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Phosphorus availability in relation to soil properties and forest productivity in *Pinus sylvestris* L. plantations

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Abstract

• *Key message Pinus sylvestris* L. productivity in Spanish plantations is driven by P availability, which, in turn, is determined by the activity of soil microorganisms, responsible for inorganic P solubilization; Fe and Al contents, responsible for P retention; and organic matter, which is source of organic P, inhibits its precipitation as insoluble compounds, and reduces P retention.

• *Context* Phosphorus is often a limiting nutrient in forest ecosystems mainly due to the low solubility of P compounds and the sorption processes occurring in soils.

• *Aims* The main aims of this work were to evaluate soil P availability, to assess which soil properties are driving P availability, and to study whether soil P availability is determining forest productivity in *Pinus sylvestris* L. plantations in Northern Spain.

• *Methods* Soil properties and forest productivity were studied in 34 plots located in monospecific *P. sylvestris* plantations. Tiessen and Moir (Canadian Society of Soil Science 75–86, 1993) sequential fractionation method was carried out to determine different forms of soil P and to provide a comprehensive assessment of available P in soils. To explore the relationships between these variables, canonical correlation analyses and Pearson's correlations were studied.

• *Results* Significant correlations were found between P fractions and soil properties related to Fe and Al contents, organic matter, and microbial biomass. Besides, significant correlations were found between site index and the studied P fractions except for P extracted with anion exchange membrane (P_{AEM}) and the recalcitrant P fraction.

• *Conclusion* In the studied soils, P availability is low and the predominant fractions of P are the recalcitrant forms. Aluminum and iron contents in the soils studied play an important role in sorption processes related to the highly and moderately labile P

This article is part of the topical collection on Mediterranean pines

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Contribution of the co-authors Teresa Bueis designed the experiment, carried out the field and laboratory work except P fractionation, run the data analysis, discussed the results, and wrote the paper.

Felipe Bravo designed the experiment, coordinated the research project, and corrected the manuscript.

Valentín Pando supported the statistical analysis and corrected the manuscript.

Yaovi-Abel Kissi carried out part of the laboratory work (P fractionation) and corrected the manuscript.

María-Belén Turrión designed the experiment, supervised the laboratory analysis, and corrected the manuscript.

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fractions and the organic phosphorus. P availability seems to be regulated by both processes: biochemical mineralization, where phosphatase activity is relevant, and biological mineralization of the soil organic matter. Phosphorus availability affects forest productivity in the *Pinus sylvestris* plantations studied.

Keywords Site index \cdot Phosphorus fractionation \cdot Sequential extraction \cdot Labile phosphorus \cdot Recalcitrant phosphorus \cdot Microbial biomass

1 Introduction

Phosphorus is known as "the key to life" because of its implication in most vital processes of organisms (Troeh and Thompson 1993) such as energy transfer, metabolic processes, and genetic codification (Marschner 1995). Phosphorus limitation is common in terrestrial ecosystems (Vitousek 1984) and forest productivity is also commonly P-limited (Achat et al. 2010; Bueis et al. 2016; Chen et al. 2008; Slazak et al. 2010).

Even when there is abundant total P in soil, P deficiencies are very common because it is the nutrient with the greatest problem in relation to its solubility, which is strongly dependent on the soil pH. Phosphorus in soils appears both in organic and inorganic forms. Inorganic forms of P in soils come from rocks as phosphates, most of which are not soluble.

Plants take up phosphates dissolved in soil solution, mainly as $H_2PO_4^-$ and $HPO_4^{2^-}$. Phosphate ions can also be adsorbed by the soil anion exchange complex. The phosphates dissolved in the soil solution and those phosphates weakly adsorbed by the exchange complex are considered available for plants and microorganisms. Part of the phosphates is more strongly retained by the exchange complex and can become available in the short term. These fractions are considered labile forms of P (Troeh and Thompson 1993). Other forms of P are included in primary Ca minerals with low solubility. Finally, the most highly recalcitrant forms of P are the stable forms, which are also in form of Ca minerals, and the residual forms, which are thought to be mainly organic forms of P associated with clays and Fe and Al oxides (Turrión et al. 2000b; Zamuner et al. 2008).

Organic matter plays a major role in P availability in forest soils. Organic matter is a direct source of available P in soils through the mineralization from dead organic matter and through the release from microbial biomass (Achat et al. 2010). Therefore, microbial biomass P is a very active part of soil organic matter. Besides, microbial biomass has the ability to immobilize P when P availability increases acting as a nutrient pool which is released into the soil when microorganisms die (Jenkinson and Ladd 1981). Furthermore, the symbiosis established between plant roots and mycorrhizal fungi increase P uptake (da Silva et al. 2017; Desai et al. 2014). Organic matter also presents indirect effects on P availability, especially in highly acidic soils. On one hand, organic matter can complex Fe and Al ions responsible for P precipitation as insoluble Fe and Al phosphates, reducing P retention



in soils (Haynes and Mokolobate 2001). In addition, the organic acids originated from organic matter decomposition enhance the solubility of calcium phosphates (Troeh and Thompson 1993) and are adsorbed by Fe and Al oxides, blocking the sorption of P (Earl et al. 1979). Finally, soil microbial activities, which are responsible for the production of phosphatase enzymes that hydrolyze organic P into inorganic P, benefit from the energy and C supply from organic matter (Allison and Vitousek 2005).

Pinus sylvestris L. has the widest natural distribution among pine species in the world. It extends from Western Spain to Eastern Russia and from Southern Spain to Northern Scandinavia. Therefore, Spanish stands constitute the southern and western limit of *P. sylvestris* distribution where it occupies more than one million hectares (Serrada et al. 2008). During the second half of the twentieth century, many *Pinus* plantations were carried out in Castilla y León region to recover the vegetal cover, to protect soils against erosion and to produce wood. In Castilla y León, *P. sylvestris* plantations occupy near 300,000 ha.

