



Universidad de Valladolid
Campus de Palencia

**ESCUELA TÉCNICA SUPERIOR
DE INGENIERÍAS AGRARIAS**

Máster en Ingeniería de Montes

Soil phosphorus availability as an indicator of forest productivity in *Pinus halepensis* Mill. plantations. Relationship between P pools and soil properties.

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September, 2020



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Disponibilidad de fósforo en el suelo como
indicador de la productividad forestal en
plantaciones de *Pinus halepensis* Mill.
Relación entre las reservas de P y las
propiedades del suelo.

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September, 2020

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Outline of the study

The main objective of this master's thesis is to assess the state and availability of phosphorus (P) in limestone soils in northern Spain. To do this, we studied the organic and inorganic extractable P fractions as well as the more stable ones that are usually more abundant in limestone soils. We tried to uncover which soil properties determine the edaphic P availability and state in *Pinus halepensis* Mill. plantations and we studied whether the P availability affects the productivity of these forests.

This research has been developed using part of the soil samples taken during the Ph.D. thesis of Dr. Teresa de los Bueis Mellado, co-tutor of this work, titled: "Relationships between the dynamics of *Pinus halepensis* Mill. and *Pinus sylvestris* L. plantations and environmental parameters: a basis for sustainable management of stands" defended in 2017. For this reason, the physiographic, climatic and stand data, as well as various soil parameters, have been obtained from this previous doctoral thesis. The author of this master's thesis carried out the laboratory analysis for the determination of soil phosphorus and the statistical study necessary to achieve the objectives of this work.

Abstract

Although phosphorus is an essential element for life and vegetation growth it is not always found in labile forms, hampering its absorption by plants. Consequently, P is often a limiting nutrient in forest ecosystems mainly due to the low solubility of P compounds and the sorption processes occurring in soils. The main objectives of this work were to evaluate soil P status and availability, to determine which soil processes are affecting the different soil P pools and which are leading P availability, and to assess which soil parameters are influencing forest productivity in *Pinus halepensis* Mill. plantations in Northern Spain and whether soil P availability is driving that productivity. The different phosphorus fractions were studied in the soil surface horizon (0-10 cm) according to their lability and their organic or inorganic nature, and were related to different soil parameters and to the site index in 32 plots of the National Spanish Forest Inventory located in monospecific plantations of *P. halepensis*. A 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 P fractions and soil parameters, canonical correlation analyses and Spearman's correlations were used. To study the relationship between P fractions, soil parameters and forest productivity, Spearman's correlations and partial least squares regression were done. Significant correlations were found between different P fractions and soil properties related to soil texture, carbonates content, soil water retention capacity, organic matter, and enzyme activity. Available P was mainly affected by organic matter-related parameters while the predominant P fraction in these calcareous soils, the primary P, was linked to the carbonates content. Besides, our results indicated that total P, carbonates content, and soil texture as well as parameters related to soil chemical fertility and to soil water retention largely drove site index in the studied plots. In conclusion, the biological mineralization processes played a key role in the P cycle of the studied soils and thus, parameters related to soil organic matter mainly led available P. The concentration of assimilable P, although low, may be sufficient to satisfy the demand of the vegetation in these calcareous soils. Moreover, although the soil parameters considered allowed explaining part of the variation in the site index of *P. halepensis* in the studied plots, it would be necessary to include climatic parameters to better explain the factors that prompt forest productivity of these plots.

Keywords: calcareous soils, forest productivity, Mediterranean environment, phosphorus availability, phosphorus fractionation, sequential extraction, site index.

Resumen ampliado

Aunque el fósforo es un elemento esencial para la vida y el crecimiento de la vegetación, no siempre se encuentra en formas lábiles debido a su baja solubilidad, fuertemente dependiente del pH del suelo, a su lenta difusión y a su alta fijación en los suelos. Esto dificulta su absorción por las plantas, por lo que se considera uno de los nutrientes más limitantes para su crecimiento y en consecuencia, también para la productividad forestal.

El P del suelo se encuentra principalmente como parte de la fracción sólida del suelo y aparece en varias formas químicas, incluido el P orgánico (P_o) y el P inorgánico (P_i). El fósforo del suelo proviene de la meteorización química de los minerales que contienen P, del polvo atmosférico y de la lluvia seca que es rica en $H_2PO_4^-$, además de la descomposición de los residuos vegetales (hojarasca, raíces, troncos, etc.). Durante esta descomposición, los microorganismos liberan P vía mineralización y captan P vía inmovilización, controlando las transformaciones de este elemento entre formas orgánicas e inorgánicas (estas últimas son las formas de fósforo disponibles para las plantas). Estos procesos forman un subciclo biológico en el ciclo general del fósforo del suelo. Otras salidas de P del sistema son producidas por erosión hídrica y eólica, procesos de lixiviación y escorrentía que provocan el lavado del P más soluble y por la absorción de P por las plantas. Los procesos de precipitación-disolución y adsorción-desorción que controlan la transferencia abiótica de P entre la fase sólida y la solución del suelo forman el subciclo geoquímico en el ciclo del fósforo del suelo.

En relación a la nutrición de las plantas, el P del suelo se puede considerar en términos de diversas reservas con diferente disponibilidad para las plantas. El ion fosfato que se encuentra en la solución del suelo es completamente accesible para las plantas, pero representa sólo una fracción diminuta del P total del suelo. La mayor parte del P del suelo es prácticamente inaccesible, puede describirse como no lábil y está presente como formas fijas e insolubles, incluidos los minerales de fosfato primario o el fosfato insoluble de Ca, Fe y Al, y el P fijado por óxidos y minerales de tipo silicatos.

Los bosques tienen un alto valor ecológico, económico y social, además de desempeñar un papel clave en el mantenimiento de la biodiversidad y como sumideros de carbono. Las comunidades vegetales influyen en la generación, renovación y mantenimiento del suelo, modificando sus propiedades físicas, químicas y biológicas. A su vez, el suelo influye en la productividad del ecosistema, así como en su desarrollo y consolidación en el tiempo. Cada

especie vegetal del ecosistema afecta al suelo de manera individual y singular, generando características propias que favorecen sus procesos de regeneración y reproducción. Además, por su alto dinamismo, los suelos responden rápidamente a los cambios de manejo en los ecosistemas. Por tanto, la gestión del suelo es una herramienta poderosa contra la degradación de los ecosistemas y en la lucha contra el cambio climático antropogénico.

Pinus halepensis Mill. se encuentra en una amplia variedad de bioclimas en la región mediterránea, pero debido a su alta tolerancia al estrés por sequía y su baja demanda de nutrientes, generalmente se ubica en áreas más secas y cálidas que otras especies de pinos mediterráneos. Esta especie vive en zonas con gran variabilidad de precipitaciones, crece a altitudes variables y en todos los sustratos. Sin embargo, la presencia de *P. halepensis* es más frecuente en suelos calizos porque encuentra menos competencia ya que la mayoría de las especies forestales no son capaces de habitar este tipo de suelos.

La productividad forestal de *P. halepensis* bajo diferentes condiciones ambientales parece estar estrechamente relacionada con la disponibilidad de agua y la cantidad de materia orgánica o elementos como nitrógeno, magnesio o potasio. Sin embargo, no se suelen considerar en estos estudios la disponibilidad y el estado del fósforo del suelo como un factor que pueda afectar la productividad forestal. Por tanto, es de gran interés evaluar la relación entre las distintas fracciones de P y la productividad forestal en nuestros rodales calcáreos. Además, aunque se conocen los procesos generales involucrados en la bioquímica y disponibilidad de P, se necesitan más estudios para aclarar qué parámetros del suelo son los que más afectan cada proceso en suelos calcáreos de sistemas forestales mediterráneos, en los que apenas existen estudios. Para comprender mejor los procesos involucrados en la disponibilidad de P en suelos calcáreos bajo condiciones mediterráneas, utilizamos el método de fraccionamiento secuencial de P de Hedley et al. (1982) modificado por Tiessen & Moir (1993). Este método permite la determinación de los diferentes depósitos de P en los suelos en relación con su disponibilidad para las plantas y la absorción microbiana.

Este trabajo se enmarca en el campo de la edafología y su objetivo principal fue evaluar el estado y la disponibilidad de fósforo en suelos calcáreos en plantaciones monoespecíficas de *P. halepensis* ubicadas en la región de Castilla y León (meseta norte de España), tratando también de que las preguntas de investigación planteadas aquí mejoren el conocimiento sobre los procesos involucrados en la disponibilidad de P en

suelos forestales calcáreos desarrollados bajo condiciones mediterráneas. En concreto, nos hemos centrado en evaluar el estado y la disponibilidad de P en el suelo, determinar qué procesos del suelo están afectando a los diferentes depósitos de este elemento y cuáles intervienen principalmente en su disponibilidad, y evaluar qué parámetros del suelo están influyendo en la productividad forestal de plantaciones monoespecíficas de *P. halepensis* en el norte de España, descifrando si la disponibilidad de P en el suelo de dichas parcelas está impulsando esa productividad. Se estudiaron las diferentes fracciones de fósforo en el horizonte superficial del suelo (0-10 cm) atendiendo a su labilidad y a su naturaleza orgánica o inorgánica y se relacionaron con diferentes parámetros del suelo y con el índice de sitio en 32 parcelas del Inventario Forestal Nacional ubicadas en plantaciones monoespecíficas de *P. halepensis*. Se llevó a cabo un método de fraccionamiento secuencial para determinar diferentes formas de P del suelo y proporcionar una evaluación completa del P disponible en los suelos.

Encontramos evidencias del efecto significativo de las propiedades del suelo relacionadas con la textura del suelo, el contenido de carbonatos, la capacidad de retención de agua del suelo, la materia orgánica y la actividad enzimática en las diferentes fracciones de P del suelo. El P disponible se vio afectado principalmente por parámetros relacionados con la materia orgánica, mientras que la fracción de P predominante en estos suelos calcáreos, el P primario, se vinculó con el contenido de carbonatos. Además, nuestros resultados indicaron que el P total, el contenido de carbonatos y la textura del suelo, así como los parámetros relacionados con la fertilidad química del suelo y la retención de agua del suelo, impulsaron en gran medida el índice de sitio en las parcelas estudiadas.

Los resultados obtenidos confirmaron que los procesos de mineralización biológica juegan un papel clave en el ciclo del P de los suelos calcáreos estudiados y, por tanto, los parámetros relacionados con la materia orgánica del suelo promueven principalmente la disponibilidad de P. La concentración de P asimilable, aunque baja, puede ser suficiente para satisfacer la demanda de la vegetación de estos suelos calcáreos. Además, si bien los parámetros edáficos considerados permitieron explicar parte de la variación en el índice de sitio de las parcelas estudiadas, sería necesario incluir parámetros climáticos para explicar mejor los factores que impulsan la productividad forestal de estas parcelas.

Palabras clave: ambiente mediterráneo, disponibilidad de fósforo, extracción secuencial, fraccionamiento del fósforo, índice de sitio, productividad forestal, suelos calizos.

1. INTRODUCTION

1.1. Soil phosphorus

Phosphorus in soils occurs almost exclusively in the form of orthophosphate with total P concentrations usually in the range of 100-3000 mg/kg soil (Frossard et al., 2000), although in forest soils lower total P concentrations have been found, ranging from 42 to 2300 mg/kg soil (Niederberger et al., 2019 in German forest soils). Soil phosphorus (P) is considered one of the most limiting nutrients for plant growth and thus, for forest productivity (Achat et al., 2010; Bueis et al., 2019a; Slazak et al., 2010, among others). Besides, P also has important implications in the vital processes of organisms (Troeh & Thompson, 1993) such as metabolic processes, genetic coding, or energy transfer (Marschner, 1995). Even when there is abundant total P in the soil, P availability for plants is usually low due to its slow diffusion and solubility, strongly dependent on the pH of the soil, and its high fixation in the soils (Tapia-Torres & García-Oliva, 2013). Plant available P in soil is a theoretical concept and consists of those P pools that in principle, can be utilized by plants during their life cycle (Whalen & Sampedro, 2010). Various chemical P-forms contribute to the size of the plant-available P pool. In summary, soil phosphorus comes from the chemical weathering of P-bearing minerals such as apatite or phosphorites (Filippelli, 2002, 2008; Haynes, 1982; Kruse et al., 2015; Smil, 2000), from the atmospheric dust and dry rain that is rich in H_2PO_4^- (although comprises a minor fraction of P inputs –Newman, 1995–) and from the decomposition of plant residues (litter, roots, trunks, etc.). During this decomposition, microorganisms release P via mineralization and absorb P via immobilization (Jalali & Ranjbar, 2009; Schachtman et al., 1998). Other P outputs from the system are produced by water and wind erosion, leaching and runoff processes that cause the washing of the more soluble P and by the absorption of P by plants. Soil P is mainly part of the solid fraction of the soil and appears in various chemical forms, including organic P (P_o) and inorganic P (P_i). These forms of P differ in their behaviour and location in soils (Hansen et al., 2004; Benjamin L. Turner et al., 2007) Inorganic forms of P in soils generally represent 35% to 70% of total P (*sensu* Harrison, 1987). This P_i in the form of phosphates comes from primary P minerals such as apatites, stengite and variscite, which are very stable and mostly not soluble. Hence, the release of available P from these rocks through weathering is generally too slow to satisfy plant demand. Plants mostly absorb soil P in the form of inorganic orthophosphates dissolved in soil solution (Fox et al., 2011; Stevenson, 1986), mainly as H_2PO_4^- and HPO_4^{2-} , but these phosphate ions can also be adsorbed by the soil anion exchange complex.

Phosphates concentrations in the soil solution are low in most natural soils but exist equilibrium relationships with labile P_i (easily soluble) and relatively insoluble P from secondary minerals (adsorbed). Thus, the phosphates dissolved in the soil solution and those weakly adsorbed by the exchange complex are considered available for plants and microorganisms. Some of the phosphates are more strongly retained by the exchange complex and may be available in the short term. These fractions are considered labile forms of P (Troeh & Thompson, 1993). Other forms of P are included in primary Ca minerals with low solubility. Finally, the most recalcitrant forms of P are the stable forms, which are also found in Ca secondary minerals, and the residual forms, which are considered primarily organic forms of P associated with clays and Fe and Al oxides (Turrión et al., 2000a; Zamuner et al., 2008). These secondary mineral P phosphates vary in their dissolution rates, depending on the size of the mineral particles and the pH of the soil (Oelkers & Valsami-Jones, 2008; Pierzynski et al., 2005). With increasing soil pH, the solubility of Fe and Al phosphates increases, but the solubility of Ca phosphates decreases (Hinsinger, 2001). All these forms of P exist in complex equilibria with each other, ranging from very stable deposits of P, scarcely available and representing a long-term P availability, to deposits of available P to plants, such as labile P and P in the soil solution which represent an immediate or short-term soil fertility (Sharpey & Smith, 1985). Therefore, the availability of soil P for plants depends on the replenishment of labile P and P in the soil solution from the other P fractions within the solid phase (M. A. Beck & Sanchez, 1994). Thereby, the amount of P in the soil solution represents a small fraction of the plant's needs, and the rest must be obtained from the solid phase through a combination of complex abiotic and biotic processes. The processes involved in the transformation of soil P are precipitation-dissolution and adsorption-desorption that control the abiotic transfer of P between the solid phase and the soil solution (Figure 1). These processes form, on the one hand, a geochemical sub-cycle in the soil P cycle. On the other hand, the biological processes of immobilization-mineralization driven by microorganisms control the transformations of P between inorganic and organic forms, which influence the orthophosphates concentration in the soil solution (Stevenson, 1986).

From the viewpoint of plant nutrition, soil P can be considered in terms of 'pools' with different availability to plants. Phosphates in the soil solution are completely accessible but this constitutes only a minute fraction of the total soil P. The bulk of soil P is virtually inaccessible. Most total P can be described as non-labile and it is present as insoluble and fixed forms including primary phosphate minerals, humus P, insoluble phosphates of Ca, Fe

and Al, and P fixed by hydrous oxides and silicate minerals. A proportion of insoluble phosphates is more accessible than that of the bulk reserves. In this labile fraction, solid phosphates is present in phosphate precipitates and is also held on soil surfaces. The labile phosphates is in rapid equilibrium with soil solution phosphates. Removal of phosphates from the soil solution by plant roots disturbs the equilibrium between the soil solution and the labile pool at the solid soil phase which leads to a release of P into the soil solution.

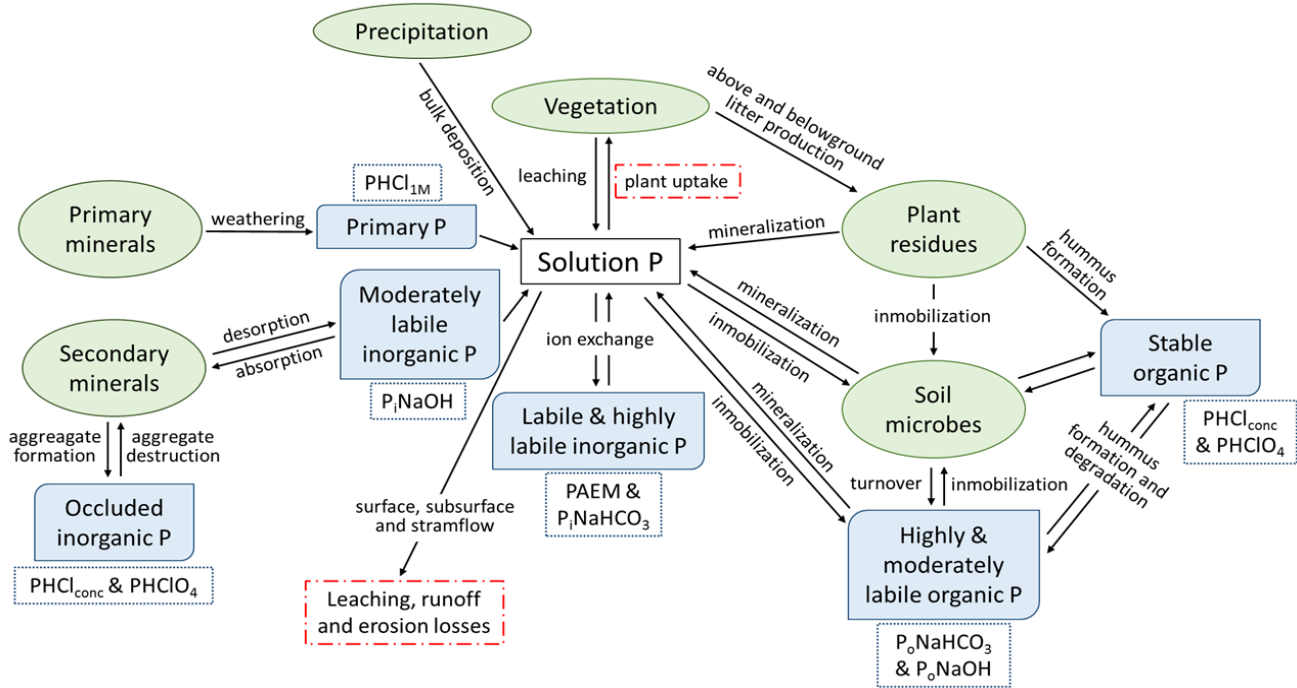


FIGURE 1 | Conceptual model of the soil phosphorus cycle. The main inputs of P into the ecosystem are indicated in green and the main outputs are framed in red. The blue boxes represent phosphorus deposits and the fractions that compose them (according to the Tiessen & Moir (1993) fractionation method). The arrows indicate the equilibria and processes that govern the P content in the soil solution. Modified from Walbridge (1991).

