnson, A.H.; Johnson, C.M.; Vieira, I.C.G.; Zarin, D.J. Labile phosphorus in soils of forest fallows and primary forest in the Bragantina region, Brazil. *Biotropica* **2003**, *35*, 2–11. [[CrossRef](#)]
20. Gatiboni, L.C.; Brunetto, G.; Rheinheimer, D.S.; Kaminsk, J. Fracionamento químico das formas de fósforo do solo: Uso e limitações. *Tóp. Ciênc. Solo* **2013**, *8*, 141–187.
21. Gama-Rodrigues, A.C.; Sales, M.V.S.; Silva, P.S.D.; Comerford, N.B.; Cropper, W.P.; Gama-Rodrigues, E.F. An exploratory analysis of phosphorus transformations in tropical soils using structural equation modeling. *Biogeochemistry* **2014**, *118*, 453–469. [[CrossRef](#)]
22. Barros, N.F.; Novais, R.F. Eucalipto. In *Recomendações para uso de Corretivos e Fertilizantes em Minas Gerais*, 5th ed.; Ribeiro, A.C., Guimarães, P.T.G., Alvarez, V.V.H., Eds.; UFV: Viçosa, Brazil, 1999; pp. 303–305.
23. Empresa Brasileira de Pesquisa Agropecuária—Embrapa. *Manual de Análises Químicas de Solos, Plantas e Fertilizantes*; Empresa Brasileira de Pesquisa Agropecuária: Brasília, Brazil, 1999.
24. Alvarez, V.V.H.; Ribeiro, A.C. Calagem. In *Recomendação Para Uso de Corretivos e Fertilizantes em Minas Gerais*, 5th ed.; Ribeiro, A.C., Guimarães, P.T.G., Alvarez, V.V.H., Eds.; UFV: Viçosa, Brazil, 1999; pp. 43–60.

25. Murphy, J.; Riley, J.P. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.* **1962**, *27*, 31–36. [[CrossRef](#)]
26. Dick, W.A.; Tabatabai, M.A. Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. *J. Environ. Qual.* **1977**, *6*, 82–85. [[CrossRef](#)]
27. Zaia, F.C.; Gama-Rodrigues, A.C.; Gama-Rodrigues, E.F.; Moço, M.K.S.; Fontes, A.G.; Machado, R.C.R.; Baligar, V.C. Carbon, nitrogen, organic phosphorus, microbial biomass and N mineralization in soils under cacao agroforestry systems in Bahia, Brazil. *Agrofor. Syst.* **2012**, *86*, 197–212. [[CrossRef](#)]
28. Lima, A.M.N.; Silva, I.R.; Neves, J.C.L.; Novais, R.F.; Barros, N.F.; Mendonça, E.S.; Smyth, T.J.; Moreira, M.S.; Leite, F.P. Soil organic carbon dynamics following afforestation of degraded pastures with eucalyptus in Southeastern Brazil. *For. Ecol. Manag.* **2006**, *235*, 219–231. [[CrossRef](#)]
29. Barreto, P.A.B.; Gama-Rodrigues, A.C.; Gama-Rodrigues, E.F.; Barros, N.F. Nitrogen balance in soil under eucalyptus plantations. *Rev. Bras. Ciênc. Solo* **2012**, *36*, 1239–1248. [[CrossRef](#)]
30. Friesen, D.K.; Rao, I.M.; Thomas, R.J.; Oberson, A.; Sanz, J.I. Phosphorus acquisition and cycling in crop and pasture systems in low fertility tropical soils. *Plant Soil* **1997**, *196*, 289–294. [[CrossRef](#)]
31. Araújo, M.S.B.; Schaefer, C.E.R.; Sampaio, E.V.S.B. Soil phosphorus fractions from toposequences of semi-arid Latosols and Luvisols in northeastern Brazil. *Geoderma* **2004**, *119*, 309–321. [[CrossRef](#)]
32. Beck, M.A.; Sanchez, P.A. Soil-phosphorus fraction dynamics during 18 years of cultivation on a typic paleudult. *Soil Sci. Soc. Am. J.* **1994**, *58*, 1424–1431. [[CrossRef](#)]
33. Tiessen, H. Phosphorus dynamics in tropical soils. In *Phosphorus: Agriculture and the Environment, Agronomy Monograph*; Sims, J.T., Sharpley, A.N., Eds.; SSSA: Madison, WI, USA, 2005; pp. 253–326.