Bueis et al. (2016) found that forest productivity was related to Al content in soils under *P. sylvestris* plantations of the acidic plateau in the north of Castilla y León. Phosphorus availability is related to Fe and Al contents in acidic soils such as the ones under *P. sylvestris* plantations in the study area. Therefore, it is crucial to assess the relationship between P availability and forest productivity in these stands.

Although the general processes involved in P availability are known, further studies are necessary to clarify the importance of each process on specific ecosystems (Hou et al. 2014). For that purpose, the Hedley et al. (1982) P sequential fractionation method modified by Tiessen and Moir (1993) was applied in this study. This method allows the determination of the different P pools in soils in relation to their availability for plants and microbial uptake (Cross and Schlesinger 1995).

Our aims were (1) to assess the soil P availability in the soils studied, (2) to determine which soil processes are driving P availability in the soils studied, and (3) to determine whether the soil P availability is driving forest productivity and which soil P fractions are the most determinant ones in the forest productivity of the stands studied. We hypothesize that (1) there is a relationship between the available forms of P in the forest soils studied and other labile or non-labile P fractions which may act as P source in the short, mid, or long term; (2) amorphous A1 and Fe forms, exchangeable A1, and

organically bound Al and Fe in soils are the most determining soil parameters for P availability in the acidic soils studied; and (3) forest productivity is related to P availability in the *P. sylvestris* plantations studied.

2 Material and methods

2.1 Study area and sampling plots

The study area was located in the acidic plateau in the north of the region of Castilla y León, Spain (Annex Table 8), where 34 plots were set up covering the widest range of stand characteristics (age and stocking) in *P. sylvestris* plantations. These plots had rectangular shape $(30 \times 40 \text{ m})$ and the average distance between them is 14 km. The main characteristics of these plots are presented in Tables 1 and 2. Lithology was composed of quarzitic gravels with a sandy-clay matrix (IGME 1975). Soils were from strongly acidic to moderately acidic with loam or sandy-loam texture (Bueis et al. 2019) and were classified as Inceptisols (Herrero et al. 2016).

2.2 Soil sampling and analysis

Soil sampling focused on the 0- to 10-cm mineral topsoil. That mineral topsoil corresponds with the Ah horizon in all the studied soils. On each plot, four sampling points located 5-m distance from the center of the plot in N, S, E, and W directions were established. The four subsamples were mixed to get a composite sample per plot. Soil samples were air-dried and sieved (2 mm) and physical, chemical, and biochemical parameters were determined (Bueis et al. 2016) including the following: pH in a 1:2.5 (soil/water) suspension, using pH meter; soil particle size distribution (pipette method); cation exchange capacity and exchangeable acidity (Bascomb 1964); easily oxidizable C (Walkley and Black 1934); exchangeable bases (Schollenberger and Simon 1945); Al and Fe within amorphous Al and Fe oxyhydroxides (Blakemore et al. 1987); organically bound Fe and Al (Bascomb 1968); exchangeable Al (Bertsch and Bloom 1996). Biochemical parameters analyzed were microbial biomass C and P (Cmic, Pmic, Murphy and Riley 1962, Vance et al. 1987),

 Table 1
 Main characteristics of the plots

Parameters	Mean	SD	Min	Max
Elevation (m.a.s.l.)	1069	72	926	1180
Latitude (°)	42.7	0.1	42.6	42.8
Longitude (°)	-4.7	0.3	- 5.2	-4.1
Slope (%)	2.4	3.2	0.0	12.0
Total precipitation (mm)	738	79	597	943
Mean annual temperature (°C)	9.6	0.4	8.8	10.4

Table 2 Main characteristics of the stand

Parameters	Mean	SD	Min	Max
Age (years)	39.7	7.4	28.0	54.0
Stocking (trees ha ⁻¹)	1098	428	400	2083
Mean height (m)	13.6	3.8	7.3	22.9
Basal area (m ² ha ⁻¹)	32.8	8.2	16.8	53.6
Site index (m at 50 years age)	18.0	2.8	12.6	22.6

mineralizable C (Cmin, Isermeyer 1952), and acid phosphatase activity (AcPhos; Tabatabai and Bremner 1996). More detailed information about the procedures carried out to determine microbial biomass and other biological parameters analyzed can be obtained in Bueis et al. (2018b).

Phosphorus fractionation was carried out following the method developed by Hedley et al. (1982) and modified by Tiessen and Moir (1993). The first fraction, extracted with anion exchange membranes (PAEM), is considered the readily available P (Turrión et al. 2008). Then, sequential extractions were carried out on the residue of the membrane extraction with progressively stronger extractants. The following extraction was carried out with 0.5 M NaHCO₃, to determine the highly labile P adsorbed in the soil exchange complex (mainly to the surface of Fe and Al crystalline oxides), which can be easily transformed into available forms (Schoenau and Huang 1991; Schoenau et al. 1989; Turrión et al. 2000a). The organic and inorganic fractions of highly labile P were determined (P_iNaHCO₃, P_oNaHCO₃) through the determination of total P in NaHCO₃ extracts after digesting with 0.5 M H₂SO₄ and potassium persulfate at 120 °C in an autoclave (Tiessen and Moir 1993) to transform the organic P into inorganic forms. The organic P in these extracts was determined as the difference between the total P and the inorganic P. The moderately labile P, strongly retained by chemisorption (Schoenau et al. 1989; Turrión et al. 2000a), was determined by the extraction with 0.1 M NaOH and organic and inorganic fractions were also determined to distinguish between the soil P associated with the humic compounds and the soil P adsorbed by Fe and Al oxides (Schoenau et al. 1989; Turrión et al. 2000b). The primary P (PHCl_{1M}), inorganic P included in Ca primary minerals of low solubility (Schoenau et al. 1989; Shen et al. 2011; Turrión et al. 2000b), was extracted with 1 M HCl. The most recalcitrant fractions are the stable and the residual P. The stable P (PHCl_{conc}) was determined by the extraction with concentrated HCl at 90 °C and the residual P was extracted with HClO₄ and digested at 230 °C. Residual P (PHClO₄) is thought to be composed of inorganic P tied up to amorphous Fe and Al oxides and a large proportion of resistant organic P associated with clay and Fe and Al oxides (Schoenau et al. 1989; Zamuner et al. 2008). For P determination in the extracts, the method by Murphy and Riley (1962) modified by Watanabe and Olsen (1965) was applied, using an ultraviolet/