1.1.1. Organic phosphorus (P_o)

Soil organic P is linked in some way with carbon and usually represents 30% to 65% of the total P in soils (A. Harrison, 1987), although this percentage can greatly vary among different soil types. Cross & Schlesinger (1995) reviewed P fractionation results from different soils and found that the percentage of organic P in entisol-, aridisol- or inceptisol-type soils was much lower than that proposed by Harrison (1987), representing around 5, 8 and 16% of total P, respectively.

Soil P_o originates from the animal and plant remains as well as from the humic substances that are formed in the soil. Mineralization processes driven by soil microorganisms (Figure 1) transform the organic P forms into inorganic forms which are the ones assimilable by plants. Soil P_o exists mainly in stabilized forms such as inositol phosphates and phosphonates, and active forms such as organic orthophosphates (monoesters and diesters) and polyphosphates (Leo M. Condron et al., 2005; Benjamin L. Turner et al., 2002). Mineralization processes mediated by soil organisms and plant roots allow the release of organic forms to inorganic ones which are available to plants. Therefore, the quantity, fractions, and dynamics of P_o in the soil are determined by a combination of biological, chemical and physical factors that in turn are influenced by environmental conditions (such as temperature and humidity), by physicochemical properties of the soil surface, by its pH and by the history and intensity of land use. For example, long-term continuous inputs of P as mineral fertilizer increase the amount of organic P in the soil (Ottobong et al., 1997; Zhang & MacKenzie, 1997). Whereas soil organic P can decrease in response to long-term cultivation (Tiessen et al., 1982, 1992), or after afforestation of grasslands (Chen et al., 2000; Condron et al., 1996). Hence, the transformation of P_o in the soil is complex and highly determines the general bioavailability of P in the soil (Benjamin L. Turner et al., 2007).

Soil organic matter is key for soil structure or for the regulation of nutrients quantity and cycles (Tate, 1984), also playing a major role in the availability of P in forest soils. Organic matter is a direct source of P organic forms that are transformed into available P forms for vegetation through the mineralization of dead organic matter and humus and through the release of microbial biomass (David L. Achat et al., 2010; Nannipieri et al., 2002). Therefore, the microbial biomass is a particularly important source of P since it can be incorporated directly into the system, shortening the P release and distribution time. Microbial biomass can host an average of 4% of the total P in temperate coniferous forests and an average of 9% of the total P in temperate hardwood forests (Xu et al., 2013). Furthermore, microbial biomass can immobilize P when its availability increases. Through this transformation, carried out by microorganisms, organic P is taken up into microbial biomass (Leo M. Condron et al., 2005; Dalai, 1977; Jenkinson & Ladd, 1981). Then, microbially-synthesized organic P compounds can be stabilized through incorporation into humic substances or sorption with soil minerals, leading to their accumulation (Celi & Barberis, 2004; Benjamin L. Turner et al., 2005). Likewise, the symbiosis between plant roots and mycorrhizal fungi increases P absorption (da Silva et al., 2017; Desai et al., 2014). Organic matter also has

indirect effects on P availability, particularly in highly acidic soils. On one hand, organic matter can form complexes of Fe and Al ions responsible for the precipitation of P as insoluble phosphates of Fe and Al, reducing the retention of P in soils (Haynes & Mokolobate, 2001). Moreover, organic acids originated from the decomposition of organic matter increase the solubility of calcium phosphates (Troeh & Thompson, 1993) and are adsorbed by Fe and Al oxides, blocking P sorption (Earl et al., 1979). Finally, soil microbial activities benefit from the energy and C supply of soil organic matter (Allison & Vitousek, 2005) and are responsible for enzymatic activities and the consequent production of different enzymes, including phosphatase. These enzymatic activities can substantially affect the transformation of P organic forms to inorganic or more labile and soluble P forms (Trasar-Cepeda et al., 2003). Phosphatase enzymes, as well as the mineralization of organic matter, are directly related to soil pH. Soils with basic pH tend to have lower concentrations of P_o (Tiessen et al., 1984) but, in turn, have higher mineralization rates than soils with acidic pH (Harrison, 1982). Rainfall and temperature are other parameters that affect the concentration of P_o in the soil (Gallardo et al., 2009). Whereas the influence of mean annual rainfall on soil P_o seems to vary among climates and ecosystems (see, for example, Harrison, 1987; Sumann et al., 1998; Turner et al., 2003), mean annual temperature has a key role in the soil P_o concentration since the immobilization processes are inversely related to temperature, while mineralization increases with it (Arenberg & Arai, 2019; Gallardo et al., 2009).

1.1.2. Inorganic phosphorus (P_i)

Inorganic phosphorus is the prime form in which plants absorb P from the soil. The low mobility of P_i in the soil is due to the high reactivity of phosphate ions in relation to other components of the soil and to the subsequent strong holdback of most of the phosphorus forms on their surface. Accordingly, only a marginal proportion of the soil phosphorus is present as P ions in the soil solution, which directly depends on the type of parent material, the soil pH, the vegetation cover, and the time of vegetation establishment (Arenberg & Arai, 2019; Binkley, 1982). The shortage of labile P is of special concern in the subtropical and tropical regions of the world with highly weathered soils (Ae et al., 1990; Batjes, 1997) and in the Mediterranean basin that is largely dominated by alkaline and calcareous soils (Matar et al., 1992). This could be due to the mineralogy and environmental geochemistry of these soils that favour strong retention of P ions in the solid soil components and maintain low

levels of P ions in the solution. Inorganic P can be present in various forms, such as apatite, phosphate, or orthophosphate precipitates, among others (Dou et al., 2009; Kruse et al., 2015), which are firstly affected by the pH of the solution (Figure 2).

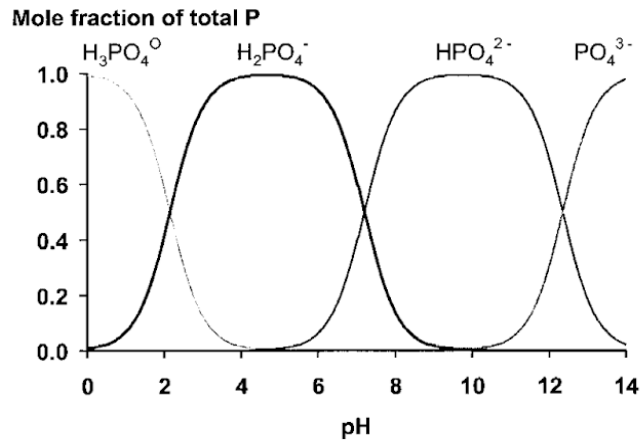


FIGURE 2 | Forms of orthophosphate ions (expressed as mole fraction of total P) in soil solution as a function of pH. From Hinsinger (2001).

Phosphorus ions are also important inorganic ligands in soil solution because they show a strong proneness to form pairs of ions or complex species with different cations, mainly Ca^{+2} and Mg^{+2} , Fe^{+2} , Fe^{+3} and Al^{+3} (Lindsay & Norvell, 1978). In acidic soils, P can be dominantly adsorbed by Al/Fe oxides and hydroxides, such as gibbsite, hematite, and goethite (Parffit, 1989). P can be first adsorbed on the surface of clay minerals and Fe/Al oxides by forming various complexes. In neutral-to-calcareous soils, P retention is dominated by precipitation reactions, although P can also be adsorbed on the surface of Ca carbonate and clay minerals (Arenberg & Arai, 2019; Devau et al., 2010; Shen et al., 2011). The presence of these cations in the soil solution depends again on the pH. Besides, the forms in which P appears also depend on the presence of other organic ligands that can form stable complexes with Ca, Fe, and Al in the soil solution, such as citrate or oxalate. These inorganic forms can also suffer reverse transformations, in which they are released back into the soil solution (Arenberg & Arai, 2019).

1.2. Factors influencing P cycle

In this section, the main factors that affect the soil P cycle will be reviewed. On the one hand, factors affecting mineralization and immobilization of phosphorus will be revised which are temperature, humidity and aeration, soil pH, the type of soil, and the parent material. On

the other hand, the effect of the environmental system on phosphorus biochemistry will be discussed.

1.2.1. Factors influencing phosphorus mineralization and immobilization

Phosphorus mineralization and immobilization rates vary with temperature due to the temperature sensitivity of microorganisms that accomplish these processes (Dalai, 1977). Mineralization increases with temperature while immobilization decreases with it (Arenberg & Arai, 2019; Dalai, 1977; Foster & Bhatti, 2006; Thompson & Black, 1947). Soil moisture and aeration also play a significant role in P immobilization, although there are no clear conclusions about its effect on mineralization (Brannon & Sommers, 1985; Dalai, 1977). Moisture is vital for phosphatases production and for the survival of immobilizing microorganisms (Dalai, 1977). Increasing soil moisture can improve mineralization in forest systems, especially those that experience wet and dry cycles (Campo et al., 1998; Dalai, 1977; Devi & Yadava, 2006). However, P immobilization has also been shown to be sensitive to drought, decreasing P diffusion, and succeeding microbial uptake (He et al., 1997). In other ecosystems, it has also been demonstrated that surplus rainfall increases inorganic P leaching, reducing its absorption in the microbial biomass (Arunachalam & Arunachalam, 2000; Dalai, 1977). The role of aeration in these processes is even more complex and can occur under both aerobic and anaerobic conditions (Suzumura & Kamatani, 1995). Phosphorus mineralization rates are also positively influenced by increasing soil pH (Arenberg & Arai, 2019; Dalai, 1977; Harrison, 1982; Haynes, 1982). Hence, soils that developed from limestone usually show higher mineralization rates (Harrison, 1982), because calcium promotes microbial activity and improves soil structure through aggregation. Although pH represents only a small part of the variation in organic phosphorus concentrations in soils around the world (Harrison, 1987), opposite effects of this parameter on organic phosphorus have been found. Thus, some authors have found that organic phosphorus concentrations in the soil were higher in acid soils such as grasslands and semi-arid agricultural soils (A. Harrison, 1987; Hawkes et al., 1984; P. N. C. Murphy et al., 2009; Tiessen et al., 1984; Benjamin L. Turner et al., 2003) while others have reported positive correlations between pH and various organic phosphorus reserves in tropical humid forests or temperate forests (Benjamin L. Turner & Engelbrecht, 2011). In addition, soil pH strongly influences the availability of inorganic P that can be assimilated by the microbial biomass (Haynes, 1982). Above pH 8, calcium phosphates precipitate decreasing the availability of P (Haynes, 1982). On the contrary, at lower pH values, iron

and aluminum phosphates are formed where P is adsorbed, limiting its bioavailability (Haynes, 1982). Therefore, between pH 6 and 7, immobilization and availability of P are optimal. Furthermore, contrasting effects of pH on different P pools have been found (Niederberger et al., 2019; Sugihara et al., 2012; Turner & Blackwell, 2013). This could be explained by the different processes that influence P availability and fixation in mineral soils at different pH levels (De Schrijver et al., 2012; Hinsinger, 2001). Additionally, the parent material, the pedogenesis, and the resulting soil type (order) also affect the fractionation and transformations of P (Arenberg & Arai, 2019; Augusto et al., 2017; Bourennane et al., 2003; Hahm et al., 2014; Tiessen et al., 1984; Walker & Syers, 1976; Zou et al., 1992). The influence of soil parent materials is the result of the link between actual P pools of soils and the physical-chemical properties of soil parent materials. The P content of the parent material can influence the accumulation of organic matter (Walker & Adams, 1958), and P transformations could be influenced by the size and lability of the P fractions in different soil orders. Concentrations of total P in the soil are also influenced by the soil weathering stage, but this factor is governed partly by parent materials as it is known that highly weathered soils are more likely found on acid parent materials than on calcareous ones (Augusto et al., 2017). Soil texture can also have an important effect on the distribution of P in soils around the world (Arenberg & Arai, 2019; Augusto et al., 2017; Buckingham et al., 2010; Niederberger et al., 2019; Zederer & Talkner, 2018). In forest soils, positive effects of clay minerals on P_o content have been identified (Talkner et al., 2009; Zederer & Talkner, 2018), while sandy soils tend to show a lower concentration of all P pools, probably due to the lower amount of organic matter or fewer opportunity for fixation in clay minerals. Generally, fine soil texture is related to higher total P contents due to the increase in the stable fraction of P (Niederberger et al., 2019; Tiessen et al., 1982, 1984). Finally, organic phosphorus mineralization in soils is affected by the ratio between organic carbon and organic phosphorus (C: P_o ratio) existing in soils and organic residues. Organic residues with a C: P_o ratio lower than 200 will release $H_2PO_4^-$ into the soil solution. When the C: P_o ratio is between 200 and 300, there is no net gain or loss of inorganic phosphates. Net phosphorus immobilization in microbial cells occurs when organic residues with a C: P_o ratio greater than 300 are mixed with the soil (Whalen & Sampedro, 2010).

1.2.2. *Environment and phosphorus biochemistry*

Phosphorus dynamics vary considerably among different environmental systems. In forests, the internal cycle predominates (Foster & Bhatti, 2006), so phosphorus flows

between vegetation, litter, and inorganic and organic P pools within the soil (Zou et al., 1992). In forests, the microbial biomass is a critical sink for litter P and it is a source of P for vegetation (David L. Achat et al., 2010; Srivastava & Singh, 1991). Phosphatase enzymes facilitate the mineralization of P_o . Their activities peak in the rhizosphere, increase with labile organic P availability, and are especially important in forests and grasslands (Tate et al., 1991). Moreover, vegetation type can also affect the P cycle. Within forest systems, different tree species have a distinctive influence on organic and inorganic phosphorus pools. Some studies even show that mixed forest stands present higher amounts of P_o and available P comparing to conifer monospecific stands (Slazak et al., 2010) due to the effect of the dynamics of the roots, phenological cycles, exudates, among others (Lucas-Borja et al., 2012). In addition, mycorrhizal associations increase plant P availability and P cycling efficiency (Giardina et al., 1995; Newbery et al., 1997). The C: P_o ratio of the organic litter determines whether the P of the litter will be mineralized or immobilized (David L. Achat et al., 2012). Besides, different environmental systems with various management intensities also affect P transformations. Forests are usually managed less intensively than other systems such as agricultural lands, but anthropogenic influence can still affect the P mineralization-immobilization dynamics. For example, inorganic P fertilization generally rises microbial uptake (that is, immobilization) in forests and grasslands (George et al., 2007; Schneider et al., 2010), whereas afforestation usually increases mineralization and extraction of whole trees reduces it (Campbell & Racz, 1975).

1.3. Study case: *Pinus halepensis* Mill. plantations

Forests occupy 30.6% of the Earth's surface (FAO, 2015; Figure 3) and provide fundamental ecosystem services such as water regulation and supply, purification of air, mitigation of the effects of droughts and floods, pollution control, among others (Daily, 1997; FAO, 2015). In addition, they are a source of oxygen as well as important carbon sinks, becoming key elements for the mitigation of anthropogenic climate change (Canadell & Raupach, 2008). Forest ecosystems are also useful for the economy and for the subsistence of millions of people since they are an important source of highly demanded products (food, wood, resin, cork...) and employment for the local population (Bravo-Oviedo et al., 2014).

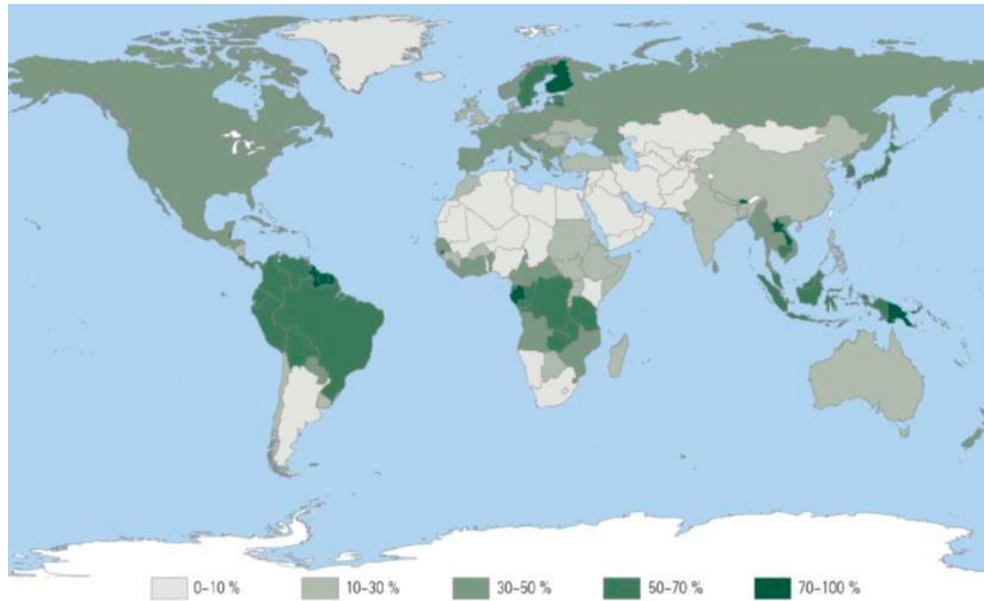


FIGURE 3 | Forest area as a percentage of total land area in 2015. From FAO (2015).

Many forest ecosystems of the Mediterranean basin and specifically, of the Iberian Peninsula, are limited by the availability of nutrients and water (Sardans & Peñuelas, 2004). This causes intense competition among forest species, which originates peculiar and complex ecosystems with a great variety of soil characteristics (Sardans et al., 2005). Plant communities influence the generation, renewal, and maintenance of soil, modifying its physical, chemical, and biological properties. In turn, soil influences the productivity of the ecosystem, as well as its development and consolidation over time (Moreno et al., 2012; Sardans & Peñuelas, 2004). Each plant species in the ecosystem affect the soil individually and singularly, generating its own characteristics that favour its regeneration and reproduction processes and consequently, creating microhabitats (Moreno et al., 2012), mainly due to the different qualities of organic matter that returns to the soil (Lucas-Borja et al., 2012). In addition, due to their high dynamism, soils respond quickly to management changes in the ecosystems (Burbano-Orjuela, 2016). Therefore, soil management is a powerful tool against the degradation of ecosystems and in the fight against anthropogenic climate change (Achat et al., 2016). However, it is essential to understand the functioning of the distinct nutrients' cycles in the soil and the factors affecting their availability, in order to subsequently establish different adequate soil management strategies that allow its improvement.

Pinus halepensis Mill. has a wide geographic distribution throughout a large part of the Mediterranean region, from Syria, Turkey, and Israel in the east to the Iberian Peninsula and Morocco in the west (Figure 4). In Spain, this species is present on the Mediterranean coast, including the Balearic Islands, and in more inland areas of the Baetic and Iberian mountain ranges, the Ebro valley and the eastern Pyrenees. The natural distribution of *P. halepensis* includes a wide variety of bioclimates in the Mediterranean region (Fady et al., 2003), but due to its high tolerance to drought stress, is usually located in drier and warmer areas than other Mediterranean pine species. This species lives in areas with wide rainfall variability, supporting from less than 400 mm per year to more than 1000 mm. It grows at variable altitudes (0-2600 m a.s.l.) and on all substrates. However, the presence of *P. halepensis* is more frequent in limestone soils because it encounters less competition since most forest species are not capable of inhabiting this type of soils (Gandullo & Sánchez Palomares, 1994). In general, it is found in easily disintegrable substrates, avoiding sandy soils due to the dryness of the upper horizons in this type of soil (Obregón, 1999).