34. Sales, M.V.S.; Gama-Rodrigues, A.C.; Comerford, N.B.; Cropper, W.P.; Gama-Rodrigues, E.F.; Oliveira, P.H.G. Respecification of structural equation models for the P cycle in tropical soils. *Nutr. Cycl. Agroecosyst.* **2015**, *102*, 347–358. [[CrossRef](#)]
35. Dieter, D.; Elsenbeer, H.; Turner, B.L. Phosphorus fractionation in lowland tropical rainforest soils in central Panama. *Catena* **2010**, *2*, 118–125. [[CrossRef](#)]
36. Xavier, F.A.S.; Almeida, E.F.; Cardoso, I.M.; Mendonca, E.S. Soil phosphorus distribution in sequentially extracted fractions in tropical coffee-agroecosystems in the Atlantic Forest biome, Southeastern Brazil. *Nutr. Cycl. Agroecosyst.* **2011**, *89*, 31–44. [[CrossRef](#)]
37. Turner, B.L. Organic phosphorus in Madagascan rice soils. *Geoderma* **2006**, *136*, 279–288. [[CrossRef](#)]
38. Oliveira, R.I.; Gama-Rodrigues, A.C.; Gama-Rodrigues, E.F.; Zaia, F.C.; Pereira, M.G.; Fontana, A. Organic phosphorus in diagnostic surface horizons of different Brazilian soil orders. *Rev. Bras. Ciênc. Solo* **2014**, *38*, 1411–1420. [[CrossRef](#)]
39. Schmitt, D.E.; Comin, J.J.; Gatiboni, L.C.; Tiecher, T.; Lorensini, F.; Melo, G.W.B.; Girotto, E.; Guardini, R.; Heinzen, J.; Brunetto, G. Phosphorus fractions in sandy soils of vineyards in southern Brazil. *Rev. Bras. Ciênc. Solo* **2013**, *37*, 472–481. [[CrossRef](#)]
40. Bellote, A.F.J.; Sarruge, J.R.; Haag, H.P.; Oliveira, G.D. Extração e exportação de nutriente pelo *Eucalyptus grandis* Hill ex-Maiden em função da idade: 1-Macronutrientes. *IPEF Piracicaba* **1980**, *20*, 1–23.
41. Poggiani, F.; Couto, H.T.Z.; Corradini, L.; Fazzio, E.C.M. Exportação de biomassa e nutrientes através da exploração dos troncos e das copas de um povoamento de *Eucalyptus saligna*. *IPEF Piracicaba* **1983**, *25*, 37–39.
42. Santana, R.C.; Barros, N.F.; Neves, J.C.L. Biomassa e conteúdo de nutrientes de procedências de *Eucalyptus grandis* e *Eucalyptus saligna* em alguns sítios florestais do Estado de São Paulo. *Sci. For.* **1999**, *56*, 155–169.
43. Santana, R.C.; Barros, N.F.; Novais, R.F.; Leite, H.G.; Comerford, N.B. Alocação de nutrientes em plantios de eucalipto no Brasil. *Rev. Bras. Ciênc. Solo* **2008**, *32*, 2723–2733. [[CrossRef](#)]
44. Laclau, J.P.; Deleporte, P.; Ranger, J.; Bouillet, J.P.; Kazotti, G. Nutrient dynamics throughout the rotation of *Eucalyptus* clonal stands in Congo. *Ann. Bot.* **2003**, *91*, 879–892. [[CrossRef](#)] [[PubMed](#)]
45. Silva, J.V.; Nogueira, G.S.; Santana, R.C.; Leite, H.G.; Oliveira, M.L.R.; Almado, R.P. Produção e acúmulo de nutrientes em povoamento de eucalipto em consequência da intensidade do desbaste e da fertilização. *Pesq. Agropec. Bras.* **2012**, *47*, 1555–1562. [[CrossRef](#)]
46. Gama-Rodrigues, A.C.; Barros, N.F. Ciclagem de nutrientes em floresta natural e plantios de eucalipto e de dandá no sudeste da Bahia, Brasil. *Rev. Árvore* **2002**, *26*, 193–207.