visible spectrophotometer Hitachi U2001. The sum of the stable and the residual P fractions is considered the highly recalcitrant P fraction (Turrión et al. 2000b) which is also referred to as highly resistant, unavailable, refractory, or occluded forms of P (Richter et al. 2006).

2.3 Forest productivity

Site index (dominant height at a reference age) is a useful tool for forest productivity estimation (Skovsgaard and Vanclay 2008) and it is strongly correlated with wood production. Site index was calculated according to the equation developed by Río et al. (2006):

$$H_{02} = \frac{40,3331}{1 - \left[\left(1 - \frac{40,3331}{H_{01}} \right) \left(\frac{T_1}{T_2} \right)^{1,5003} \right]}$$
(1)

where H_{01} is the current dominant height in meters (at current age T_1) and H_{02} is the site index, the dominant height at the reference age of 50 years (T_2). Summary of the characteristics of the plots studied is presented in Table 2.

2.4 Statistical analyses

To explore the relationships between P fractions considered (Tiessen and Moir 1993), Pearson's correlation coefficients (in R: cor.test; method: Pearson) were calculated and principal component analysis (PCA) was performed (in R: princomp). To assess the relationships between P fractions and soil parameters, Pearson's correlation coefficients were calculated, and canonical correlation analyses (CCA) were performed using CCA and CCP packages in R (González and Déjean 2012; Menzel 2012). The high amount of soil parameters of interest made it troublesome to interpret an only canonical correlation analysis. Therefore, canonical correlation analyses were carried out to study (a) the relationships between P fractions and soil parameters related to Fe and Al, (b) P fractions and soil parameters related to organic matter, and (c) P fractions and soil parameters related to Ca. Finally, to study the relationships between site index (as an estimation of forest productivity) and P fractions, Pearson's correlation coefficients were calculated, and canonical correlation analysis was performed. Due to the high correlation found between the different groups of variables studied, some of them have been removed from PCA and CCA and others have been grouped together (PtNaHCO₃ and PtNaOH). Kaiser's Measure of Sampling Adequacy (in R: KMO; psych package; Revelle 2018) was calculated to test whether the sample size was adequate or not for PCA and CCA (Cerny and Kaiser 1977). The studied variables were tested for linearity, outliers, and normality (in R: shapiro.test) and those variables not normally distributed were transformed $(\log(x))$ and \sqrt{x} before the





analyses. More specifically, the following transformations were needed to achieve normality: $log(P_iNaHCO_3)$, $\sqrt{P_oNaHCO_3}$, $log(P_TNaHCO_3)$, $log(P_iNaOH)$, $log(PHCl_{1M})$, $log(Ca^{2+})$, \sqrt{Pmic} , log(Cmin), and \sqrt{AcPhos} . In order to enhance the readability of the results and discussion sections, we will only refer to the variables themselves in the following text, unless it affects the interpretation. Statistical analyses were performed with R software (TeamR 2015).

3 Results

3.1 Phosphorus fractions and relationships between them

Summary of the characteristics of the soils studied and their P fractions is shown in Tables 3 and 4, respectively (Bueis et al. 2018a).

The stable P fraction (PHCl_{conc}) is the biggest fraction in the soils studied followed by the moderately labile organic fraction. The smallest P fraction in these soils is the primary P that includes P forms in primary Ca minerals, only accounting for 2% of total P, followed by the P_{AEM} (less than 3% of total P). The most recalcitrant forms of P (P_{rec}) which include the stable (PHCl_{conc}) and residual P (PHClO₄) account for more than 42% of total P.

In the principal component analysis of P fractions, two principal components accounted for 83.4% of total variance and presented eigenvalues higher than 1. Kaiser's Measure of Sampling Adequacy (MSA) was 0.7 and, therefore, this analysis was considered statistically acceptable. The first principal component was negatively correlated to the highly labile P fraction (PtNaHCO₃; Annex Table 9 and Fig. 1), the moderately labile fraction (P_tNaOH), and the primary P (PHCl_{1M}). The second principal component was positively correlated to the PAEM and negatively correlated to the recalcitrant forms of P (Prec). According to the principal component analysis and the Pearson correlations (Table 5), the P_{AEM} fraction was positively correlated to the inorganic highly labile P (PiNaHCO₃) and to the primary P (PHCl_{1M}). The highly and moderately labile fractions were also significantly correlated with each other. Positive correlations were also found between the most recalcitrant forms of P (Prec) and the organic highly labile (P_oNaHCO₃), the organic moderately labile P (P_oNaOH), and the primary forms of P (PHCl_{1M}).