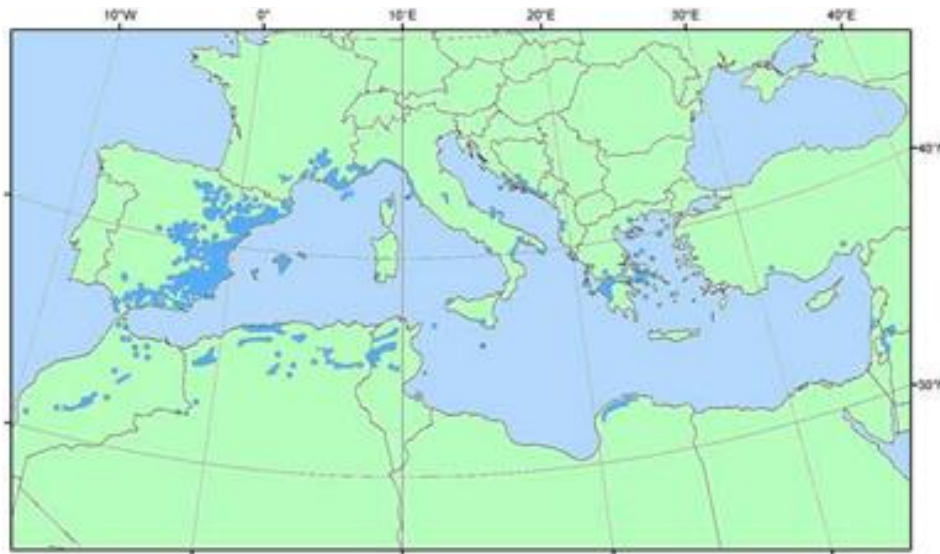


FIGURE 4 | *Pinus halepensis* distribution map. Blue shaded areas correspond to the species' natural distribution. From www.euforgen.org.

Pinus halepensis growth varies greatly within its distribution due to the different bioclimates in which it inhabits. In general, this species grows less in Spain than in other places such as Italy or France. Various studies have shown that depending on the peculiarities of the bioclimate, different parameters would mostly influence its growth. Thus, in very dry areas, water availability parameters will mainly govern the establishment, growth,

and reproduction of this species, while in areas with lower temperatures, this parameter will mostly influence (Condés & García-Robredo, 2013; Martín-Sanz et al., 2016).

Due to its high resistance to drought and its low nutrient demand, *P. halepensis* has been extensively used in afforestation so it is widespread in planted stands across Mediterranean dry zones (Gil et al., 1996). These reforestations mainly targeted ecological restoration and less frequently wood production. This species frequently used in low-demanding ornamental plantations under conditions unsuitable for other species (Chambel et al., 2013) and has been also used for the afforestation of former agricultural lands, although it's great colonizing ability allows it to expand to these areas naturally (Vallejo et al., 2012). In the region of Castilla y León (Spain), *P. halepensis* plantations occupy near 24,000 ha on the dry slopes of the Valladolid, Palencia, and Soria moors mainly (Figure 5). This study was carried out in pure *P. halepensis* plantations in Castilla y León growing in calcareous basic soils. Calcareous soils are most frequently found in semi-arid and arid regions and thus, water availability is the main limiting factor for plant growth, although the availability of P is also an important limiting factor.



FIGURE 5 | Distribution map of the *Pinus halepensis* plantations in Castilla y León.
From Gil Sánchez & Torre Antón (2007).

1.4. Research justification

Several studies found that the forest productivity of *P. halepensis* in natural stands and plantations under different environmental conditions was closely related to the availability of water and the amount of organic matter or elements such as nitrogen, magnesium or potassium (Bueis et al., 2017a; Fernández et al., 2016). However, these studies did not consider the availability and state of soil phosphorus as a factor that can affect forest productivity. Bueis et al. (2019a) showed that the P availability in the short- and medium-term was decisive in the forest productivity of *P. sylvestris* plantations in acid soils in Castilla y León. Therefore, evaluating the relationship between the different P fractions and forest productivity in our calcareous stands is highly interesting.

Although the general processes involved in the biochemistry and availability of P are known, more studies are needed to clarify which soil parameters are the ones that most affect each process in calcareous soils and to know the importance of each process in different ecosystems (Hou et al., 2014). On the one hand, some studies have reported that P sorption in calcareous soils is more related to its Fe oxide content than to CaCO_3 (Ryan et al., 1985; Solis & Torrent, 1989), while other studies have shown that labile P concentration in calcareous soils is negatively correlated with CaCO_3 content (Afif et al., 1993; Sharpley & Smith, 1985). On the other hand, several authors have studied P availability in different ecosystems, both in forests systems (De Schrijver et al., 2012; Niederberger et al., 2019; Richter et al., 2006) and in other types of ecosystems (Akhtar et al., 2005; Harrell & Wang, 2006; Zhang & MacKenzie, 1997), but there are very few studies referring to forests calcareous soils developed under Mediterranean conditions. These studies in Mediterranean soils have mainly been carried out on Vertisols, Entisols or Aridisols (Lopez-Pineiro & Garcia-Navarro, 2001; Saavedra & Delgado, 2005; Shaheen et al., 2007), which are quite different from the inceptisols that are studied here. These soils differ both in their formation and in some of their properties such as soil texture, carbonate content, organic matter, mineralogical composition, and content of Fe and Al hydrated oxide. Therefore, it is expected that the behaviour and state of the soil P differ in our soils compared to those previous studies. In order to better understand the processes involved in the availability of P in calcareous soils under Mediterranean conditions, the sequential fractionation method of P from Hedley et al. (1982) modified by Tiessen & Moir (1993) was used. This method allows to determine the different P pools in soils in relation to their availability to plants and microbial uptake (Cross & Schlesinger, 1995).

2. Objectives and hypothesis

The main objective of this master's thesis is to evaluate the state and availability of phosphorus in calcareous soils under *Pinus halepensis* plantations located in Castilla y León region (northern plateau of Spain). We also intended that the research questions posed here would improve the knowledge about the processes involved in P availability in forests calcareous soils developed under Mediterranean conditions. The specific objectives were (1) to assess soil P status and its availability in the studied soils, (2) to determine which soil processes are affecting the different soil P pools and which are driving P availability, and (3) to determine which soil parameters are influencing the forest productivity of the studied stands and whether soil P availability is leading this productivity. We hypothesized 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; (2) soil parameters related to organic matter and carbonates content are the most determining soil parameters for P availability in the basic soils studied; and (3) forest productivity is mainly related to P availability and organic matter-related parameters in the *P. halepensis* plantations studied.

3. Materials and Methods

3.1. Study area and sampling plots

This study was carried out with soils sampled in 32 plots belonging to the Spanish National Forest Inventory (SNFI; DGCN, 2002) and located in pure *P. halepensis* plantations. These stands are in the south of Palencia and Valladolid provinces, in the Castilla y León region (northern half of Spain; Figure 6 and Supplementary Information 9.1., Table S9.1.1. for geographical coordinates of the stands).



FIGURE 6 | Location of the *Pinus halepensis* studied plots. Modified from (Bueis et al., 2019b)

The altitude of the studied plantations ranges from 769 to 915 m a.s.l. and their slopes from 0 to 55%. Stands age range from 45 to 61 years; this age was established as the difference between the year of plantation and the year when the sampling was carried out. The mean annual temperature of the area is 11.7°C and the mean annual precipitation is 456 mm, suffering from summer drought (mean hydric deficit of 254 mm; Bueis et al., 2017a). The climate is classified as arid/sub-humid according to the Lang (Lang 1915), Martonne (De-Martonne, 1926) and the Annual Hydric (Thorntwaite, 1949) Indexes (Bueis et al., 2017a). Lithology in these *P. halepensis* stands is formed of limestone and marl (IGME, 1975). The calcareous soils in this area, originated from those carbonate-rich parent materials, are classified as Calcixerepts within the Inceptisol order (Llorente & Turrión, 2010). This type of soils shows basic pH (from 8 to 8.9). The soils studied have a clay-loam

texture and they show a high amount of organic matter but low total nitrogen, as usually occur in forest soils. The understory of the stands is dominated by *Quercus ilex* L., *Quercus faginea* Lam., *Genista scorpius* (L.) D.C., *Dorycnium pentaphyllum* Scop., *Stachelina dubia* L. *Lithodora fruticosa* L. and *Salvia lavandulifolia* Vahl. The main characteristics of the stands studied are shown in Table 1.

TABLE 1 | Main stands characteristics extracted from Bueis et al. (2017a).

Parameter	Mean	SD*	Min	Max
Elevation (m.a.s.l)	821.4	34.9	769	915
Latitude (°)	41.8	0.2	41.3	42.2
Longitude (°)	-4.7	0.3	-5.2	-4.1
Slope (%)	25.5	13.9	0	55
Total precipitation (mm)	456.1	27.6	405	548
Mean annual temperature (°C)	11.7	0.5	11	12
Age (years)	53.8	4.3	45	61
Stocking (trees/ha)	859.5	428.7	293.6	1711.8
Dominant height (m)	8.6	1.7	5.5	12
Mean height (m)	7.1	1.6	4.6	11.2
Quadratic mean diameter (cm)	16.3	3.6	10.3	26.2
Basal area (m ² /ha)	15.8	5.5	9.1	32
Site index (m at 80 years age)	10.9	2.2	6.8	15

*SD: standard deviation.

3.2. Soil sampling

In autumn 2012, the first 10 cm of mineral topsoil were sampled in the 32 *P. halepensis* plots (Bueis et al., 2019a) following the method of Jokela et al. (1988). Only those first 10 cm of soil were sampled as this layer is the fertile soil and the environmental changes are more strongly reflected in those first centimeters of soil. At each plot, four points located 5 m from the center of the plot were sampled (in N, S, E, and W directions). This sampling method allows to obtain representative samples which reflect the soil variability. At each of these points, one disturbed soil mineral sample was taken. The four disturbed samples from each plot were lumped together to attain a composite mineral soil sample per plot (for more details on soil sampling see Bueis et al., 2019a). Disturbed soil mineral samples were air-dried (Figure 7) and sieved (2 mm) before laboratory analyses (done in duplicate).

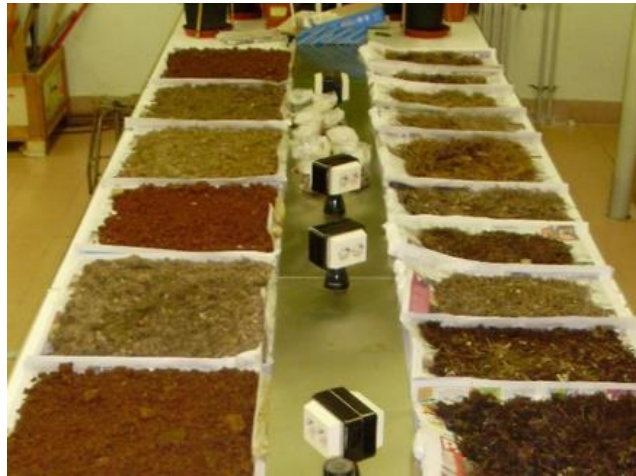


FIGURE 7 | Air drying of soil samples in the laboratory. Photo by @T. Bueis.

3.3. Laboratory methodology for edaphic phosphorus determination

Phosphorus sequential fractionation was carried out following the method developed by Hedley et al. (1982) and modified by Tiessen & Moir (1993). This fractionation was designed to remove progressively less available P with each subsequent soil extraction. It is useful to separate plant- available or labile forms of P from several refractory P pools in soils (Figure 8). Furthermore, with this procedure, the organic and inorganic P fractions can be differentiated, which is key to understanding the P cycle in forest soils. Considering that the P cycle has a biological component in which organic forms play an important role, and a geological or geochemical component in which that role is played by inorganic forms, it is necessary to differentiate between these fractions. Other procedures also widely used in the study of the soil P do not differentiate between organic and inorganic forms (see for example the P fractionation by Chang & Jackson (1957), thus being the procedure by Tiessen & Moir (1993) more interesting. Therefore, with this procedure the fractions chemically less resistant to hydrolysis, showing higher bioavailability and turnover in the soil system, are separated from stable organic and inorganic fractions. Because our soils showed basic pH and have a high amount of carbonates, we found several problems when following the fractionation steps, problems that almost did not occur when working with acid soils, and that significantly lengthened and hindered the procedure. Forthcoming, each one of the steps of the fractionation of P will be explained in detail, also indicating the difficulties encountered.

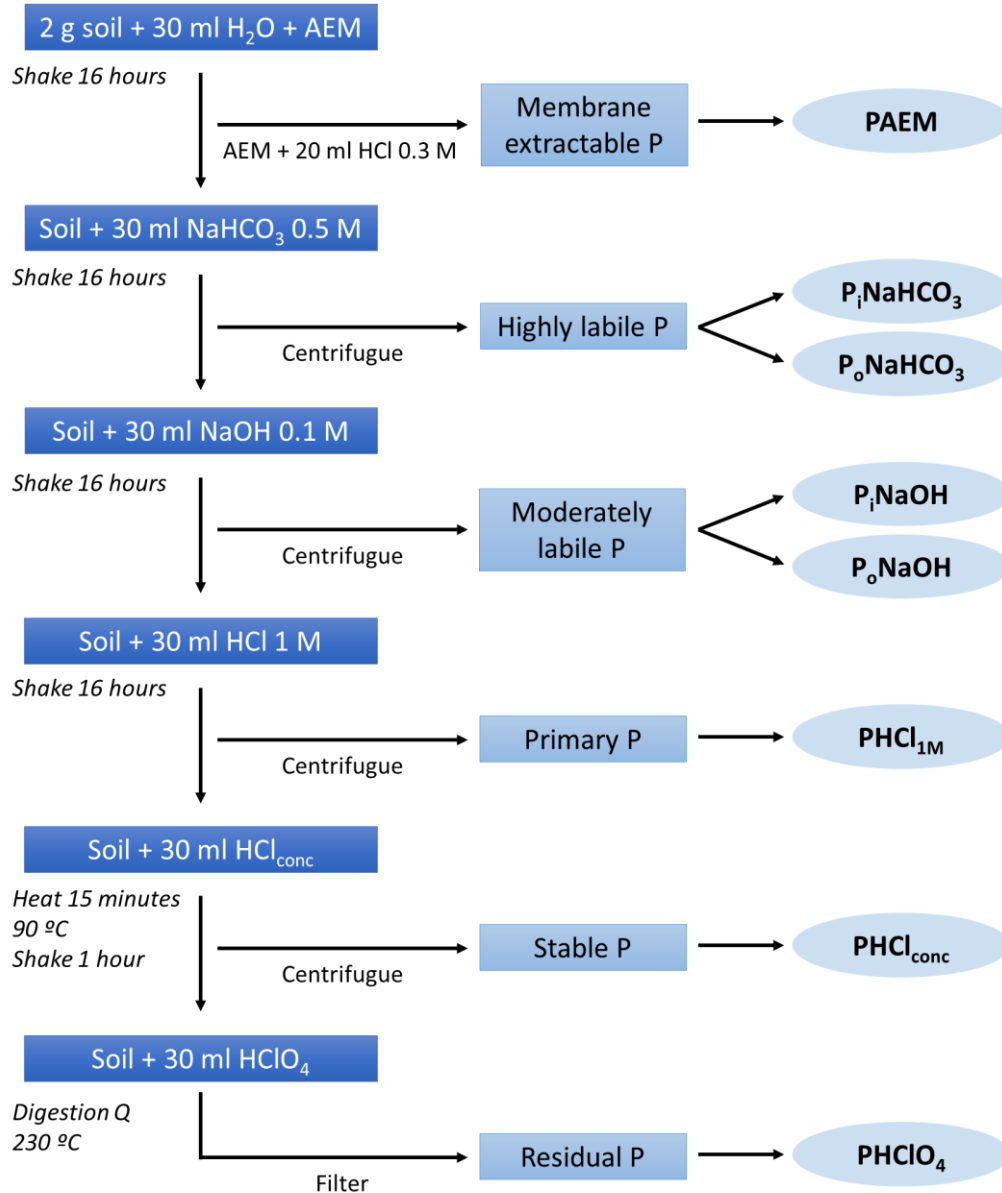


FIGURE 8 | Sequential phosphorus extraction procedure modified from Tiessen & Moir (1993).
AEM: anion exchange membrane; P_i: inorganic P; P_o: organic P; HCl_{conc}: concentrated HCl.

For the final determination of P_i concentrations in the extracts obtained, we applied the molybdenum-blue colorimetric method described by Murphy & Riley (1962) and modified by Watanabe & Olsen (1965), using an ultraviolet/visible spectrophotometer Thermo Genesys 20 (Figure 9; the detailed procedure is shown in Supplementary Information 9.2.). The pH of the extracts was adjusted to a range between 5.4 and 6.6 with HCl or NaOH and using p-nitrophenol as an indicator (Supplementary Information 9.3.). Analytical determinations were performed in duplicate on all soil samples and P concentration was expressed in mg P kg⁻¹

in oven-dried soil bases. The coefficient of variation (CV) was calculated between the replicates of the same soil for each of the analyzed fractions. When the CV between the two replicates was greater than 10%, two new replicates were performed to improve the measurement.

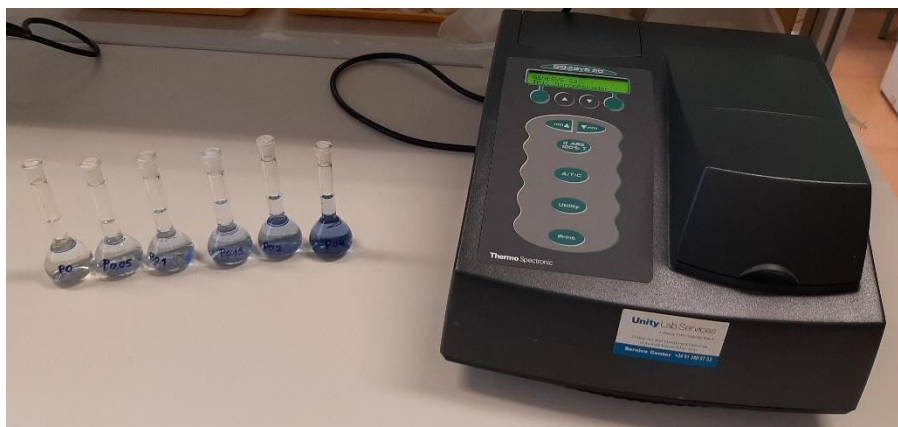


FIGURE 9 | Spectrophotometer and volumetric flasks corresponding to the calibration curve for absorbance measurement. Photo by ©R.C. Martín-Sanz.

3.3.1. Membrane extractable P (PAEM)

The first fraction extracted corresponds to the labile or readily exchangeable inorganic P (P_i) and P that is easily dissolved from solid phases in the soil (Tiessen & Moir, 1993; Turrión et al., 2008). For this first step, we used anion exchange membranes (PAEM; see complete procedure for this step in Supplementary Information 9.5.1.). Flat exchange membranes were used for this study (Figure 10). Once the exchange membranes have adsorbed on their surface the phosphate ions existing in the soil solution, desorption of these ions must be performed using 0.3 M HCl; is in this solution where soil P is analyzed. Exchange membranes can be reused once they have been reconditioned back to the form $-HCO_3^-$. Then, sequential extractions were carried out on the residue of this first extraction with progressively stronger extractants.