47. Gatiboni, L.C.; Kaminski, J.; Rheinheimer, D.S.; Flores, J.P.C. Biodisponibilidade de formas de fósforo acumuladas em solo sob sistema plantio direto. *Rev. Bras. Ciênc. Solo* **2007**, *31*, 691–699. [[CrossRef](#)]
48. Jones, D.L. Organic acids in the rhizosphere—A critical review. *Plant Soil* **1998**, *205*, 25–44. [[CrossRef](#)]

49. Hinsinger, P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. *Plant Soil* **2001**, *237*, 173–195. [[CrossRef](#)]
50. Celi, L.; Barberis, E. Abiotic stabilization of organic phosphorus in the environment. In *Organic Phosphorus in the Environment*; Turner, B.L., Frossard, E., Baldwin, D., Eds.; CABI Publishing: Wallingford, WI, USA, 2005; pp. 113–132.
51. Richardson, A.E.; Lynch, J.P.; Ryan, P.R.; Delhaize, E.; Smith, F.A.; Smith, S.E.; Harvey, P.R.; Ryan, M.H.; Veneklaas, E.J.; Lambers, H.; *et al.* Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil* **2011**, *349*, 121–156. [[CrossRef](#)]
52. Guppy, C.N.; Menzies, N.W.; Moody, P.W.; Blamey, F.P.C. Competitive sorption reactions between phosphorus and organic matter in soil: A review. *Aust. J. Soil* **2005**, *43*, 189–202. [[CrossRef](#)]
53. Lambers, H.; Raven, J.A.; Shaver, G.R.; Smith, S. Plant nutrient-acquisition strategies change with soil age. *Trends Ecol. Evol.* **2008**, *23*, 95–103. [[CrossRef](#)] [[PubMed](#)]
54. Lambers, H.; Brundrett, M.C.; Raven, J.A.; Hopper, S.D. Plant mineral nutrition in ancient landscapes: High plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* **2010**, *334*, 11–31. [[CrossRef](#)]
55. Radersma, S.; Grierson, P. Phosphorus mobilization in agroforestry: Organic anions, phosphatase activity and phosphorus fractions in the rhizosphere. *Plant Soil* **2004**, *259*, 209–219. [[CrossRef](#)]
56. Grierson, P.F.; Adams, M.A. Plant species affect acid phosphatase, ergosterol and microbial P in a Jarrah (*Eucalyptus marginata* Donn ex Sm.) forest in south-western Australia. *Soil Bio. Biochem.* **2000**, *32*, 1817–1927. [[CrossRef](#)]
57. Vitousek, P.M.; Porder, S.; Houlton, B.Z.; Chadwick, O.A. Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol. Appl.* **2010**, *20*, 5–15. [[CrossRef](#)] [[PubMed](#)]
58. Zou, X.; Binkley, D.; Caldwell, B.A. Effects of dinitrogen-fixing trees on phosphorus biogeochemical cycling in contrasting forests. *Soil Sci. Soc. Am. J.* **1995**, *59*, 1452–1458. [[CrossRef](#)]
59. Barto, E.K.; Alt, F.; Oelmann, Y.; Wilcke, W.; Rillig, M. Contributions of biotic and abiotic factors to soil aggregation across a land use gradient. *Soil Biol. Biochem.* **2010**, *42*, 2316–2324. [[CrossRef](#)]



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