3.2 Relationships between P fractions and Fe and Al in soils

The first two canonical factors in the canonical correlation analysis of P fractions and Fe and Al-related parameters were significant according to Wilk's lambda test (P < 0.5) and presented canonical correlations of 0.86 and 0.68, respectively.

 Table 3
 Main characteristics of soils

Soil variables	Mean	SD	Min	Max
Clay (%)	16.0	3.3	9.0	26.4
Sand (%)	70.6	7.3	55.1	83.7
Silt (%)	13.4	5.2	6.1	24.5
pH	4.5	0.5	3.7	5.6
Exchangeable acidity $(\text{cmol}_{(+)} \text{ kg}^{-1})$	13.4	4.6	5.6	23.6
Total organic C/total organic P	1114	896	338	4311
$Ca^{+2} (cmol_{(+)} kg^{-1})$	0.9	0.7	0.2	2.9
$K^{+} \left(\operatorname{cmol}_{(+)} kg^{-1} \right)$	0.17	0.05	0.08	0.34
$Mg^{+2} (cmol_{(+)} kg^{-1})$	0.13	0.13	0.01	0.52
Cation exchange capacity $(\text{cmol}_{(+)} \text{kg}^{-1})$	18.8	3.1	11.8	24.2
Base saturation (%)	7.3	5.3	2.4	21.1
Easily oxidizable carbon (%)	2.2	0.6	1.3	3.4
Al within amorphous oxyhydroxides (mg g^{-1})	6.6	2.5	2.5	11.6
Exchangeable Al $(\text{cmol}_{(+)} \text{kg}^{-1})$	6.8	3.8	0.0	14.3
Organically bound Al (mg g^{-1})	9.1	3.9	2.7	17.9
Fe within amorphous oxyhydroxides (mg g^{-1})	4.0	1.5	1.7	7.9
Organically bound Fe (mg g^{-1})	4.6	1.7	1.8	7.7
Microbial biomass C (mg kg ⁻¹)	113.4	47.4	45.5	232.9
Mineralizable C (mg kg ^{-1} week ^{-1})	56.3	21.6	22.0	112.1
Microbial biomass P (mg kg ^{-1})	11.0	5.8	4.6	32.2
Acid phosphatase activity ($\mu g g^{-1} h^{-1}$)	8.8	6.0	0.5	24.4

Al_A, Fe_A, Al and Fe within amorphous Al and Fe oxyhydroxides

Kaiser's Measure of Sampling Adequacy (MSA) was 0.77 and, therefore, this analysis was considered statistically acceptable. The first canonical factor was negatively correlated to the amorphous Fe and Al (Annex Table 10 and Fig. 2), the exchangeable Al, the organically bound Fe and Al, the highly labile P fraction (P_t NaHCO₃), and the moderately labile P fraction (P_t NaOH). The first canonical factor was also positively correlated to the readily available P fraction (P_{AEM}) and

Table 4Phosphorus fractions (mg kg $^{-1}$) in soils studied

P fractions	Mean	SD	Min	Max	% of P _{Total}
P _{AEM}	4.0	1.7	1.4	7.3	2.6
P _i NaHCO ₃	5.6	2.9	2.3	12.2	3.6
P _o NaHCO ₃	14.7	7.4	4.5	37.9	9.5
P _i NaOH	23.6	10.2	11.6	53.6	15.2
P _o NaOH	37.5	20.9	0.9	84.7	24.3
PHCl _{1M}	3.3	3.0	0.5	12.8	2.1
PHCl _{conc}	56.1	22.0	13.8	101.5	36.3
PHClO ₄	9.8	4.1	2.6	19.1	6.3
P _{Total}	154.5	55.5	59.7	280.7	100

 P_{AEM} , readily available P; P_iNaHCO_3 , P_oNaHCO_3 , inorganic and organic highly labile P; P_oNaOH , P_iNaOH , inorganic and organic moderately labile P; $PHCl_{IM}$, primary P; $PHCl_{conc}$ stable P; $PHClO_4$, residual P; P_{Total} , addition of all previous P fractions analyzed

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the primary P (PHCl_{1M}). The recalcitrant P fraction (P_{rec}) was strongly and negatively correlated to the second canonical factor. As shown by the canonical correlation analysis (Annex Table 10 and Fig. 2) and Pearson's correlations between P fractions and soil Fe and Al (Table 6), the labile forms of P presented positive correlations with the amorphous and organically bound Fe and Al and exchangeable acidity in soils. Besides, P_{AEM} was negatively correlated to exchangeable Al and positively correlated to soil pH.

3.3 Relationships between P fractions and organic matter

The first two canonical factors in the canonical correlation analysis of P fractions and organic matter–related variables were significant (Wilk's lambda test; P < 0.05) and their canonical correlations were 0.87 and 0.75 respectively. Kaiser's Measure of Sampling Adequacy (MSA) was 0.55 and, therefore, this analysis was considered statistically acceptable. The first canonical factor (Annex Table 11 and Fig. 3) was negatively correlated to the easily oxidizable C, the microbial biomass C, the acid phosphatase activity, the highly labile P (P_tNaHCO₃), the moderately labile P (P_tNaOH), and the recalcitrant P (P_{rec}). The second canonical factor was negatively correlated to the mineralizable C (C_{min}), the P_{AEM}, and the primary P (PHCl_{1M}).