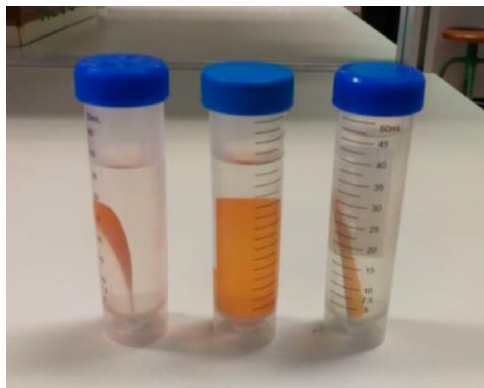


FIGURE 10 | Anion exchange membranes in contact with the 0.3 M HCl solution in the desorption step. Photo by ©R.C. Martín-Sanz.

3.3.2. Highly labile P ($P_i\text{NaHCO}_3$ and $P_o\text{NaHCO}_3$)

The following extraction was performed with 0.5 M NaHCO_3 with pH 8.5, to determine the highly labile P adsorbed in the soil exchange complex (Figure 11(a)). Using basic extractants such as NaHCO_3 or NaOH –utilized in the next step of the fractionation– implies the extraction of organic and inorganic forms of P. The determination of the P concentration corresponding to the organic forms is carried out by difference between the total P extracted, determined after performing digestion of the organic matter of the extract, and the existing inorganic P in the extract. The alkaline solution of NaHCO_3 when applied to soils rich in organic matter, as the studied forest soils, will extract a significant amount of humic substances that interfere in the subsequent colorimetric determination of P. Therefore, to remove those humic substances before determining the inorganic P, it is necessary to use active carbon (Figure 11 (b)). Here, we encounter a problem when performing the analytical methodology in the laboratory. The active carbon for this analysis should be phosphorus-free, but we found that it had amounts of phosphorus that interfered with our analyses. Thus, we needed to remove the phosphorus from the active carbon before continuing with the P fractionation in our soil samples (the methodology followed to remove P from active carbon is shown in Supplementary Information 9.4.).

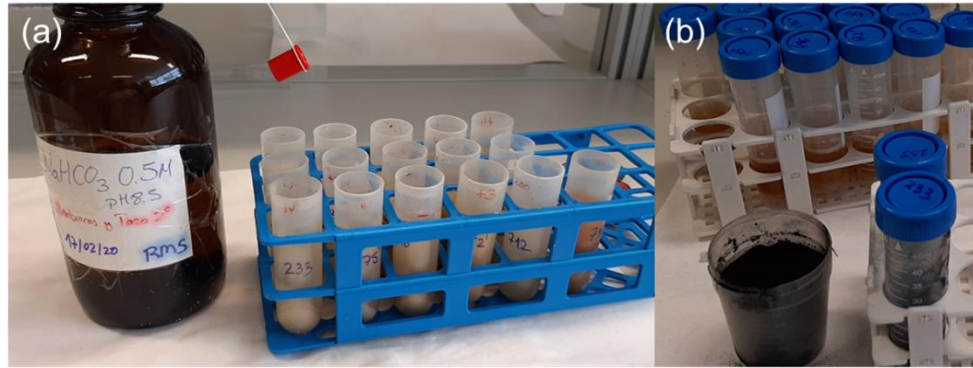


FIGURE 11 | (a) Highly labile P extraction with 0.5 M NaHCO₃. (b) Addition of active carbon to the NaHCO₃ extracts to eliminate interferences produced by organic matter.
Photos by @R.C. Martín-Sanz.

The total P was determined to obtain the inorganic and organic fractions of highly labile P (P_iNaHCO₃, P_oNaHCO₃). This total P was determined in NaHCO₃ extracts after digesting with 0.5 M H₂SO₄ and potassium persulfate at 120 °C for 45 minutes in an autoclave (Tiessen & Moir, 1993; Figure 12). This digestion is done 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 (see the detailed procedure for this complete step in Supplementary Information 9.5.2.). In this step, we found another drawback. When adding the sulfuric acid, a very virulent reaction occurs, because the extractant used is basic (NaHCO₃) and because our soils have many carbonates. Therefore, the sulfuric acid should be added very slowly, leaving time for the reaction to reduce. This also happened in the next step when determining the total P extracted by NaOH.

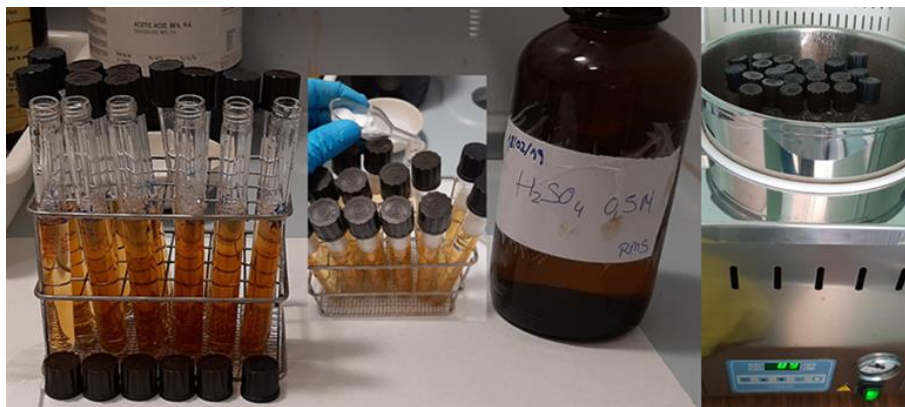


FIGURE 12 | Determination of total P in NaHCO₃ extracts by digestion in autoclave.
Photos by @R.C. Martín-Sanz.

3.3.3. Moderately labile P (P_i NaOH and P_o NaOH)

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 (Figure 13). This P fraction is thought to be associated with the surface of amorphous and some crystalline Al and Fe minerals and is probably available in the mid-term. As in the previous step, the alkaline NaOH solution extracts a big amount of humic substances in soils rich in organic matter. To eliminate those humic substances that interfere with the colorimetric determination of P, it is necessary to use active carbon with the consequent problem previously described. Total P was determined in the NaOH extracts after digesting with 0.5 M H_2SO_4 and potassium persulfate at 120°C for 45 minutes in an autoclave (Tiessen & Moir, 1993). As in the previous step, this was done to transform the organic P into inorganic forms. The organic P in these extracts was obtained as the difference between total P and inorganic P (procedure detailed in Supplementary Information 9.5.3.). The organic P in this extract is involved in the mid-term P transformations in soils and is a more stable P than the organic P in the bicarbonate extract (Cross & Schlesinger, 2001).

The remaining P extracts (explained below) represent soil P that is available for long time periods (Cross & Schlesinger, 1995).



FIGURE 13 | Moderately labile P extraction with 0.1 M NaOH. Photo by ©R.C. Martín-Sanz.

3.3.4. Primary P (PHCl_{1M})

The primary P (PHCl_{1M}) was extracted with 1 M HCl (Figure 14 (a); detailed methodology is shown in Supplementary Information 9.5.4.). In this step, we also encountered serious difficulties. Our soils showed high amounts of carbonates so the reaction that occurred when adding 1 M HCl to the soil was very violent and took a long time to reduce (Figure 14 (b)). Therefore, in addition to adding the HCl gradually and moving the soil each time (to separate it from the bottom of the centrifuge tube), we had to stop for at least two days so that the reaction ended and we could continue with the procedure without danger of losing sample.

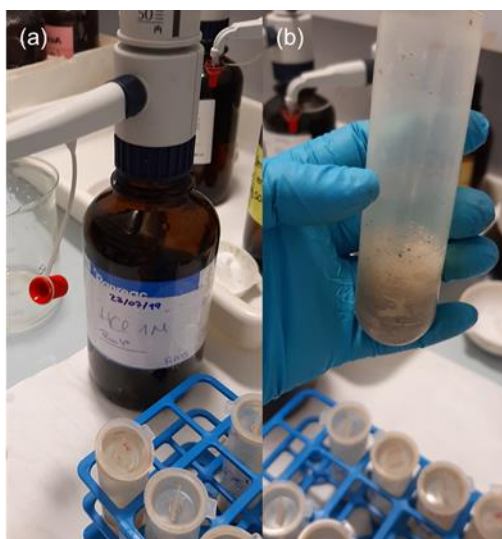


FIGURE 14 | (a) Primary P extraction with 1 M HCl. (b) Detail of the violent reaction that occurs when pouring 1 M HCl into the soil sample. Photos by @R.C. Martín-Sanz.

3.3.5. Stable and residual P (PHCl_{conc} and PHClO_4)

Finally, the procedure has two other steps to extract the most recalcitrant fractions which are the stable and the residual P. The sum of these two fractions is considered the highly recalcitrant P fraction (Turrión et al., 2000b) and could be also named as highly resistant, unavailable, refractory, or occluded P (Richter et al., 2006). These fractions are derived from non-alkali-extractable organic debris (Magid et al., 1996; Tiessen & Moir, 1993). On one hand, the stable P (PHCl_{conc}) was obtained by the extraction with concentrated HCl at 90 °C in a water bath (Figure 15) and corresponds with P_i and P_o bound in the interior of Fe and Al minerals and apatite (Tiessen & Moir, 1993; procedure detailed in Supplementary Information 9.5.5.). On the other hand, the residual P (PHClO_4) was extracted with HClO_4 and digested at 230 °C in a digestion block (Figure 16; procedure detailed in Supplementary

Information 9.5.6.) and represents the most stable form of P that is available only in the long term (Cross & Schlesinger, 1995).

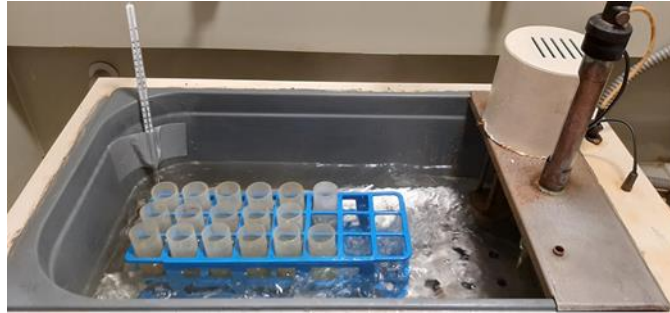


FIGURE 15 | Stable P extraction with concentrated HCl in a water bath. Photos by ©R.C. Martín-Sanz.



FIGURE 16 | Residual P extraction with HClO_4 in the digestion block. Photos by ©R.C. Martín-Sanz.

Again, when pouring the perchloric acid in the soil samples a highly intense reaction appeared (Figure 17 (a)). Thus, the HClO_4 had to be added gradually, firstly in the centrifuge tubes and then into the digestion tubes (Figure 17 (b)).

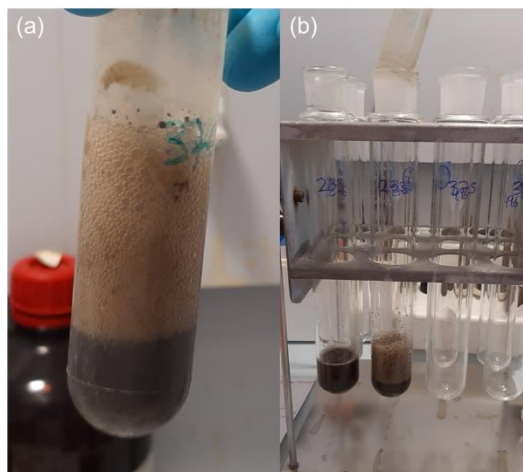


FIGURE 17 | (a) Detail of the reaction that occurs when pouring HClO_4 into the soil samples. (b) Digestion tubes used for the residual P extraction. Photos by @R.C. Martín-Sanz.

3.4. Other soil parameters

As previously stated, formerly available data from Dr. Bueis' doctoral thesis was used in this study (Bueis, 2017). During this doctoral thesis, a large number of soil analyses were carried out, creating extensive databases with more than 35 physical, chemical, and biochemical soil parameters (see Bueis et al., 2017a, 2018, 2019b, for more information on the analysis carried out and the databases in Bueis et al., 2016, 2017b). Some of these parameters analyzed were the particle size distribution by the pipette method (MAPA, 1993) and the successive determination of the clay, sand and silt content according to international criteria (ISSS) and silt and sand following the USDA criteria (physical property), pH using a 1:2.5 (soil/water) suspension (MAPA, 1993) or easily oxidizable carbon (EOC) through the Walkley & Black (1934) method (chemical properties) and mineralizable C (Isermeyer, 1952) as an example of biochemical parameters. We extracted the edaphic parameters data for this study from those two databases previously indicated (Bueis et al., 2016, 2017b) and we discarded some soil parameters because they were obtained as quotients of other selected parameters, thus providing redundant information. Therefore, we rejected the total organic carbon to total nitrogen ratio (quotient of both parameters), soil available water (quotient of field capacity and permanent wilting point), the ratio between mineralizable carbon or microbial biomass and total organic carbon, and the microbial metabolic ratio. We also discarded the sand and silt content according to international criteria (ISSS) since we use sand and silt percentages following the USDA criteria due to the particle size considered in this second criterion has higher ecological significance. Furthermore, the bulk density was

included in the analysis because it is an indicator of compaction and soil health, it affects infiltration, rooting, water retention capacity, porosity, plant nutrients availability, and soil microorganisms' activity. Taking into account that the high number of available soil parameters (33 selected parameters) and the number of plots studied (n = 32) can greatly hinder the statistical study, the parameters were clustered into five groups related (1) to soil physical fertility, (2) to chemical fertility, (3) to soil organic matter, (4) to soil composition, and (5) to the enzymatic activity of the soil (Table 2).

TABLE 2 | Summary of reported soil parameters used in this study and clustered into five groups (extracted from Bueis et al., 2016, 2017b).

Group	Parameter abbreviation	Description and units	Methodology
Soil composition	Clay	Clay content (%)	Pipette method (MAPA, 1993)
	SiltUS	Silt content USDA criteria (%)	Pipette method (MAPA, 1993)
	SandUS	Sand content USDA criteria (%)	Pipette method (MAPA, 1993)
	Carb	Total carbonates (%)	Bundy & Bremner (1972)
	ActiveC	Active carbonates (%)	Bashour & Sayegh (2007)
	Gypsum	CaSO ₄ .2H ₂ O (%)	Richards (1954)
Physical fertility	BD	Bulk density (g/cm ³)	Field determination with cylindrical metal sampler
	Por	Soil porosity (%)	Using bulk and real densities
	FC	Field capacity (%)	Eijkelkamp pF equipment (½ atm)
	PWP	Permanent wilting point (%)	Eijkelkamp pF equipment (15 atm)
Chemical fertility	pH	Soil pH value	PHmeter determination in 1:2.5 soil/water suspension (MAPA, 1993)
	CEC	Cation exchange capacity (cmol+/kg soil)	Schollenberger & Simon (1945)
	Fe	Available iron (mg/kg soil)	DTPA-TEA method (Lindsay & Norvell, 1978)
	Cu	Available copper (mg/kg soil)	DTPA-TEA method (Lindsay & Norvell, 1978)
	Mn	Available manganese (mg/kg soil)	DTPA-TEA method (Lindsay & Norvell, 1978)
	Zn	Available zinc (mg/kg soil)	DTPA-TEA method (Lindsay and Norvell 1978)
	K	Exchangeable potassium (cmol+/kg soil)	Extraction with 1M ammonium acetate (pH 7) and AAS/AES determination
	Mg	Exchangeable magnesium (cmol+/kg soil)	Extraction with 1M ammonium acetate (pH 7) and AAS determination
	Na	Exchangeable sodium (cmol+/kg soil)	Extraction with 1M ammonium acetate (pH 7) and AAS determination

AAS/AES: Atomic absorption/emission spectroscopy. DTPA-TEA: diethylenetriaminepentaacetic acid–triethanolamine. U (μmol/min) is defined as the amount of the enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method.

TABLE 2 (cont.) | Summary of reported soil parameters used in this study and clustered into five groups (extracted from Bueis et al., 2016, 2017b).

Group	Parameter abbreviation	Description and units	Methodology
Organic matter	EOC	Easily oxidizable carbon (%)	Walkley & Black (1934)
	TN	Total nitrogen (%)	Dry combustion using LECO CHN 2000
	TOC	Total organic carbon (%)	Dry combustion using LECO CHN 2000
	Cmin	Mineralizable carbon (mg/kg soil week)	Isermeyer (1952)
	Cmic	Microbial biomass carbon (mg/kg soil)	Fumigation-extraction method, Vance et al. (1987)
	Nmic	Microbial biomass nitrogen (mg/kg soil)	Fumigation-extraction method, Brookes et al. (1982)
	Pmic	Microbial biomass phosphorus (mg/kg soil)	Fumigation-extraction method, Brookes et al. (1982)
Enzymatic activity	FDA	Fluorescein diacetate hydrolysis reaction (µg/g*h)	Alef & Nannipieri (1995)
	DHA	Dehydrogenase activity (µg/g*h)	Casida et al. (1964)
	Ure	Urease activity (µg/g*h)	Hofmann (1963)
	Cat	Catalase activity (O ₂ /3min*g)	Beck (1971)
	AlkP	Alkaline phosphatase activity (µg/g*h)	Tabatabai & Bremner (1969)
	AcP	Acid phosphatase activity (µg/g*h)	Tabatabai & Bremner (1969)
	WSP	Water soluble phenols (mg/kg soil)	Box (1983)

AAS/AES: Atomic absorption/emission spectroscopy. DTPA-TEA: diethylenetriaminepentaacetic acid-triethanolamine.

3.5. Forest productivity

Site index (dominant height at a reference age) is a useful tool for forest productivity estimation (Skovsgaard & Vanclay, 2008) as it is strongly correlated with wood production. Here, we used the site index calculated by Bueis et al. (2017a) for the same *Pinus halepensis* plots. In this previous work, site index (SI) was calculated according to the equation developed by Montero et al. (2001):

$$H_0 = a * (1 - e^{-0.203954*t})^{\frac{1}{1.046295}}$$

where H_0 is the dominant height in meters and t is the age in years. The value of a was determined for every plot from the previous equation and the current H_0 and t values. To determine site index for every plot (dominant height at the reference age of 80 years), the

value of a for each plot and the reference age ($t = 80$ years) were introduced into the equation and the resulting value of H_0 was the site index.