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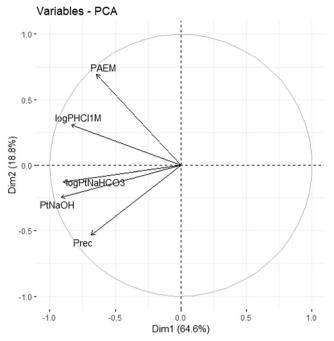


Fig. 1 Variables plot for the first (Dim1) and second (Dim2) components of the principal component analysis performed to the P fractions (P_{AEM} , available P; P_t NaHCO₃, highly labile P; P_t NaOH, moderately labile P; PHCl_{1M}, primary P; P_{rec} , recalcitrant P)

According to the canonical correlation analysis (Annex Table 11 and Fig. 3) and the Pearson correlations between P fractions and organic matter–related variables (Table 6), the easily oxidizable C was positively correlated to the inorganic moderately labile P form (P_i NaOH). The microbial biomass P was positively correlated to all P fractions considered and microbial biomass C was positively correlated to all P fractions considered in the analysis except with P_i NaHCO₃ and P_i NaOH. Mineralizable C was negatively correlated to the inorganic moderately labile P fraction (P_i NaOH) and acid phosphatase activity was only correlated (positively) to the organic moderately labile P fraction (P_o NaOH).

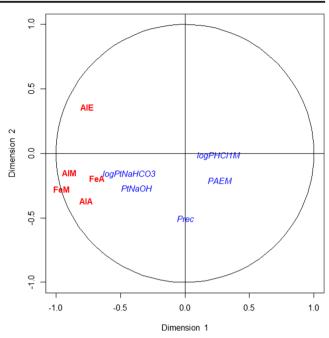


Fig. 2 Variables plot for the first and second canonical factors of the canonical correlation analysis performed to the P fractions and Fe- and Al-related variables (Al_A, Fe_A, Al and Fe within amorphous Al and Fe oxyhydroxides; Al_E, exchangeable Al; Al_M, Fe_M, organically bound Al and Fe; P_{AEM} , available P; P_t NaHCO₃, highly labile P; P_t NaOH, moderately labile P; PHCl_{1M}, primary P; P_{rec} , recalcitrant P)

3.4 Relationships between P fractions and Ca

The first two canonical factors in the canonical correlation analysis of P fractions and Ca-related variables were significant (Wilk's lambda test; P < 0.05) and their canonical correlations were 0.80 and 0.71, respectively. Kaiser's Measure of Sampling Adequacy (MSA) was 0.67 and, therefore, this analysis was considered statistically acceptable. The first canonical factor (Annex Table 12 and Fig. 4) was negatively correlated to the exchangeable Ca, the P_{AEM}, the highly labile P fraction (P_tNaHCO₃), the moderately labile P fraction (P_tNaOH), and the primary P (PHCl_{1M}). The second canonical factor was positively correlated to the soil pH and the recalcitrant P fraction

Table 5	Pearson's correlations			
between	P fractions in soils ("-"			
means n	ot significant)			

	P _{AEM}	log(P _i NaHCO ₃)	$\sqrt{P_oNaHCO_3}$	log(P _i NaOH)	P _o NaOH	log(PHCl _{1M})
log(P _i NaHCO ₃)	0.45**					
√P _o NaHCO ₃	-	-				
log(PiNaOH)	-	0.62***	0.45**			
P _o NaOH		-	0.82***			
log(PHCl _{1M})	0.66***	-	0.54**	0.44**	0.57***	
P _{rec}	-	-	0.54***	-	0.72***	0.45**

P < 0.01; *P < 0.001. P_{AEM} , available P; P_iNaHCO_3 , P_oNaHCO_3 , inorganic and organic highly labile P; P_iNaOH , P_oNaOH , inorganic and organic moderately labile P; $PHCl_{IM}$, primary P; P_{rec} , recalcitrant P fraction (PHCl_{conc} + PHClO₄)



	P _{AEM}	log(P _i NaHCO ₃)	$\sqrt{(P_oNaHCO_3)}$	log(P _i NaOH)	P _o NaOH	log(PHCl _{1M})	P _{rec}
pН	0.50**	-	-	_		0.51**	0.50**
EOC	-			0.44**		-	-
EA	-	-	-	0.53**	-	-	-
log(Ca ²⁺)	0.73***	-	-	-	0.46**	0.62***	
Al_E	-0.46**	-	-	-	-	-0.46**	-
Fe _A	-	-	0.54**	0.45**	0.60***	-	-
Cmic	0.70***	0.53**	-		0.54**	0.47**	
√Pmic	0.60***		0.49**		0.60***	0.44**	0.44**
log(Cmin)	-	-	-	-0.51**	-	-	-
Site index	-	0.54***		0.58***	0.47**	0.50**	-

 Table 6
 Pearson's correlations between P fractions and physical, chemical, and biochemical parameters in soils and site index ("-" means not significant)

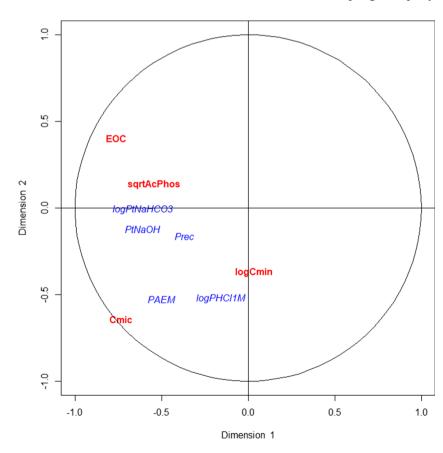
P < 0.01; *P < 0.001. P_{AEM} , available P; P_iNaHCO_3 , P_oNaHCO_3 , highly labile inorganic and organic P; P_iNaOH , P_oNaOH , moderately labile inorganic and organic P; $PHCl_{IM}$, primary P; P_{rec} , recalcitrant P; EOC, easily oxidizable C; EA, exchangeable acidity; Fe_A , Fe within amorphous Fe oxyhydroxides; Al_E , exchangeable Al; *Cmic*, microbial biomass C; *Pmic*, microbial biomass P; *Cmin*, mineralizable C

 (P_{rec}) . According to the canonical correlation analysis (Annex Table 12 and Fig. 4) and the correlations between P fractions and Ca-related variables (Table 6), the P_{AEM} fraction was positively correlated to the soil pH and the exchangeable Ca. The organic moderately labile fraction of P (P_oNaOH) and the primary P fraction (PHCl_{1M}) showed a positive correlation with Ca and the recalcitrant P fraction with the pH.

3.5 Relationships between soil P fractions and forest productivity

Finally, in the canonical correlation analysis performed on P fractions in relation to forest productivity (estimated through the site index of stands), the only canonical factor was significant (Wilk's lambda; P < 0.05) and presented a canonical correlation of 0.70. Kaiser's Measure of Sampling Adequacy

Fig. 3 Variables plot for the first and second canonical factors of the canonical correlation analysis performed to the P fractions and organic matter–related variables (EOC, easily oxidizable C; Cmic and Pmic, microbial biomass C and P, respectively; Cmin, mineralizable C; AcPhos, acid phosphatase activity; P_{AEM}, available P; PtNaHCO₃, highly labile P; PtNaHCO₃, highly labile P; PHCl_{1M}, primary P; P_{rec}, recalcitrant P)





(MSA) was 0.72 and, therefore, this analysis was considered statistically acceptable. The canonical factor corresponds to the site index (Table 7) and is positively correlated to the P_{AEM} , to the highly labile P (P_t NaHCO₃), to the moderately labile P (P_t NaOH), and to the primary P (PHCl_{1M}).

According to the canonical correlation analysis (Table 7) and the Pearson correlations (Table 6) between P fractions and site index, the site index was strongly and positively correlated to the inorganic both highly and moderately labile P fractions (P_iNaHCO_3 , P_iNaOH), the organic highly labile P (P_oNaHCO_3), the organic moderately labile P (P_iNaOH), and the PHCl_{1M}.

4 Discussion

4.1 Distribution of phosphorus fractions and relationships among them

The stable (PHCl_{conc}) and the organic moderately labile (P_o NaOH) fractions were the predominant forms of P in the soils studied as also observed by Turrión et al. (2007). The low soil pH in the soils studied is related to the presence of exchangeable Al responsible for the decrease in P solubility and for the increase in the non-labile P pools (Cross and Schlesinger 1995; Hou et al. 2014).

The positive correlations observed between the PAEM and the inorganic highly labile P (P_iNaHCO₃) indicate that this fraction in the soils studied could act as source of available P in the short term. Tiessen et al. (1984) found that bicarbonate extracted P explained 80% of the variability of available P. In our soils, the organic bicarbonate extractable fraction (P₀NaHCO₃) was not significantly correlated to available P even when this fraction includes easily exchangeable P compounds and it is considered a very active fraction of soil organic P (Zamuner et al. 2008). Even when the soils studied are acidic, the PHCl_{1M}, which is associated with Ca, was strongly correlated to PAEM indicating that this P fraction in primary minerals could act as source of available P in the mid-term (Cross and Schlesinger 1995; Richter et al. 2006). In addition, the most refractory forms of P were also positively correlated to organic and inorganic less resistant fractions P (PoNaHCO3 and PoNaOH) and to primary P (PHCl_{1M}) clarifying the role of P fractions with slower cycles as source of labile and available P in the long term (Richter et al. 2006).

4.2 Phosphorus fractions in relation to soil properties

In natural soils, some microorganisms are effective at releasing P through the mineralization of organic P (Bhattacharyya and Jha 2012) and the solubilization of mineral P by the production of dissolving compounds (Sharma et al. 2013). Results shown in Fig. 3 indicate that the first canonical factor

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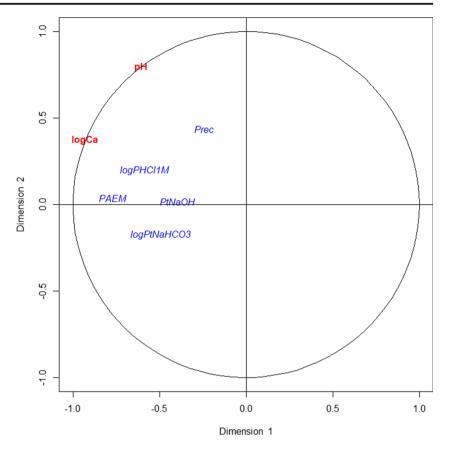
of the CCA was highly correlated to the microbial biomass C and the acid phosphatase activity which plays an important role in the P biochemical mineralization where P is released from organic compounds regulated by P demand. The second canonical factor was highly correlated to the microbial biomass C and to the mineralizable C which represents the biological mineralization of P from organic compounds during soil organic matter oxidation by soil organisms and regulated by the energy demand. Both biochemical and biological P mineralization seem to be relevant in the soils studied. Acid phosphatase activity is known to be inhibited in strongly acidic soils by exchangeable Al and protons (Bueis et al. 2018b; Trasar-Cepeda and Gil-Sotres 1987) which dominate the exchange complex in the soils studied. Microbial populations not only take part in P mineralization and solubilization of soil P but also are a pool of organic P, which is released when microorganisms die (Nannipieri et al. 2002). Our results showed that the microbial biomass C and the microbial biomass P were significantly correlated to PAEM. Lack of consistent relationships between available P and phosphatase activities cannot be interpreted as lack of a role for these enzymes, because they are controlled by end product inhibition and repression by phosphate (McGill and Cole, 1981).