3.6. Statistical analysis

Firstly, to determine the relationships between P fractions (Tiessen & Moir, 1993), Spearman's correlation coefficients (done with *Hmisc* package in R software; Harrell et al., 2018) were calculated and principal component analysis (PCA) was performed (in R: *prcomp*). To assess the relationships between P fractions and groups of edaphic parameters, Spearman's correlation coefficients were calculated, and canonical correlation analysis (CCA) were carried out using CCA and CCP packages in R (González et al., 2008; Menzel, 2012). Therefore, canonical correlation analyses were used to explore the joint association between P fractions and soil parameters related to (a) physical fertility, (b) chemical fertility, (c) soil composition, (d) organic matter, and (e) soil enzymatic activity. Canonical correlation analysis identifies linear combinations between two data matrices that are highly correlated (response matrix Y and explanatory matrix X). In our case, the Y matrix is formed by the different phosphorus fractions studied in the 32 plots (8 × 32 matrix) and we have several X matrices formed by the different groups of soil parameters previously explained (see Table 2). This procedure allowed us to assess which combinations of soil parameters were most correlated with variation in P fractions. Kaiser's Measure of Sampling Adequacy (in R: *KMO*; *psych* package; Revelle, 2017) was calculated to test whether the sample size was adequate or not for CCA (Cerny & Kaiser, 1977). This test can vary from 0 to 1 and indicates the degree to which each variable in a set is predicted without error by the other variables. Lastly, we used Spearman's correlations and general partial least squares regression (PLS; in R: *pls* from the *pls* package; Wehrens & Mevik, 2007) to study the relationship between the site index (as an estimate of forest productivity), the P fractions, and all the edaphic parameters included in this study. PLS is a multivariate statistical technique that combines principal component analysis (PCA) and multiple linear regression which allows us to explain both the variability of the matrix of independent variables and the vector of the dependent variable. PLS is particularly useful when the number of predictor variables is greater than the number of observations and when there are many highly related explanatory variables (Kassambara, 2018; Mateos-Aparicio, 2011). The PLS regression, like PCA, seeks to find components that maximize the variability of the predictors (the edaphic parameters and the P fractions in this study) but both techniques differ in that PLS requires that the components have a maximum correlation with the outcome variable (in this

case the site index), while the PCA ignores the response variable (Abdi, 2003). Therefore, PLS is a supervised procedure by the outcome variable whereas PCA is unsupervised. However, although this method is a more objective way to select the predictors the model obtained requires all the variables that intervene in its specification. Therefore, from a practical point of view and with a predictive purpose, it is of limited application in management. In addition, to describe the soil type of the studied plots, descriptive analysis and Spearman's correlations were carried out among all soil parameters used in this work. Before performing all the PCA, CCA and PLS analyses, the studied variables were tested for linearity, outliers, and normality (in R: shapiro.test), and those variables not normally distributed were transformed. Statistical analyses were performed with R software (R Core Team, 2020).

4. Results

4.1. Exploratory data analysis

In statistics, exploratory data analysis is an approach to analyze data sets to summarize their main characteristics, to check the assumptions, and to look for patterns or for spot anomalies, among others. Descriptive statistics and visual methods are generally used, without formal models or hypothesis testing.

As was indicated in the statistical analysis section, the studied variables were tested for linearity, outliers, and normality (in R: `shapiro.test`). Those variables not normally distributed were transformed before the analyses (Supplementary Information 9.1., Table S9.1.2.). To enhance the understandability of the results and discussion sections, we will only refer to the variables themselves in the following text, unless it affects the interpretation.

4.2. Main characteristics of the studied soils

As can be seen in Table 3, the studied soils are basic (pH values between 8.0 and 8.9) and rich in carbonates, with total carbonates content higher than 30% (except for plots 717, 718, and 723 with 3.0%, 1.4%, and 9.4% active carbonates, respectively; see Bueis et al., 2016, 2017b). Active carbonates content in all the studied soils is less than 3%, so although they have a high total carbonate content, they have low chlorinating power. Gypsum concentrations in the studied soils range from 9 to 51% so they can be considered as moderately gypsic and strongly gypsic soils (Table 3; IUSS Working Group WRB, 2006). Soil texture can be considered as fine (clay loam, sandy clay loam, silty clay, and silty clay loam) and medium (loam, silt, and silt loam; Supplementary Information 9.1., Table S9.1.1.).

TABLE 3 | Main characteristics of the studied soils.

Soil parameter and units	Mean	SD	Min	Max
Clay (%)	22.4	10.0	4.5	43.2
SiltUS (%)	53.2	18.5	13.3	88.6
SandUS (%)	24.4	14.7	1.3	61.1
Carbonates (%)	54.3	19.5	1.4	79.1
ActiveC (%)	1.6	0.8	0.0	3.2
Gypsum (%)	25.3	10.6	8.9	50.6
BD (g/cm ³)	1.1	0.2	0.8	1.4
Porosity (%)	46.1	6.0	37.1	58.7
FC (%)	23.4	4.8	15.0	31.9
PWP (%)	15.1	5.4	4.0	26.6
pH	8.4	0.2	8.0	8.9
CEC (cmol+/kg soil)	21.0	4.8	14.7	38.4
Fe (mg/kg soil)	7.8	4.6	3.2	26.7
Cu (mg/kg soil)	0.5	0.2	0.2	1.6
Mn (mg/kg soil)	16.0	6.5	7.0	32.8
Zn (mg/kg soil)	0.6	0.3	0.2	1.5
K (cmol+/kg soil)	0.8	0.2	0.5	1.4
Mg (cmol+/kg soil)	3.1	1.9	0.6	7.6
Na (cmol+/kg soil)	0.1	0.0	0.0	0.2
EOC (%)	1.7	0.8	0.9	4.3
TN (%)	0.1	0.1	0.0	0.4
TOC (%)	2.1	1.1	0.2	5.1
Cmic (mg/kg soil)	209.7	82.5	96.0	445.3
Cmin (mg/kg soil week)	34.2	11.7	17.3	62.4

TABLE 3 (cont.) | Main characteristics of the studied soils.

Soil parameter and units	Mean	SD	Min	Max
Nmic (mg/kg soil)	26.9	11.6	10.4	50.1
Pmic (mg/kg soil)	7.7	4.3	1.4	17.2
FDA ($\mu\text{g/g}\cdot\text{h}$)	20.4	8.8	5.4	38.2
DHA ($\mu\text{g/g}\cdot\text{h}$)	9.4	4.9	1.9	23.5
Ure ($\mu\text{g N/g}\cdot\text{h}$)	76.8	42.0	17.9	196.6
Cat ($\text{O}_2/3\text{min}\cdot\text{g}$)	1.2	0.6	0.5	3.7
AlkP ($\mu\text{g/g}\cdot\text{h}$)	5.9	3.0	0.2	12.6
AcP ($\mu\text{g/g}\cdot\text{h}$)	4.6	2.4	0.7	10.3
WSP (mg/kg soil)	27.0	18.0	5.0	80.0

SD: standard deviation. See explanation of parameters' abbreviations in Table 2.

As expected, the strongest significant correlations were generally found among the soil parameters belonging to the same groups of soil properties previously commented (see Supplementary Information 9.1., Table S9.1.3. for details on these correlations). Cation exchange capacity was mainly correlated to organic matter-related parameters such as EOC (Spearman $\rho = 0.80$, $P < 0.001$), TOC ($\rho = 0.62$, $P < 0.001$), TN ($\rho = 0.67$, $P < 0.001$), Cmic ($\rho = 0.62$, $P < 0.001$), Cmin ($\rho = 0.56$, $P = 0.001$) and Nmic ($\rho = 0.63$, $P < 0.001$) and to several available micronutrients and exchangeable macronutrient content such as Mn ($\rho = 0.54$, $P = 0.001$) and K ($\rho = 0.50$, $P = 0.004$). CEC was also highly correlated with enzyme activity-related parameters: DHA ($\rho = 0.52$, $P = 0.003$), Urease ($\rho = 0.67$, $P < 0.001$), Catalase ($\rho = 0.76$, $P < 0.001$) and AlkP ($\rho = 0.50$, $P = 0.003$) among others. However, cation exchange capacity was not significantly correlated to soil clay content as could be expected. Highly significant correlations were also obtained between the variables related to organic matter and the enzymatic activities of the soil. Thus, the EOC showed highly significant correlations with all the enzymes studied, even with acid phosphatase, and Cmin was also similarly correlated with the enzyme activity-related parameters. The TOC, TN, Cmic and Nmic also showed highly significant and positive correlations with all the studied enzyme activities except for acid phosphatase.

4.3. Soil phosphorus fractionation

The soils studied showed a mean total P content of 289 mg/kg, varying between 116 and 611 mg/kg. Organic P represented around 12% of the P_{total} (Table 4). As expected, the primary P fraction ($\text{PHCl}_{1\text{M}}$) was the biggest fraction in the soils studied followed by the stable P fraction ($\text{PHCl}_{\text{conc}}$; Table 4). The primary P includes P forms in primary Ca minerals, accounting for 47% of total P. The $\text{PHCl}_{\text{conc}}$ accounted for more than 27% of total P. The smallest P fraction in these soils was the labile or readily exchangeable P fraction (PAEM) that accounted for 1.2% of total P.

TABLE 4 | Phosphorus fractions (mg kg^{-1}) in the studied soils.

P fraction	Mean	SD	Min	Max	% of P_{Total}
PAEM	3.4	1.9	1.0	10.6	1.2
P_iNaHCO_3	6.9	2.8	1.6	15.2	2.4
P_oNaHCO_3	5.5	3.6	0.2	15.6	1.9
P_iNaOH	11.4	9.5	3.2	46.9	3.9
P_oNaOH	30.5	20.2	8.6	120.8	10.5
$\text{PHCl}_{1\text{M}}$	137.0	94.3	3.7	387.1	47.3
$\text{PHCl}_{\text{conc}}$	78.6	44.4	18.9	235.5	27.2
PHClO_4	16.1	5.6	6.5	32.0	5.6
P_{Total}	289.4	120.8	116.8	611.6	100

SD: standard deviation.

In the principal component analysis of P fractions, three principal components accounted for 74% of total variance. The first principal component was mainly positively correlated to the organic highly labile P fraction (P_oNaHCO_3), the second principal component was positively correlated to the stable P fraction ($\text{PHCl}_{\text{conc}}$) and the third principal component was positively correlated to the primary forms of P ($\text{PHCl}_{1\text{M}}$; Figure 18 and Table 5).

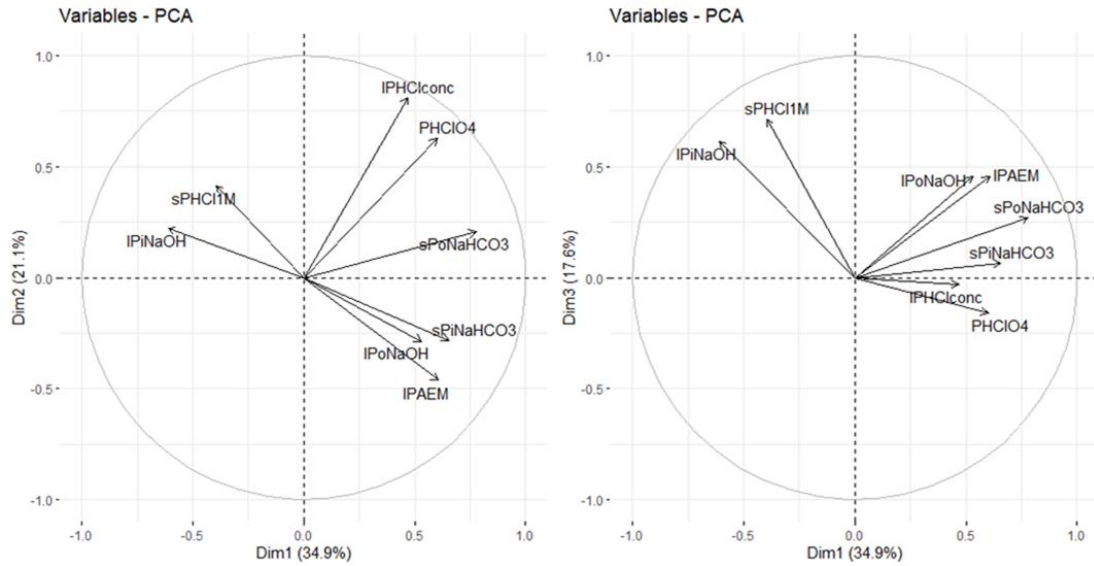


FIGURE 18 | Variables plot for the first (Dim1), second (Dim2) and third (Dim3) components of the Principal Component Analysis based on the soil P fractions.

TABLE 5 | Results from the Principal Component Analysis applied to phosphorus fractions data from 32 *Pinus halepensis* plots studied. Variables with loadings > |0.70| in bold case.

P fraction	PC1	PC2	PC3
log(PAEM)	0.61	-0.46	0.46
$\sqrt{P_iNaHCO_3}$	0.65	-0.28	0.07
$\sqrt{P_oNaHCO_3}$	0.78	0.21	0.27
log(PiNaOH)	-0.61	0.22	0.62
log(PoNaOH)	0.53	-0.29	0.46
$\sqrt{PHCl_{1M}}$	-0.40	0.41	0.71
log(PHCl _{conc})	0.47	0.81	-0.03
PHClO ₄	0.60	0.63	-0.16
<i>Importance of components</i>			
Proportion of variance explained	0.35	0.21	0.18
Cumulative proportion of variance	0.35	0.56	0.74

According to the Spearman's correlations among all P fractions, the PAEM fraction was positively correlated to the inorganic highly labile P (P_iNaHCO_3 ; Spearman rho = 0.47, $P = 0.007$) and to the organic moderately labile P (P_oNaOH ; rho = 0.54, $P = 0.001$; Table 6). The organic highly labile fraction (P_oNaHCO_3) was significantly correlated with the organic

moderately labile P (P_oNaOH ; $\rho = 0.44$, $P = 0.013$) and with the stable P fraction ($PHCl_{conc}$; $\rho = 0.39$; $P = 0.027$). The highest positive significant correlations were found between the inorganic moderately labile P (P_iNaOH) and the primary P forms ($PHCl_{1M}$; $\rho = 0.60$, $P < 0.000$) and between the stable ($PHCl_{conc}$) and the residual ($PHClO_4$) P forms ($\rho = 0.69$, $P < 0.000$). Besides, total P was only significantly related to P_iNaOH , $PHCl_{1M}$ and $PHCl_{conc}$ (Table 6).

TABLE 6 | Spearman's correlations among P fractions ("-" means not significant). Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	PAEM	P_iNaHCO_3	P_oNaHCO_3	P_iNaOH	P_oNaOH	$PHCl_{1M}$	$PHCl_{conc}$
P_iNaHCO_3	0.47**						
P_oNaHCO_3	-	-					
P_iNaOH	-	-	-				
P_oNaOH	0.54**	-	0.44*	-			
$PHCl_{1M}$	-	-	-	0.60***	-		
$PHCl_{conc}$	-	-	0.39*	-	-	-	
$PHClO_4$	-	-	-	-	-	-	0.66***
P_{Total}	-	-	-	0.44*	-	0.82***	0.49**

4.4. Relationship between P fractions and soil parameters

4.4.1. P fractions and soil composition parameters

In the canonical correlation analysis performed on P fractions in relation to soil composition parameters, we firstly included six parameters (see Table 2), but the Kaiser's Measure of Sampling Adequacy (MSA) was 0.40 and thus, this analysis was not considered statistically acceptable. Therefore, we decided to eliminate one parameter related to soil texture (i.e. clay, silt or sand content). Clay content was highly correlated to silt content ($r = -0.66$, $P < 0.000$), while it was not significantly correlated to sand content ($r = 0.06$, $P = 0.732$). Silt and sand contents were also highly significantly correlated ($r = -0.74$, $P < 0.001$). Thus, we decided to keep in the analysis the clay percentage and we checked the individual MSA for silt content and the individual MSA for sand content trying to discard one of these two variables. As the MSA of sand was higher than that of silt (0.39 and 0.38, respectively) we discarded silt content in the analysis. Clay and sand contents give good information about the texture of the studied soils. The Kaiser's Measure of Sampling Adequacy without silt content (MSA = 0.60) indicated that the analysis was statistically acceptable. So, this CCA was performed using five soil parameters related to soil composition: percentage of

clay, percentage of sand according to USDA criteria, carbonates, active carbonates, and gypsum content. Two canonical dimensions were significant (Wilk's lambda; $P < 0.001$ and $P = 0.002$, apiece) and presented correlations of 0.87 and 0.81, respectively (Supplementary Information 9.1., Table S9.1.4.). The first canonical dimension was mainly influenced by the sand content ($r = 0.97$) and the primary P (PHCl_{1M} ; $r = -0.91$; Figure 19 and Table 7). The second canonical dimension was negatively correlated to carbonates ($r = -0.77$), active carbonates content ($r = -0.69$) and the inorganic highly labile P (P_iNaHCO_3 ; $r = -0.54$), and was positively correlated to the residual P (PHClO_4 ; $r = 0.54$).

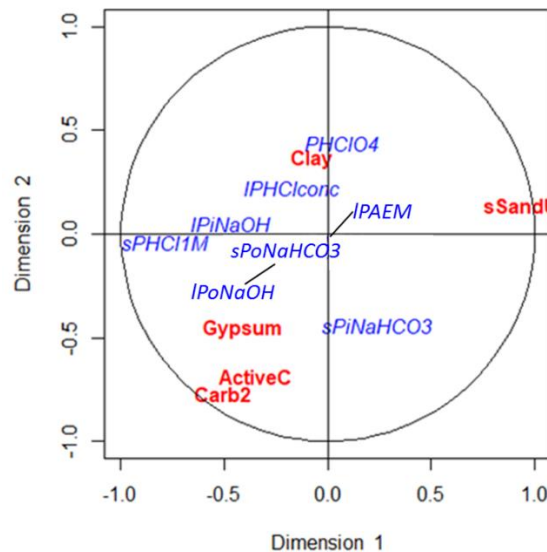


FIGURE 19 | Variables plot for the first and second canonical dimensions of the canonical correlation analysis performed to the P fractions and the soil composition parameters. Abbreviations for soil parameters as in Supplementary Information 9.1., Table S9.1.2.

TABLE 7 | Factor loadings (simple correlations between each canonical factor and the variables) of canonical correlation analysis performed on P fractions in relation to soil composition parameters. Variables with higher importance in each component in bold case.

Variable	Dimension 1	Dimension 2
Clay	-0.08	0.36
√SandUS	0.97	0.14
ActiveC	-0.35	-0.69
Gypsum	-0.41	-0.46
Carbonates ²	-0.51	-0.77
log(PAEM)	0.02	-0.06
√PiNaHCO ₃	0.28	-0.54
√P _o NaHCO ₃	-0.16	-0.10
log(PiNaOH)	-0.54	0.06
log(P _o NaOH)	-0.32	-0.13
√PHCl _{1M}	-0.91	-0.06
log(PHCl _{conc})	-0.20	0.27
PHClO ₄	0.08	0.54

Clay content was positively correlated with the residual P (PHClO₄) but negatively with PAEM (Table 8). Sand content was highly negatively correlated with the primary P (PHCl_{1M}) and the inorganic moderately labile P (PiNaOH). The amount of gypsum was positively related to the highly labile P and the organic moderately labile P, and finally, carbonates content was negatively correlated with PHClO₄.

TABLE 8 | Spearman's correlations between P fractions and soil composition parameters ("-" means not significant). Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	PAEM	PiNaHCO ₃	P _o NaHCO ₃	PiNaOH	P _o NaOH	PHCl _{1M}	PHCl _{conc}	PHClO ₄
Clay	-0.40*	-	-	-	-	-	-	0.42*
SiltUS	-	-	-	-	-	0.54**	-	-0.35*
SandUS	-	-	-	-0.52**	-	-0.81***	-	-
ActiveC	-	-	-	-	-	-	-	-
Gypsum	-	0.39*	0.46**	-	0.48**	-	-	-
Carbonates	-	-	-	-	-	-	-	-0.62***

4.4.2. P fractions and soil physical fertility parameters

The first canonical dimension in the canonical correlation analysis of P fractions and physical fertility-related parameters was significant according to Wilk's lambda test ($P = 0.0005$) with a canonical correlation of 0.89 (Supplementary Information 9.1., Table S9.1.5.). Kaiser's Measure of Sampling Adequacy (MSA) was 0.63 and, therefore, this analysis was considered statistically acceptable. The first canonical dimension was negatively correlated to field capacity, permanent wilting point, soil porosity, and to all P fractions, and it was just positively related to bulk density (Figure 20 and Table 9).

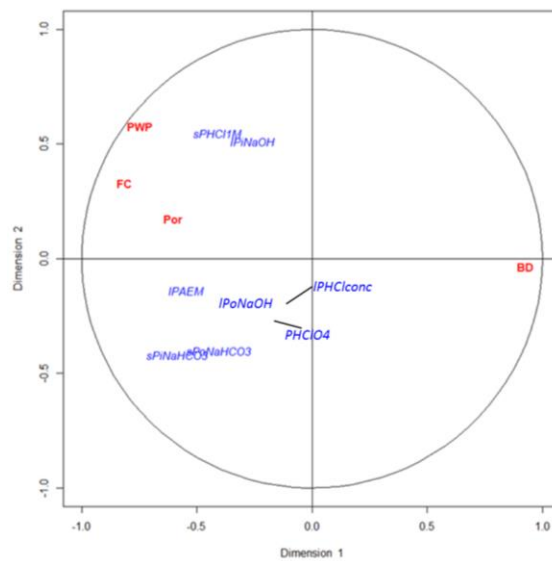


FIGURE 20 | Variables plot for the first canonical dimension of the canonical correlation analysis performed to the P fractions and the physical fertility-related parameters. Abbreviations for soil parameters as in Supplementary Information 9.1., Table S9.1.2.

TABLE 9 | Factor loadings (simple correlations between each canonical factor and the variables) of canonical correlation analysis performed on P fractions in relation to soil physical fertility parameters.

Variable	Dimension 1
BD	0.93
Porosity	-0.60
FC	-0.81
PWP	-0.75
log(PAEM)	-0.61
$\sqrt{P_iNaHCO_3}$	-0.66
$\sqrt{P_oNaHCO_3}$	-0.46
log(P_iNaOH)	-0.29
log(P_oNaOH)	-0.32
$\sqrt{PHCl_{1M}}$	-0.46
log($PHCl_{conc}$)	-0.17
$PHClO_4$	-0.14

Strong positive correlations between the primary P forms and field capacity and permanent wilting point were found (Table 10). The inorganic moderately labile P fraction and PAEM were also positively correlated with the previously referred soil parameters. Porosity only showed significant correlations with the inorganic highly labile P fraction and was the parameter least correlated with the first canonical root in the CCA. Bulk density was significantly and negatively related to PAEM and to the inorganic highly labile fraction, respectively.

TABLE 10 | Spearman's correlations between P fractions and soil physical fertility-related parameters ("-" means not significant). Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	PAEM	P_iNaHCO_3	P_oNaHCO_3	P_iNaOH	P_oNaOH	$PHCl_{1M}$	$PHCl_{conc}$	$PHClO_4$
BD	-0.36*	-0.58**	-	-	-	-	-	-
Porosity	-	0.37*	-	-	-	-	-	-
PWP	0.36*	-	-	0.45*	0.38*	0.49**	-	-
FC	-	-	-	0.44*	-	0.49**	-	-

4.4.3. P fractions and soil chemical fertility parameters

The canonical correlation analysis (CCA) between the P fractions and soil chemical fertility-related parameters produced two canonical dimensions that captured significant relationships between the two data sets ($r = 0.95$, $P < 0.001$ and $r = 0.92$, $P = 0.011$, respectively; Supplementary Information 9.1., Table S9.1.6.). Kaiser's Measure of Sampling Adequacy (MSA) was 0.61 and, therefore, this analysis was considered statistically acceptable. For the chemical fertility-related parameters, the first canonical dimension was most strongly influenced by the reciprocal cation exchange capacity (1/CEC; $r = 0.81$) and the second canonical dimension by the available manganese ($r = -0.72$), followed by the reciprocal of the available iron (1/Fe; $r = 0.62$) and the exchangeable magnesium ($r = 0.61$). For the P fractions, the first dimension was mainly correlated to PAEM ($r = -0.63$) and P_oNaHCO_3 ($r = -0.59$), and for the second dimension, the primary forms of P ($PHCl_{1M}$) was the dominating variable ($r = 0.87$; Figure 21 and Table 11).

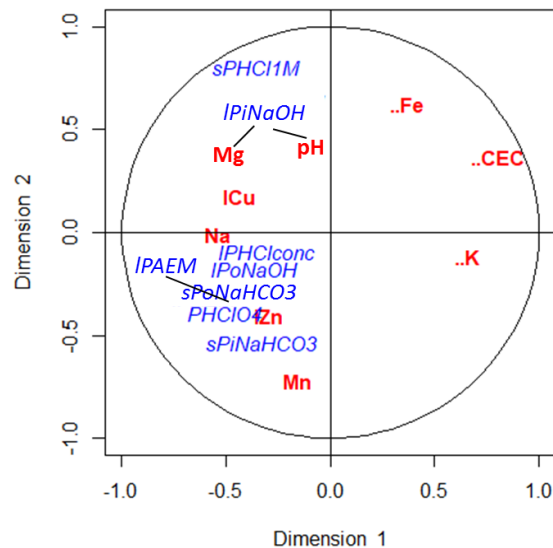


FIGURE 21 | Variables plot for the first and second canonical dimensions of the canonical correlation analysis performed to the P fractions and the chemical fertility-related parameters. Abbreviations for soil parameters as in Supplementary Information 9.1., Table S9.1.2.

TABLE 11 | Factor loadings (simple correlations between each canonical factor and the variables) of canonical correlation analysis performed on P fractions in relation to soil chemical fertility parameters.

Variable	Dimension 1	Dimension 2
pH	-0.20	0.61
1/CEC	0.81	0.37
1/Fe	0.37	0.62
log(Cu)	-0.43	0.18
log(Zn)	-0.29	-0.40
Mn	-0.15	-0.72
Mg	-0.27	0.61
1/K	0.66	-0.12
Na	-0.54	-0.008
log(PAEM)	-0.63	-0.34
$\sqrt{\text{PiNaHCO}_3}$	-0.35	-0.58
$\sqrt{\text{PoNaHCO}_3}$	-0.59	-0.30
log(PiNaOH)	-0.23	0.70
log(PoNaOH)	-0.38	-0.20
$\sqrt{\text{PHCl1M}}$	-0.37	0.87
log(PHClconc)	-0.32	-0.10
PHClO ₄	-0.54	-0.43

According to the canonical correlation analysis and the Spearman's correlations between P fractions and soil parameters related to chemical fertility (Table 12), the most labile P forms were positively correlated with the cation exchange capacity and with the amount of available manganese. Moreover, the readily exchangeable P extracted with membranes was also positively correlated with the exchangeable potassium. Organic highly labile P fraction was also positively correlated with the amount of available zinc and iron, as was the residual P. Organic moderately labile P was also positively related to the cation exchange capacity, available zinc and exchangeable potassium. Inorganic moderately labile P was negatively correlated with the available manganese and positively with the exchangeable magnesium, as the primary P fraction that was also positively related to pH and negatively to available iron.

TABLE 12 | Spearman's correlations between P fractions and soil chemical fertility-related parameters ("- " means not significant). Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	PAEM	P _i NaHCO ₃	P _o NaHCO ₃	P _i NaOH	P _o NaOH	PHCl _{1M}	PHCl _{conc}	PHClO ₄
pH	-	-	-	-	-	0.48**	-	-
CEC	0.55**	0.53**	0.36*	-	0.45*	-	-	-
Fe	-	-	0.40*	-	-	-0.40*	-	0.49**
Cu	-	-	-	-	-	-	-	-
Zn	-	-	0.52**	-	0.44*	-	-	-
Mn	0.43*	0.52**	-	-0.43*	-	-0.55**	-	-
Mg	-	-	-	0.48**	-	0.61***	-	-
K	0.54**	-	-	-	0.39*	-	-	-
Na	-	-	-	-	-	-	-	-

4.4.4. P fractions and organic matter parameters

A biplot of the first two dimensions of the CCA analysis based on P fractions and soil organic matter variables is shown in Figure 22. The explained correlations are 0.92 ($P < 0.001$) and 0.81 ($P = 0.041$) for the first and the second canonical correlation, respectively. Kaiser's Measure of Sampling Adequacy (MSA) was 0.78 and thus, this analysis was considered statistically acceptable (Supplementary information 9.1., Table S9.1.7.). The first canonical dimension (Figure 22 and Table 13) was negatively correlated with all the organic matter-related parameters considered, as well as with all P fractions except to the inorganic moderately labile P (P_iNaOH) and with the primary P (PHCl_{1M}), two fractions strongly related to each other. The second canonical dimension was mainly negatively related to the easily oxidizable C and the microbial biomass N, and positively to the most recalcitrant P forms (PHCl_{conc} and PHClO₄).

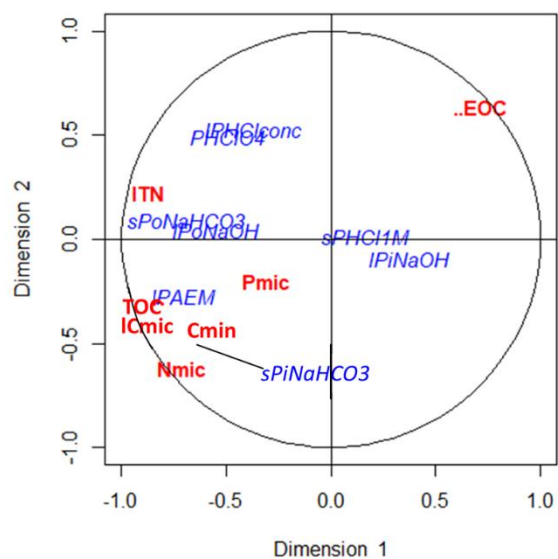


FIGURE 22 | Variables plot for the first and second canonical dimensions of the canonical correlation analysis performed to the P fractions and the soil parameters related to organic matter. Abbreviations for soil parameters as in Supplementary Information 9.1., Table S9.1.2.

TABLE 13 | Factor loadings (simple correlations between each canonical factor and the variables) of canonical correlation analysis performed on P fractions in relation to soil organic matter parameters.

Variables	Dimension 1	Dimension 2
TOC	0.89	-0.33
1/EOC	0.72	0.63
log(TN)	-0.87	0.22
log(Cmic)	-0.82	-0.35
Cmin	-0.55	-0.43
Nmic	-0.71	-0.62
Pmic	-0.31	-0.20
log(PAEM)	-0.77	-0.34
$\sqrt{P_iNaHCO_3}$	-0.70	-0.53
$\sqrt{P_oNaHCO_3}$	-0.75	0.11
log(P_iNaOH)	0.40	-0.12
log(P_oNaOH)	-0.59	0.05
$\sqrt{PHCl_{1M}}$	0.18	0.01
log($PHCl_{conc}$)	-0.40	0.65
$PHClO_4$	-0.54	0.61

We found strong correlations between the plant-available P forms and almost all organic matter-related parameters (Table 14). These correlations were all positive. The most recalcitrant P forms (PHCl_{conc} and PHClO₄) were just positively correlated to total nitrogen and the primary P form (PHCl_{1M}) did not show any significant correlation.

TABLE 14 | Spearman's correlations between P fractions and soil parameters related to organic matter ("-" means not significant). Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	PAEM	P _i NaHCO ₃	P _o NaHCO ₃	P _i NaOH	P _o NaOH	PHCl _{1M}	PHCl _{conc}	PHClO ₄
TOC	0.54**	0.64***	0.40*	-	0.45*	-	-	-
EOC	0.73***	0.70***	-	-	0.45*	-	-	-
TN	0.60***	-	0.49**	-	0.51**	-	0.36*	0.49**
Cmic	0.51**	0.62***	0.43*	-	0.50**	-	-	-
Cmin	0.46**	0.61***	-	-	-	-	-	-
Nmic	0.59***	0.74***	-	-	0.40*	-	-	-
Pmic	0.36*	-	-	-	-	-	-	-

4.4.5. P fractions and soil enzymatic activity

The first canonical dimension in the canonical correlation analysis based on P fractions and soil enzymatic activity-related variables was significant (Wilk's lambda test; $P < 0.001$) and its canonical correlation was 0.93 (Supplementary Information 9.1., Table S9.1.8.). Kaiser's Measure of Sampling Adequacy (MSA) was 0.65 and, therefore, this analysis was considered statistically acceptable. The canonical dimension was negatively correlated to all soil enzymatic activity parameters (Figure 23 and Table 15). Besides, most of the phosphorus fractions were negatively correlated with the first canonical dimension except the inorganic moderately labile P (P_iNaOH) and the primary P (PHCl_{1M}).

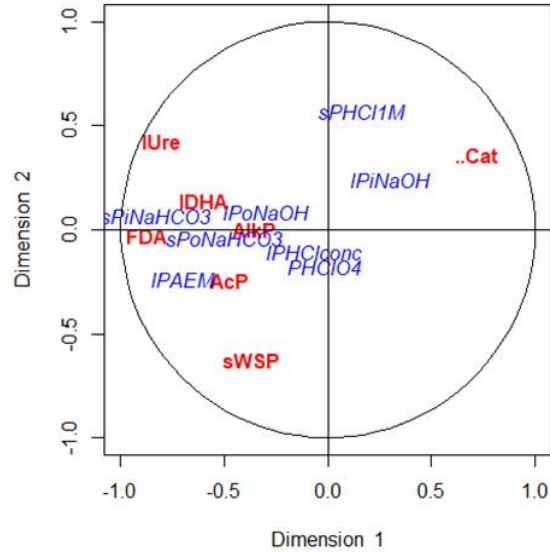


FIGURE 23 | Variables plot for the first canonical root of the canonical correlation analysis performed to the P fractions and the soil enzymatic activity parameters. Abbreviations of soil parameters as in Supplementary Information 9.1., Table S9.1.2.

TABLE 15 | Factor loadings (simple correlations between each canonical factor and the variables) of canonical correlation analysis performed on P fractions in relation to soil enzymatic activity parameters.

Variables	Dimension 1
FDA	-0.87
AlkP	-0.35
AcP	-0.47
log(DHA)	-0.60
log(Ure)	-0.80
1/Cat	0.72
\sqrt{WSP}	-0.37
log(PAEM)	-0.75
$\sqrt{P_iNaHCO_3}$	-0.89
$\sqrt{P_oNaHCO_3}$	-0.54
log(P_iNaOH)	0.33
log(P_oNaOH)	-0.32
$\sqrt{PHCl_{1M}}$	0.18
log($PHCl_{conc}$)	-0.07
$PHClO_4$	-0.02

As expected, fundamentally strong significant correlations were found between soil parameters related to enzyme activity and the most labile and plant-available phosphorus fractions (Table 16). The primary, stable and residual P fractions (PHCl_{1M}, PHCl_{conc} and PHClO₄, respectively) did not show significant correlations with these soil parameters, as well as the inorganic moderately labile P fraction (highly related to the primary P).

TABLE 16 | Spearman's correlations between P fractions and soil enzymatic activity parameters ("-" means not significant). Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	PAEM	P _i NaHCO ₃	P _o NaHCO ₃	P _i NaOH	P _o NaOH	PHCl _{1M}	PHCl _{conc}	PHClO ₄
FDA	0.43*	0.69***	0.41*	-	-	-	-	-
DHA	0.55**	0.51**	0.40*	-	0.44*	-	-	-
Urease	0.47**	0.76***	-	-	0.41**	-	-	-
Catalase	0.77***	0.54**	0.38*	-	0.36*	-	-	-
AlkP	-	-	-	-	0.36*	-	-	-
AcP	0.46**	-	-	-	-	-	-	-
WSP	0.39*	-	-	-	-	-	-	-

4.5. Relationship between forest productivity, soil P fractions and soil parameters

In the soils studied, the site index did not correlate with any soil parameter and only showed significant correlation with moderately labile organic phosphorus (P_oNaOH; rho = 0.38, $P = 0.034$). In order to study the importance of the different edaphic parameters considered in the productivity of the *P. halepensis* stands a PLS analysis was performed. The PLS analysis included the site index as the response variable and all the soil parameters and all the phosphorus fractions as predictive variables. The complete model with the maximum number of components was carried out, looking for the optimal number of components that allow us to explain the highest variance of the site index (Y) with the lowest number of predictor variables (X). The PLS resulted in the selection of two components that explain 48% of the variation of the site index using 26% of the information of the soil parameters and P fractions (Supplementary Information 9.1., Table S9.1.9.). Therefore, with about a quarter of the predictor variables almost half of the site index is explained. Taking the third component, the explained variance of the site index would only increase by 11%, but the number of necessary predictor variables would increase by more than 23%.

The results showed that the first component was mostly related to the composition of the soil since the soil parameters with the highest relevance were carbonates, active

carbonates, and soil texture (75% of the component). Soil pH and the permanent wilting point (PWP) were also important variables in this component as well as the primary P fraction (PHCl_{1M}). Component 1 only used 14.4% of information from predictors and was able to predict 21.2% of site index variability. The second component of the PLS was associated with variables related to the water in the soil (bulk density, field capacity, and PWP; 43% of the component) and those related to the soil chemical fertility (exchangeable potassium and magnesium, and cation exchange capacity; 41% of the component; Supplementary Information 9.1., Table S9.1.10.). In addition, this second component was also related to the organic moderately labile P fraction (P_oNaOH). In the second component, the gain of explanation of predictors variability was low (11.4%) when comparing to the large percentage of site index variability gained (26.5%). Therefore, these results indicated that the most important factors that influenced the growth and productivity of *P. halepensis* in our stands were soil texture, carbonates and active carbonates content, and primary P, as well as soil water retention-related parameters.

5. Discussion

5.1. Main characteristics of the studied soils

The studied calcareous soils, although rich in carbonates, showed low content of active carbonates meaning that their chlorinating power is low. Active carbonates are an indirect measurement of the particle size distribution or surface area of the CaCO_3 so they give an estimate of the CaCO_3 in the clay and fine silt fraction and it is indirectly responsible for plant growth reductions and physiological disorders such as chlorosis (Callot & Dupuis, 1980). Besides, these soils are moderate to strongly gypsic, fact that can also hinder plant growth. Gypsum mineral causes high concentrations of Ca and sulfate ions in soil solution, which may hamper the solubility of other nutrients in the soil, such as P, Fe, and Mn. Moreover, the high concentrations of Ca^{+2} and SO_4^{-2} , as well as the associated salinity, may have a physiological impact on root absorption sites, reducing nutrients uptake and plant growth. Soil texture at the studied plots can be considered as fine or medium (Table 3 and Supplementary Information 9.1., Table S9.1.1.). The base saturation percentage in these soils is 100% (data not shown) and is governed mainly by the Ca^{+2} cation. This could be explained because the studied soils show a composition with high concentrations of carbonate minerals as well as gypsum. The latter exerts a dominating influence because of its relatively high solubility, and the carbonates because of their alkalinity and pH buffering properties (Loeppert & Suarez, 1996). Interestingly, the cation exchange capacity is mainly due to the organic matter content in the soils and it is not related to the clay content. This is because in soils with high carbonates content and with active carbonates, such as the studied soils, part of the granulometric clays correspond to materials that do not show the behaviour of mineralogical clays in terms of their ion exchange properties.