Organic P in Inceptisols contributes by 40–45% to total P (Yang and Post 2011); however, in the soil analyzed in our study, the organic P represented between 12 and 51%; thus, we cannot affirm that the organic P amount in the studied soils is high. In addition, it has been observed that the organic P (P_o NaOH) decreases with decreasing P availability. Normally, high C to Po ratios (200 or more), as those found in the studied soils, are associated with P-deficient soils, whereas soils well supplied with available P have C to Po ratios which are generally < 100 (Yang and Post 2011).

The positive correlation found between the primary P fraction (PHCl_{1M}) and pH and the exchangeable Ca indicates that this P pool in soils could be related to Ca primary minerals (Schoenau et al. 1989; Shen et al. 2011; Turrión et al. 2000a).

Correlations found between highly and moderately labile P fractions (PiNaHCO₃, PoNaHCO₃, PiNaOH, PoNaOH) and soil parameters such as exchangeable acidity and amorphous Fe content are in accordance with the general belief that these P fractions are controlled by sorption processes which, in acidic soils as the ones studied, are mainly carried out by Fe oxides (Hedley et al. 1982; Schoenau et al. 1989). Sorption processes are considered to be the most important process controlling P availability in soils (Lajtha et al. 1999). Phosphate sorption maintains very low concentrations of P in soil solutions, particularly in mineral soils. Since sorption is reversible to some degree, sorbed P may be a source of plant-available P either immediately or in the mid or long term. However, the degree of reversibility is not well understood, and there is no single easy measurement of P sorption. The low pH and the high exchangeable acidity in soils studied

Fig. 4 Variables plot for the first and second canonical factors of the canonical correlation analysis performed to the P fractions and calcium-related variables (Ca, exchangeable Ca; P_{AEM}, available P; P_tNaHCO₃, highly labile P; P_tNaOH, moderately labile P; PHCl_{1M}, primary P; P_{rec}, recalcitrant P)



are related to high amounts of Fe and Al, responsible for P sorption (Turrión et al. 2008). Crystalline and amorphous Fe and Al oxides are the main phosphate "sorbents" in acidic soils (Hsu 1989). However, amorphous Fe and Al oxides are responsible for major P sorption because of their high specific surface area (Achat et al. 2010). Besides, amorphous Fe and Al oxides become more reactive with soil acidification (Richter et al. 2006). Slazak et al. (2010) also found a significant and positive correlation between P_iNaOH and Fe and Al oxides in soils under forest stands in Germany.

Organic matter is source of available P by direct and indirect means. Organic matter could act as source of

Table 7	Factor loadings
for cano	nical correlation
analysis	performed on
site inde	x in relation to P
fractions	s in soils

Variable	Factor 1		
P _{AEM}	0.460		
log(PtNaHCO3)	0.857		
P _t NaOH	0.819		
log(PHCl _{1M})	0.710		
P _{rec}	0.166		
Site index	1		

P_{AEM}, readily available P; *P_tNaHCO₃*, highly labile P; *P_tNaOH*, moderately labile P; *PHCl_{1M}*, primary P; *P_{rec}*, recalcitrant P

inorganic labile P in the soils studied as reflected in the positive correlation found between the easily oxidizable C and the moderately labile inorganic P fractions (P_i NaOH). Hou et al. (2014) also found a direct relation between soil organic C and P associated with Al and Fe. Besides, they observed an increment in inorganic soluble P and labile inorganic P with soil organic C as these soluble and labile forms of P come from the mineralization of the organic matter. The positive correlation found between the microbial biomass P and P_{AEM} is consistent with previous studies (Zamuner et al. 2008) and with the fact that microbial biomass regulates P availability through the mineralization of the organic P contained in their own structures after their death.

Besides, organic matter complexes Fe and Al ions blocking P precipitation and also occupies exchangeable sites in Fe and Al oxides reducing their sorption of P (Earl et al. 1979; Haynes and Mokolobate 2001). Organically bound Fe and Al presented positive correlations with labile P fractions indicating that the higher the amount of organically bound Fe and Al, the lower the P precipitation in these soils.

The recalcitrant P fraction (P_{rec}) was also positively correlated to soil pH indicating that higher soil pH is related to higher amounts of P occluded in Ca compounds (Richter et al. 2006).



4.3 Phosphorus fractions in relation to forest productivity

The site index of the studied plots is within the range found for *P. sylvestris* stands by Río et al. (2006) all over the Castilla y León region and by Palahi et al. (2004) in stands located in north-east Spain.

In the soils studied, available P and highly and moderately labile and primary P fractions were positively correlated to site index, estimator of forest productivity in the P. sylvestris plantations studied. In the short term, the available and the most labile fractions of P determine the productivity of ecosystems (Chen et al. 2008). However, in the mid-term, less labile P fractions make important contributions to P availability (Cross and Schlesinger 1995; Richter et al. 2006) as reflected in the correlations found. Turrión et al. (2000a) studied the effect of P fertilization in forests located in the Spanish Central System and found a forest productivity increment after fertilization. In previous studies, Bueis et al. (2016) developed a discriminant model to predict forest site index for *P. sylvestris*, using the plots studied in the present work. This model included soil, climatic, and physiographic parameters. The model selected had as predictors: latitude, porosity, inorganic Al, and microbial biomass, parameters which were strongly related with precipitations and water surplus, soil water availability, phosphorus immobilization, and the turnover rate of nutrients (Bueis et al. 2016).

5 Conclusions

In the studied acidic soils, P availability for plants and microorganisms is low and the predominant fractions of P are the recalcitrant forms. In these soils, both biological and biochemical mineralization processes play an important role in P cycling. Aluminum and specially iron contents in the soils studied also provoke phosphorus immobilization processes related to the highly and moderately labile P fractions.

Finally, the short- and mid-term P availability seems to be determinant of forest productivity in the *P. sylvestris* plantations studied as previously expected.