To our knowledge, little information exists about the concentration of assimilable micronutrients (Zn, Mn, Fe and Cu) in forest soils. Concentrations of assimilable Zn extracted with DTPA in the soils studied in this work were low (mean value of 0.6 mg/kg) and approximately 50% of the forest plots studied showed values of this assimilable Zn under 0.5 mg/kg, concentration considered limiting for the development of vegetation in agricultural soils (Lanyon et al., 2004; Roca et al., 2007). For assimilable Mn in agricultural soils, concentrations lower than 5 mg/kg are usually considered very low (sensu Mitsios et al., 2003) and deficiencies can be expected when extractable concentrations with DTPA are less than 1 mg/kg (Lindsay & Norvell, 1978; Roca et al., 2007). The values obtained for this

form of Mn in the studied soils were higher than 7 mg/kg, so deficiencies would not be expected for this element. For assimilable Fe, deficiencies can be expected for extractable values with DTPA lower than 4.5 mg/kg in agricultural soils (Lindsay & Norvell, 1978). Other authors as Mitsios et al. (2003), considered very low concentrations of this element when they are below 3 mg/kg. Mean value of assimilable Fe in the studied soils was 7.8 mg/kg and none of them presented a concentration in this element extracted with DTPA lower than 3 mg/kg. Concentrations lower than 0.4 mg/kg for extractable Cu with DTPA are considered as limiting for agricultural use (Kruger et al., 1985). However, the classification of Mitsios et al. (2003) establishes a value of 0.3 mg/kg. The mean values of Cu in the studied soils were 0.5 mg/kg, with 10% of the soils showing Cu concentrations that can be classified as very low. Thus, the studied soils could present deficiencies in this element. Pines are very frugal species and their nutritional requirements cannot be compared with those of any agricultural crop. Therefore, although the studied soils may present deficiencies in some nutrients, this will not highly affect the development and growth of the *P. halepensis* trees in the studied plots.

The organic matter content determines the presence and distribution of micronutrients in available forms in soils, as well as clay and microbial activity (Roca et al., 2007). However, in the studied soils none of the assimilable metals extracted with DTPA showed significant correlations with clay content and only the assimilable forms of Mn and Zn displayed significant and positive correlations with the parameters related to the content of soil organic matter (TOC, EOC, TN). The assimilable Mn was also correlated with the microbial activity and the assimilable Zn also presented a highly significant correlation with soil gypsum content. Soil Zn is usually more available in soils with greater organic matter content (Rengel, 2015) and its extractability from the soil is negatively related to gypsum content (Iratkar et al., 2014). Therefore, low plant availability of Zn can be expected in gypsiric soils. The available forms of these micronutrients to vegetation imply their participation in the formation of chelates with organic compounds produced by the soil microbiota (Rengel, 2015). Assimilable Fe did not show any significant correlation with the parameters related to organic matter or microbial activity and as expected, it exhibited a highly significant and negative correlation with soil carbonates content. Assimilable Cu was only significantly correlated with the assimilable Zn and with the gypsum content of the soil.

Mineralizable C contents in the studied calcareous soils ranged between 17.3 and 62.4 mg C/kg soil week, which can be considered low compared to non-calcareous forest soils

(Bueis et al., 2019b). This could be due to the fact that carbonates stabilize soil organic matter by enhancing soil aggregation and binding Ca, affecting soil organic matter mineralization (Martí-Roura et al., 2019).

Soil enzymes play a key role in nutrient turnover in forest ecosystems, as they are responsible for the transformation of organic matter into available nutrients for plants. Enzyme activities found are in the range of usual values in Mediterranean ecosystems soils under dry conditions such as those of the calcareous studied soils (Caravaca et al., 2002; Uzun & Uyanoz, 2011). All the enzyme activities studied except acid phosphatase were significantly correlated to microbial biomass C and N, mineralizable C, total organic C, and total N. According to these results, several authors found also significant correlations between enzyme activities and microbial biomass C and N, and mineralizable C (A. Muscolo et al., 2015).

5.2. Soil phosphorus status and availability

The fractionation method used in this work, developed by Hedley et al. (1982) and modified by Tiessen & Moir (1993), allowed us to characterize the organic and inorganic forms of P that differ in their availability for plants and microorganisms. This sequential fractionation procedure enables analyzing the P fractions that participate in the short- and long-term transformations of the soil and helps to evaluate the availability of organic P to plants. Fractionation also allows to differentiate between organic P and inorganic P, thus distinguishing between soil P_o associated with humic and organic compounds that are more or less easily mineralized by microbes and soil P_i retained by chemisorption (Cross & Schlesinger, 2001; Schoenau et al., 1989; Tiessen et al., 1984; Turrión et al., 2000b).

Total P concentration in the studied soils was low but in the range found in previous studies on forest soils (Alt et al., 2011; Niederberger et al., 2019; Velásquez Camacho, 2019) and was also similar to that found in limestone soils of northwestern Spain (Trasar-Cepeda et al., 1991) and in inceptisol-type soils (Cross & Schlesinger, 1995).

The first step in the Tiessen & Moir procedure permits the labile P to be extracted through the use of ion exchange membranes that are based on the movement of phosphate ions from the soil particles to the soil solution (Schoenau & Huang, 1991), thus acting as an anion exchange surface to which the phosphate ions in the soil adhere. This membrane method

is independent of the type of soil and simulates the action of plant roots (Schoenau & Huang, 1991; Zamuner, 1999), so it is a good approach to study the fraction of available P to plants (PAEM). Following results from previous works, the readily exchangeable P (PAEM) was the lowest fraction in the studied soils. With this fractionation method, the highly labile P (PNaHCO₃) is also extracted, which correlates with the absorption of P by plants, can be easily transformed into available forms, and simulates the action of plant roots in dissolving P minerals (Olsen et al., 1954; Schoenau et al., 1989; Schoenau & Huang, 1991; Turrion et al., 2000a).

Moderately labile P strongly retained by chemisorption (PNaOH) is considered associated with amorphous or crystalline Al and Fe minerals and is probably available in the medium term (Schoenau et al., 1989; Turrion et al., 2000a). The inorganic form of this fraction represented in the studied calcareous soils less than 4% of the total extracted P and was the studied P fraction with the highest variation coefficient.

The primary (PHCl_{1M}) and the stable (PHCl_{conc}) fractions were the predominant forms of P in the studied soils as observed in other calcareous soils (Alt et al., 2011; Carreira et al., 2006; Cross & Schlesinger, 1995; Harrell & Wang, 2006; Lopez-Pineiro & Garcia-Navarro, 2001; Sharpley et al., 1985; Trasar-Cepeda et al., 1991; Yu et al., 2006). The primary P (PHCl_{1M}) corresponds to the inorganic P included in the primary minerals of calcium carbonate of low solubility (Schoenau et al., 1989; Shen et al., 2011; Tiessen & Moir, 1993; Turrión et al., 2000a, 2000b), stable P (PHCl_{conc}) corresponds to P_i and P_o bound inside minerals of Fe and Al and apatite (Tiessen & Moir, 1993). The predominance of PHCl_{1M} may be due to the presence of free calcium carbonate where Ca is the dominant cation under alkaline conditions (Lopez-Pineiro & Garcia-Navarro, 2001) or might be explained also by high carbonates and active carbonates content in the soil. This high primary P fraction reflects the geochemical influence on P cycling in calcareous soils which is dominated by high concentrations of calcium carbonate minerals (Cross & Schlesinger, 2001; Lajtha & Bloomer, 1988; Lindsay, 1979).

In the studied soils, the concentration of organic P calculated as the sum of P_oNaHCO₃ and P_oNaOH, represented around 12% of the total P, following the results found in other studies in inceptisols or in Mediterranean forest soils (Bueis et al., 2019a; Cross & Schlesinger, 1995). Soil organic P mainly exists in stabilized forms as phosphates and phosphonates, and active forms as orthophosphate diesters, labile orthophosphate monoesters, and organic polyphosphates (Condrón et al., 2005; Turner et al., 2002).

Residual P (PHClO_4) which is composed of inorganic P bound to amorphous Fe oxides and Al and a large proportion of resistant organic P associated with clay and Fe and Al oxides (Cross & Schlesinger, 1995; Schoenau et al., 1989; Zamuner et al., 2008) represented less than 6% of the total extracted P and this fraction had the lowest variation coefficient of all the studied P fractions.

As expected, the correlation among soil P fractions showed that the organic forms of P (P_0NaHCO_3 and P_0NaOH) were significantly correlated. Besides, the available P (PAEM) was significantly correlated with the extractable fraction of inorganic bicarbonate (P_iNaHCO_3) since this fraction includes easily interchangeable P compounds and is considered a very active fraction of organic P from the soil (Zamuner et al., 2008) so could act as source of available P in the short term. Surprisingly, primary P (PHCl_{1M}), which is associated with Ca, was strongly correlated with moderately labile inorganic P (P_iNaOH). The relationship between these two fractions deserves an in-depth study to understand their significance in the P dynamics in limestone soils with high carbonates content. Furthermore, the most refractory forms of P ($\text{PHCl}_{\text{conc}}$ and PHClO_4) were strongly correlated with each other and also positively with the organic P fraction extracted with bicarbonate (P_0NaHCO_3), clearing up the role of these P fractions with slower cycles as a source of labile and available P in the long term (Richter et al., 2006). The fact that the two most recalcitrant phosphorus fractions were highly correlated following results from other studies (see, for example, Bueis et al., 2019a) would allow to simplify the fractionation procedure, avoiding extracting the stable and residual P fractions, and directly extracting the recalcitrant P. Total P, calculated as the sum of all the P fractions extracted, was just correlated with the primary P, the stable P and the inorganic moderately labile P. Consequently, in these calcareous soils total P seems to be mostly formed by the P included in Ca minerals.

5.3. Phosphorus fractions in relation to soil parameters

Specific characteristics of each soil determine the solubility and nutrient absorption capacity of the soils (Stewart & Tiessen, 1987). The oxides of Al and Fe play an important role in the control of the dynamics of P in acid soils, while in limestone soils the cation Ca^{+2} does it, in addition, in both types of soil organic matter determines to a great extent the availability of phosphorus (Achat et al., 2016). In addition, soil properties do not affect all forms of soil P equally. Therefore, the soil processes involved in the availability of P will differ greatly between different ecosystems. Our results showed that the available and organic

fractions of P were fundamentally affected by organic matter and enzymatic activities, while the P fractions linked to Ca were mainly influenced by the sand and carbonates content.

The highly significant correlations ($P > 0.001$) of the parameters related to the composition of the soil were established with the most stable fractions of P, both the most recalcitrant fractions (PHClO_4) and those linked to calcium (PHCl_{1M}), and the organic fractions (P_0NaOH). Although important, the direct effect of texture on the distribution of P pools in forest soils has been little studied (Niederberger et al., 2019; Zederer & Talkner, 2018). Strong negative correlations were found between sand content and primary P, a fraction that represents the total P content in the studied soils. The negative influence of sand content on the concentration of primary P or total P has been previously demonstrated in soils from very different climatic zones around the world (Augusto et al., 2017; Buckingham et al., 2010; Cross & Schlesinger, 2001; McCullum, 1996; Niederberger et al., 2019; Shaheen et al., 2007; Zederer & Talkner, 2018). This negative influence of the sand on the P content could be interpreted as the decreasing amount of surfaces to which the P could be absorbed or fixed. That is, the percentage of sand is the same as '(% silt +% clay)', therefore the previously mentioned negative correlation implies a correlation of the same weight and opposite sign with the sum of silt and clay. This sum of silt and clay corresponds to the particle sizes in which the carbonates with the highest activity in the soil are found. And the most important fraction of P in these soils (the fraction linked to Ca: PHCl_{1M}) would be associated with those highly active carbonates. According to the results of previous studies, carbonates content had significant and positive influence on P bound to Ca as well as on the smaller carbonates (active carbonates; Lopez-Pineiro & Garcia-Navarro, 2001; Niederberger et al., 2019; Shaheen et al., 2007). However, the correlation between carbonates content and recalcitrant P (PHClO_4) was negative. The forms of P linked to this recalcitrant fraction are mainly formed by those related to the oxides of Fe and Al existing in the soils and those represent a small proportion in limestone soils. This would explain the low concentration of P found in this very stable and recalcitrant form. In addition, the inverse relationship between the Ca and Fe and Al content in soils is well known, which would help to explain the previously indicated negative correlation between the calcium carbonate content and the most recalcitrant forms of P in the soils studied.

Soil aeration and water retention capacity are important factors affecting P dynamics because they play important roles in mineralization and immobilization of this element (Arenberg & Arai, 2019). However, the significance of these factors in the P cycle remains

uncertain. We studied the effect of soil aeration in P fractions through bulk density and porosity. Our results indicated that the P labile fractions were reduced when increased soil bulk density, i.e. with reduced soil aeration. Lower soil aeration might imply lower soil microbial activities and subsequently reduced mineralization processes. However, contradictory results have been reported in this matter, with higher mineralization rates seen both in good aeration conditions and in bad conditions (Brannon & Sommers, 1985; Bridgham et al., 1998; Dick & Tabatabai, 1978; Suzumura & Kamatani, 1995). The reason why bulk density greatly affects the soil P available forms may be, on the one hand, to the content of organic matter, since the higher the organic matter, the lower the bulk density. And on the other hand, it may be due to aspects related to the textural class. Textural classes of the studied soils were fine and medium (several soils presented silt and silty-clay textures, and others loam, silty-loam, and clay-loam textures). Soils with silty textures showed higher values of bulk density and lower porosity, displaying worse aeration and therefore, lower concentrations of the P available fractions. As expected, parameters related to soil water retention were positively correlated with the most labile P (PAEM), since soil moisture and so water retention capacity, are essential for soil microorganisms survival and for phosphatases production (Arenberg & Arai, 2019; Bitton, 2005; Criquet et al., 2002; Dalai, 1977), increasing also the labile P fractions. However, whether the soil has more or less water depends not only on its retention capacity but also, and more importantly, on the amount of water that reaches the soil, that is, on precipitation. In this study we did not consider climatic or physiographic variables, so we did not take precipitation into account. Therefore, with the soil parameters related to water retention, we cannot determine the availability of water on the studied soils or their actual aeration level, since this will depend on precipitation. That is, the fact that the studied soils show high field capacity does not imply that they have sufficient water for the microorganisms to carry out their activities and consequently, increase the amount of labile P in the soil. In fact, the studied plots are under the Mediterranean climate where annual precipitation is low (less than 500 mm) and the summer drought is intense and long-lasting. Therefore, in the studied plots as in other forest systems, the scarcity of precipitation and low soil moisture, together with a low water retention capacity, can greatly reduce the enzymatic activities in the soil, thus reducing the mineralization of P (Bueis et al., 2018; Criquet et al., 2004; Dalai, 1977; Devi & Yadava, 2006). Interestingly, the soil water retention parameters were also positively correlated with primary P (PHCl_{1M}) and moderately labile P (PNaOH). This does not imply that there is a direct cause-effect relationship between these soil parameters and those fractions of P, but

rather that we can have a significant relationship because some other factor is affecting both parameters at the same time (this is one of the drawbacks involving the interpretation of correlations). This high correlation could be explained by the textural class of the soils studied. Soils with fine textures showed high values of field capacity and permanent wilting point and, in turn, in those soils, the granulometric fractions were governed by carbonates that largely control the primary P fraction.

Phosphorus mineralization rates as well as its solubility and fixation, are influenced by soil pH (Dalai, 1977; Haynes, 1982; Hinsinger, 2001; Shang et al., 1992; Stevenson & Cole, 1999; Trasar-Cepeda et al., 1991). Calcareous soils have higher mineralization rates because calcium improves soil structure and microbial activity (Harrison, 1982). Furthermore, pH modifies the concentrations of the different P pools in the soil (Turner & Blackwell, 2013), affecting them in contrasting ways (see for example, De Schrijver et al., 2012; Hinsinger, 2001; Niederberger et al., 2019). The positive correlation found between pH and primary P (PHCl_{1M}) indicates that this P pool in soils could be explained by the increase in the fixation of P in clay minerals and in Ca primary minerals (Hinsinger, 2001; Schoenau et al., 1989; Shen et al., 2011; Turrion et al., 2000a). Unlike previous studies, our results did not show a strong effect of pH on P pools, which is probably due to the low pH variation among the studied soils (range 8.0 - 8.9).

The results of the correlations and the CCA for the group of parameters related to the chemical fertility of the soil indicated that the different fractions of phosphorus, mainly labile P, could increase with the increase of the cation exchange capacity and the amount of exchangeable potassium. This could be explained by the strong relationship of these fertility parameters with the organic matter of the soil. In addition, primary P was negatively related to assimilable iron and manganese since when carbonates or active limestone content in the soil increases, the amount of assimilable Fe and Mn decreases, while primary P bounded in Ca-minerals increases.

As expected, the most labile fractions of P both inorganic (PAEM and P_iNaHCO_3) and organic (P_oNaHCO_3) were highly correlated with soil parameters related to organic matter and enzymatic activity. This indicates that in the soils studied the biological cycle of P has great importance in the availability of this element. The availability of P from organic forms may be due to two possible causes: (1) The first is the general mineralization of soil organic matter (Bhattacharyya & Jha, 2012). As P is part of this organic matter, it is released in the general mineralization process and becomes inorganic forms available to vegetation. This

would be the biological mineralization of P from organic compounds during the oxidation of soil organic matter by organisms and it is regulated by the energy demand of edaphic microorganisms. Results from CCA and Spearman's correlations done with the group of organic matter-related parameters showed the relevance of this biological P mineralization in the studied soils, as found in previous studies (Bueis et al., 2018). (2) The second possible cause of the release of P from organic forms could be the specific release of P due to the action of particular enzymes involved (acid phosphatase and, especially in the studied soils, alkaline phosphatase; Sharma et al., 2013). This is the biochemical mineralization of P where this element is released from organic compounds and is governed by the specific need for available P. In general, it is considered that the lower the availability of P in the soil, the higher the activity of phosphatase (either acid or alkaline, depending on the soil pH), since this enzyme is released when necessary, that is, when available P levels are limiting. Unlike in acid soils (Bueis et al., 2018), in the calcareous soils studied, alkaline phosphatase had a slightly higher concentration than acid phosphatase and did not show any correlation with assimilable forms of phosphorus. In addition, alkaline phosphatase (AlkP) did not show high relevance in the first canonical axis of the CCA performed with the group of enzyme activity-related parameters. The absence of importance in the CCA and of correlation between the available P forms and alkaline phosphatase could explain that in these calcareous soils the concentration of assimilable P, although low, may be sufficient to satisfy the demand of the vegetation and soil microorganisms, unlike what was found in acid soils in northern Spain (Bueis et al., 2018). The rest of the enzymes considered in this study (without phosphatase activity) showed highly significant correlations with the labile forms of P, both in the Spearman correlations and in the CCA. The fluorescein diacetate hydrolysis reaction (FDA), that showed a higher weight in the corresponding canonical dimension, reflects all the hydrolytic activity in the soil (Alef & Nannipieri, 1995) and is commonly used as an indicator of the general microbial activity in the soil (Bandick & Dick, 1999). Furthermore, all the enzymes were related to each other and to the microbiological parameters Cmic, Cmin, Nmic. Microbial populations, besides participating in the mineralization and solubilization of soil P, are also a reserve of organic P that is released when microorganisms die (Achat et al., 2010; Nannipieri et al., 2002). Our results showed that the microbial biomass of C, N, and P were significantly correlated with the most labile phosphorus and the microbial biomass C also correlated with organic P. The microbial biomass P in our study represented 2.7% of the total P, similar to previous results in coniferous forests (Xu et al., 2013). The positive correlation found between microbial

biomass P and PAEM is consonant with previous studies (Bueis, Bravo, et al., 2019; Zamuner et al., 2008) and with the fact that microbial biomass regulates soil P availability through the release of its P content. Therefore, all these results might indicate that the biological mineralization of P in these soils is more important than the biochemical mineralization of P due to the action of alkaline phosphatase.