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Data availability The datasets generated during and/or analyzed during the current study are available in the Zenodo repository (Bueis et al. 2018a) at https://doi.org/10.5281/zenodo.1560581.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Plot

Annex

Table 8Location of the
plots studied. (UTM
Projection in meters;
Datum ETRS89)

FIOL	U IIVI_A	UIW_I
1	356,510	4,718,046
2	346,008	4,735,864
3	345,449	4,732,431
4	356,953	4,723,227
5	352,284	4,724,256
6	370,257	4,717,777
7	371,299	4,717,225
8	371,111	4,716,897
9	372,303	4,715,356
10	356,791	4,722,980
11	358,125	4,712,512
12	356,874	4,723,451
13	353,086	4,733,717
14	353,515	4,736,657
15	347,849	4,728,273
16	374,732	4,715,297
17	341,138	4,727,330
18	343,309	4,731,280
19	344,755	4,731,657
20	344,069	4,729,889
21	344,273	4,727,795
22	343,114	4,726,676
23	340,167	4,724,006
24	340,347	4,724,323
25	341,275	4,721,130
26	344,662	4,728,832
27	345,725	4,733,054
28	343,620	4,729,463
29	341,554	4,727,760
30	344,540	4,729,354
31	345,010	4,728,213
32	344,987	4,728,181
33	345,075	4,728,213
34	345,080	4,728,126

UTM X

UTM Y

 Table 9
 Factor loadings of the principal component analysis of P fractions in soils

Variable	Component 1	Component 2
P _{AEM}	-0.359	0.715
log(PtNaHCO3)	-0.500	0.133
P _t NaOH	-0.509	-0.253
log(PHCl _{1M})	-0.465	0.320
P _{rec}	-0.381	-0.551

 P_{AEM} , available P; P_tNaHCO_3 , highly labile P; P_tNaOH , moderately labile P; $PHCl_{IM}$, primary P; P_{rec} , recalcitrant P fraction

Table 10Factorloadings (simplecorrelations betweeneach canonical factor andthe variables) ofcanonical correlationanalysis performed on Pfractions in relation to Feand Al in soils

	Factor 1	Factor 2
Al _A	-0.765	- 0.367
Al_E	-0.758	0.360
Al _M	-0.897	-0.148
Fe _A	-0.681	-0.195
Fe _M	-0.956	-0.276
P _{AEM}	0.315	-0.311
log(PtNaHCO3)	-0.505	-0.236
P _t NaOH	-0.439	-0.395
log(PHCl _{1M})	0.300	-0.026
P _{rec}	0.006	-0.740

 Al_A , Fe_A , amorphous Al and Fe; Al_E , exchangeable Al; Al_M , Fe_M , organically bound Al and Fe; P_{AEM} , readily available P extracted with anion exchange membranes; P_tNaHCO_3 , highly labile P; P_tNaOH , moderately labile P; $PHCl_{IM}$, primary P; P_{rec} , recalcitrant P

Table 11Factorloadings (simplecorrelations betweeneach canonical factor andthe variables) ofcanonical correlationanalysis performed on Pfractions in relation to

organic matter in soils

	Factor 1	Factor 2
EOC	-0.759	0.405
Cmic	-0.733	-0.640
log(Cmin)	0.034	-0.368
√AcPhos	-0.542	0.138
P _{AEM}	-0.572	-0.704
log(PtNaHCO3)	-0.697	-0.011
P _t NaOH	-0.697	-0.158
log(PHCl1M)	-0.183	-0.695
P _{rec}	-0.422	-0.214

EOC, easily oxidizable C; *Cmic*, microbial biomass C; *Cmin*, mineralizable C; *AcPhos*, acid phosphatase activity; P_{AEM} , available P; P_tNaHCO_3 , highly labile P; P_tNaOH , moderately labile P; $PHCl_{IM}$, primary P; P_{rec} , recalcitrant P Table 12Factorloadings (simplecorrelations betweeneach canonical factor andthe variables) ofcanonical correlationanalysis performed on Pfractions in relation to Cain soils

$\begin{tabular}{ c c c c c c } \hline Factor 1 & Factor 2 \\ \hline log(Ca^{2+}) & -0.927 & 0.375 \\ pH & -0.606 & 0.796 \\ P_{AEM} & -0.956 & 0.052 \\ log(P_tNaHCO_3) & -0.614 & -0.242 \\ P_tNaOH & -0.497 & 0.025 \\ \hline log(P_tNaHCO_3) & 0.051 \\ \hline log(P_tNaHCO_3) & 0.0$			
$\begin{array}{llllllllllllllllllllllllllllllllllll$		Factor 1	Factor 2
$\begin{array}{llllllllllllllllllllllllllllllllllll$	log(Ca ²⁺)	-0.927	0.375
$\begin{array}{c} \text{log}(P_t\text{NaHCO}_3) & -0.614 & -0.242 \\ P_t\text{NaOH} & -0.497 & 0.025 \end{array}$	pН	-0.606	0.796
P_t NaOH -0.497 0.025	PAEM	-0.956	0.052
	log(P _t NaHCO ₃)	-0.614	-0.242
1 (7)1(2) 0 700 0 000	P _t NaOH	-0.497	0.025
$\log(\text{PHCl}_{1\text{M}}) = -0.732 = 0.280$	log(PHCl _{1M})	-0.732	0.280
P _{rec} -0.303 0.614	P _{rec}	-0.303	0.614

 Ca^{+2} , exchangeable calcium; P_{AEM} , available P; P_tNaHCO_3 , highly labile P; P_tNaOH , moderately labile P; $PHCl_{IM}$, primary P; P_{rec} , recalcitrant P

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