Although in the studied soils, organic forms of P represented a fairly low percentage of total P compared to other studies (Fox et al., 2011; A. Harrison, 1987; Niederberger et al., 2019; Stevenson & Cole, 1999; Turner, 2008; Yang & Post, 2011), a strong influence of total soil carbon (TOC) and easily oxidizable carbon (EOC) on the distribution of P could be expected. Following previous studies, we found a positive correlation between organic P, available P and highly labile inorganic P with soil organic C (Bueis et al., 2019a; Cleveland & Liptzin, 2007; Johnson et al., 2003; Perakis et al., 2017; Zederer & Talkner, 2018), among others), since these soluble and labile forms of P come from the mineralization of organic matter (Hou et al., 2014). The positive relationship between inorganic P and easily oxidizable carbon may be due to the fact that organic matter could act as a source of labile inorganic P in the studied soils (Leo M. Condon et al., 2005; Johnson et al., 2003). In addition, CCA results performed with the organic matter-related parameters showing that the first canonical factor was highly correlated with TOC and the second canonical factor with EOC, indicating that these two parameters might be good positive predictors of labile and moderately labile P. Finally and as expected, the most recalcitrant forms of P did not correlate either with the soil parameters related to organic matter or those related to enzymatic activities.

5.4. Relationship between forest productivity, soil P fractions and soil parameters

The site index of the studied plots calculated by Bueis et al. (2017a) were within the range proposed for several authors for *P. halepensis* plantations and natural stands in Spain (del Río et al., 2014; Montero et al., 2001; Rojo Alboreca et al., 2017). Bueis et al. (2017a) developed a discriminant model to predict the forest site index for *P. halepensis*, using the plots studied in the present work. This model included climatic and physiographic parameters, in addition to some of the soil parameters used in this work, but they did not include P fractions. The selected model by Bueis et al. (2017) had as predictors three parameters related to water availability (annual water index, porosity, and slope) and a parameter related to soil organic matter (microbial biomass N).

In the soils studied in this work, the site index was not correlated with any soil parameter and within the P fractions, it was only correlated with moderately labile organic phosphorus (P_oNaOH). This may be due to the fact that the growth of *P. halepensis* (which is associated with site index and productivity) is driven mainly by soil water availability considering both its supply and its retention in the soil (Condés & García-Robredo, 2013; del Castillo et al., 2015; del Río et al., 2014; Gandullo et al., 1972; Manrique-Alba et al., 2017). Therefore, the water availability seems to be the most limiting factor for the productivity of *P. halepensis* throughout its circum-Mediterranean distribution. This higher importance of water availability than soil factors in growth and productivity has been also found for other pine species as *P. sylvestris* or *P. contorta* (Björn Berg et al., 1995; Bravo & Montero, 2001; Manimel Wadu et al., 2017). However, although we did not consider climatic variables, the objective of this work was to verify the importance of soil parameters in the productivity of the studied plots of *P. halepensis* with which we explained around 48% of the variation of the site index. Our results indicated that the site index in the studied plots was conditioned by parameters related to soil texture and carbonates content as well as to the primary P, which is the P pool that mostly represents the total P in these plots. In addition, soil chemical fertility related to the cation exchange capacity and the supply of macronutrients (K and Mg fundamentally) and the parameters related to soil water retention also explained much of the site index variance in the studied plots. These results are in line with those obtained by other authors for *P. halepensis* (Bravo & Montero, 2001; del Río et al., 2014; Fernández et al., 2016). The availability of micronutrients did not affect the forest productivity of these plots while in agricultural soils this factor is one of the most important for crops' growth (see, for example, Lindsay & Norvell, 1978; Mitsios et al., 2003; Roca et al., 2007). Generally, decomposition of organic matter affects the productivity of ecosystems, particularly in nutrient-poor forests such as Mediterranean forests (Muscolo et al., 2007). However, the parameters related to organic matter did not seem to influence the site index of the studied plots. This may be due to the fact that the release of nutrients from plant litter depends not only on the activity of the microorganisms but also and first of all, on the quality of the litter and on environmental factors. In our case, the quality of the litter is the same in all the plots: same species of *Pinus* with the same chemical composition in needles, same strategies for the conservation of nutrients in their tissues, and same decomposition rates (B. Berg, 2014; Bueis et al., 2017a; Carrasco et al., 2017; Cobertera, 1993). Furthermore, the plots are located in areas with very similar environmental conditions so environmental factors would not make great differences in the nutrient release process between plots either.

6. Conclusions

1. In the basic soils studied, the availability of P, although low, may be sufficient to satisfy the demand of the vegetation and soil microorganisms.
2. As hypothesized, highly labile inorganic P and moderately labile organic P could act as a source of available P in the short or medium term in the studied soils.
3. In these calcareous soils, the total P seems to be formed mainly by the primary P bound into Ca minerals.
4. The availability of P is mainly due to parameters related to organic matter and enzymatic activities, but not to carbonates content in the soil. However, carbonates content is linked to the main fraction in these soils, which can act as a source of available P in the short term.
5. In these soils, biological mineralization processes play a more important role than biochemical processes in the P cycle.
6. With increasingly finer soil texture, higher total P contents were observed, attributable to increases in the primary fraction.
7. In these calcareous soils with a high content of carbonates and gypsum, it must be considered that when measuring clay content, we determine soil components of less than 2 micrometers size in which not only mineralogical clay is included but also other particles that lack ion exchange capacity. This fact is important to understand the results obtained on the relationship between the different forms of P and the soil parameters.
8. The site index of *Pinus halepensis* in the studied plots is not affected by the availability of P in the soil, but it is affected by the primary P (representative of total P). Furthermore, the parameters related to organic matter are not the ones that most influence the site index which is mainly affected by carbonates content and soil

texture, and by parameters related to the chemical fertility of the soil and its water retention capacity.

9. The results indicate that the soil parameters by themselves are not the factors that most influence the productivity of the *Pinus halepensis* stands studied. As found by other authors, including climatic variables mainly related to water supply would have allowed us to further explain the variation in the site index of the studied plots.
10. The fact that the two most recalcitrant phosphorus fractions were highly correlated would make it possible to simplify the fractionation procedure, avoiding extracting both the stable and residual P fractions, and thus reducing the time and difficulty of the process.
11. It is essential to continue expanding the limited existing knowledge on forest soils, their internal dynamics, and their possible relationship with the productivity of the stands. This knowledge will allow us to contribute very valuable information to sustainable forest management, in order to increase forest productivity, avoid the degradation of ecosystems or improve the role of soils and forests in mitigating climate change.

7. Conclusiones

1. En los suelos básicos estudiados la disponibilidad de P, aunque escasa, puede ser suficiente para satisfacer la demanda de la vegetación y los microorganismos del suelo.
2. El P inorgánico altamente lábil y el P orgánico moderadamente lábil pueden actuar como fuentes de P disponible a corto o medio plazo en los suelos estudiados.
3. En estos suelos calcáreos, el P total parece estar formado principalmente por el P primario que está unido a los minerales de Ca.
4. La disponibilidad de P se debe principalmente a parámetros relacionados con la materia orgánica y las actividades enzimáticas, pero no al contenido de carbonatos en el suelo. Sin embargo, el contenido de carbonatos está ligado a la fracción principal en estos suelos, que puede actuar como fuente de P disponible a corto plazo.
5. En estos suelos, los procesos de mineralización biológica juegan un papel más importante que los procesos bioquímicos en el ciclo del P.
6. Con una textura del suelo más fina se observaron mayores contenidos de P total, lo que se podría atribuir a aumentos en la fracción primaria.
7. En estos suelos calcáreos con alto contenido de carbonatos y yeso, se debe tener en cuenta se debe tener en cuenta que al medir el contenido de arcilla estamos determinando componentes del suelo de tamaño inferior a 2 micrómetros en el que se incluye no solo arcilla mineralógica sino también partículas que carecen de capacidad de intercambio iónico. Este hecho es importante para comprender los resultados obtenidos sobre la relación entre las diferentes formas de P y los parámetros del suelo.
8. El índice de sitio de *Pinus halepensis* en las parcelas estudiadas no se ve afectado por la disponibilidad de P del suelo, pero sí por el P primario (representativo del P

total). Además, los parámetros relacionados con la materia orgánica no son los que más influyen en el índice de sitio que se ve afectado principalmente por el contenido de carbonatos y la textura del suelo, y por los parámetros relacionados con la fertilidad química del suelo y su capacidad de retención de agua.

9. Los resultados indican que los parámetros del suelo por sí mismos no son los factores que más influyen en la productividad de las parcelas de *Pinus halepensis* estudiadas. Como han encontrado otros autores, la inclusión de variables climáticas relacionadas principalmente con el suministro de agua nos habría permitido explicar más la variación en el índice de sitio de esta especie en las parcelas estudiadas.
10. El hecho de que las dos fracciones de fósforo más recalcitrantes estuvieran altamente correlacionadas permitiría simplificar el procedimiento del fraccionamiento, evitando extraer tanto la fracción de P estable como la residual, reduciendo así el tiempo y la dificultad del proceso.
11. Es fundamental seguir ampliando el limitado conocimiento existente sobre suelos forestales, sus dinámicas internas y su posible relación con la productividad de las masas. Este conocimiento nos permitirá aportar información muy valiosa a la gestión forestal sostenible, con el fin de aumentar la productividad de las masas, evitar la degradación de ecosistemas o mejorar el papel de los suelos y los bosques en la mitigación del cambio climático.

8. References

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9. Supplementary Information

9.1. Supplementary tables

TABLE S9.1.1. | Location of the 32 *Pinus halepensis* plots studied (UTM Projection in meters; Datum ED50) and soil textural class at each plot (modified from Bueis et al. 2019b). *ID plot number: National Forest Inventory plot identification number.

ID plot number*	UTM_X	UTM_Y	Textural class
6	333000	4640000	Silt
7	333000	4639000	Silty clay loam
8	337000	4637000	Loam
9	333000	4635000	Clay loam
25	332000	4633000	Clay loam
43	321000	4627000	Loam
107	322000	4618000	Clay loam
144	368000	4623000	Silt loam
156	367000	4617000	Silt loam
202	330000	4605000	Silt loam
223	349000	4613000	Silty clay loam
228	347000	4610000	Silt
233	370000	4608000	Silt loam
375	377000	4613000	Silt loam
376	394000	4612000	Silt loam
496	412000	4604000	Loam
662	373000	4656000	Silt loam
664	388000	4668000	Clay loam
712	367000	4585000	Silty clay loam
717	356000	4639000	Sandy clay loam
718	357000	4639000	Sandy clay loam
723	353000	4638000	Sandy clay loam
771	386000	4632000	Silt loam
786	404000	4631000	Silt loam
864	360000	4575000	Silty clay
1237	390000	4639000	Silt loam

TABLE S9.1.1. (Cont.) | Location of the 32 *Pinus halepensis* plots studied (UTM Projection in meters; Datum ED50) and soil textural class at each plot (modified from Bueis et al. 2019b). *ID plot number: National Forest Inventory plot identification number.

ID plot number*	UTM_X	UTM_Y	Textural class
1245	382000	4627000	Silt loam
2057	357000	4629000	Silt loam
2063	356000	4625000	Silty clay
2070	371000	4622000	Loam
2108	378000	4617000	Silt loam
2136	403000	4621000	Loam

TABLE S9.1.2. | Variable transformations required to achieve normality and their abbreviated name used in the analyses. Dashed-line separates P fractions from soil parameters.

Variable	Transformation	Abbreviated name
PAEM	logarithm (log10)	IPAEM
P _i NaHCO ₃	square root ($\sqrt{}$)	sPiNaHCO3
P _o NaHCO ₃	square root ($\sqrt{}$)	sPoNaHCO3
P _i NaOH	logarithm (log10)	IPiNaOH
P _o NaOH	logarithm (log10)	IPoNaOH
PHCl _{1M}	square root ($\sqrt{}$)	sPHCl1M
P _{Total}	logarithm (log10)	IPTotal
CEC	reciprocal (1/x)	..CEC
Fe	reciprocal (1/x)	..Fe
Cu	logarithm (log10)	ICu
Zn	logarithm (log10)	IZn
K	reciprocal (1/x)	..K
SandUS	square root ($\sqrt{}$)	sSandUS
Carbonates	square (x^2)	Carb2
EOC	reciprocal (1/x)	..EOC
TN	logarithm (log10)	ITN
Cmic	logarithm (log10)	ICmic
DHA	logarithm (log10)	IDHA
Urease	logarithm (log10)	IUre
Catalase	reciprocal (1/x)	..Cat
WSP	square root ($\sqrt{}$)	sWSP

TABLE S9.1.3. | Spearman's correlations among all the soil parameters considered in this study ("- " means no correlation).
Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	BD	Porosity	FC	PWP	pH	CEC	Fe	Cu	Mn	Zn	K	Mg	Na
Porosity	-0.84***												
FC	-0.72***	0.46**											
PWP	-0.50**	-	0.78***										
pH	-	-	-	-									
CEC	-0.67***	0.40*	0.59***	0.57**	-								
Fe	-	-	-	-	-0.54**	-							
Cu	-	-	-	-	-	-	-						
Mn	-	-	-	-	-0.57**	0.54**	0.50**	-					
Zn	-	-	-	-	-	-	0.49**	0.52**	0.39*				
K	-	-	0.47**	0.78***	-	0.50**	-	-	-	-			
Mg	-0.47**	-	0.57**	0.51**	-	-	-0.51**	-	-	-0.39*	-		
Na	-0.40*	-	0.50**	0.54**	0.49**	-	-0.39*	-	-	-	0.36*	0.48**	
EOC	-0.61***	-	0.50**	0.48**	-	0.80***	-	-	0.58**	-	0.39*	-	-
TN	-	-	0.35*	0.37*	-	0.67***	-	-	0.45*	0.37*	0.40*	-	-
TOC	-0.36*	-	-	-	-	0.62***	-	-	0.62***	0.45*	-	-	-
Cmic	-0.61***	-	0.39*	-	-	0.62***	-	-	0.37*	-	-	-	-
Cmin	-0.36*	-	0.37*	-	-	0.56**	-	-	0.65***	-	-	-	-
Nmic	-0.63***	0.36*	0.44*	-	-	0.63***	-	-	0.43*	-	-	-	-
Pmic	-	-	-	-	-	-	-	-	-	-	-	-	-
Clay	-	-	-	-	-	-	-	-	-	-	-0.43*	-	-
SiltUS	-	-	0.56**	0.70***	0.35*	-	-0.45*	-	-	-	0.52**	-	0.45*
SandUS	0.36*	-	-0.56**	-0.62***	-0.61***	-	0.42*	-	0.44*	-	-0.38*	-0.42*	-0.41*
Carbonates	-	-	0.44*	-	-	-	-0.49**	-	-	-	-	-	-
ActiveC	-	-	-	-	-	-	-	-	-	-	-	-	-
Gypsum	-	-	-	-	-	-	-	0.37*	-	0.60***	-	-	-
FDA	-	-	-	-	-	0.39*	0.37*	-	0.45*	0.41*	-	-	-
DHA	-	-	-	-	-	0.52**	-	-	0.63***	-	-	-	-
Urease	-0.71***	0.43*	0.50**	0.36*	-	0.67***	-	-	-	-	-	-	-
Catalase	-0.49**	-	-	-	-	0.76***	-	-	-	-	-	-	-
AlkP	-	-	0.35*	-	-	0.50**	-	-	0.42*	-	-	-	0.47**
AcP	-	-	-	-	-	-	-	-	-	-	-	-	-
WSP	-	-	-	-	-0.44*	0.36*	-	-	0.50**	-	-	-	-

TABLE S9.1.3. (Cont. 1) | Spearman's correlations among all the soil parameters considered in this study (“-” means no correlation).
Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	EOC	TN	TOC	Cmic	Cmin	Nmic	Pmic	Clay	SiltUS	SandUS	Carbonates
TN	0.59***										
TOC	0.87***	0.62***									
Cmic	0.80***	0.51**	0.80***								
Cmin	0.76***	0.46**	0.81***	0.69***							
Nmic	0.88***	0.42*	0.81***	0.91***	0.76***						
Pmic	0.39*	-	0.39*	0.43*	-	0.40*					
Clay	-0.39*	-	-	-	-	-0.36*	-				
SiltUS	-	-	-	-	-	-	-	-0.66***			
SandUS	-	-	-	-	-	-	-	-	-0.74***		
Carbonates	-	-	-	-	-	-	-	-	0.58**	-0.48**	
ActiveC	-	-	-	-	-	-	-	-	-	-0.49**	0.42*
Gypsum	0.44*	0.44*	0.53**	0.43*	0.39*	0.50**	-	-	-	-	-
FDA	0.70***	0.37*	0.79***	0.79***	0.56**	0.83***	-	-	-	-	-
DHA	0.73***	0.49**	0.76***	0.71***	0.68***	0.73***	0.36*	-	-	-	-
Urease	0.69***	0.45**	0.57**	0.75***	0.52**	0.77***	-	-	-	-	0.42*
Catalase	0.83***	0.65***	0.68***	0.71***	0.61***	0.72***	0.42*	-	-	-	-
AlkP	0.49**	0.37*	0.49**	0.45**	0.52**	0.47**	-	-	-	-	-
AcP	0.37*	-	-	-	-	-	-	-	-	-	-
WSP	0.40*	-	-	-	0.54**	-	-	-	-	0.43*	-

TABLE S9.1.3. (Cont. 2) | Spearman's correlations among all the soil parameters considered in this study (“-” means no correlation).
Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	ActiveC	Gypsum	FDA	DHA	Urease	Catalase	AlkP	AcP
Gypsum	-							
FDA	-	-						
DHA	-	-	0.65***					
Urease	-	0.37*	0.60***	0.45*				
Catalase	-	-	0.59***	0.68***	0.58**			
AlkP	-	0.50**	0.38**	-	-	-		
AcP	-	-	-	-	-	-	-	
WSP	-0.53**	-	-	-	-	0.44*	0.41*	-

TABLE S9.1.4. | Test of Canonical Dimensions for the CCA based on P fractions and soil composition-related parameters. Dimensions with *P* value < 0.05 in bold case.

Dimension	Canonical correlation	F-value	df1	df2	<i>P</i> value
1	0.87	2.82	40	85.6	0.00003
2	0.81	2.29	28	73.5	0.002
3	0.69	1.74	18	59.9	0.056
4	0.61	1.40	10	44.0	0.214
5	0.30	0.57	4	23.0	0.684

TABLE S9.1.5. | Test of Canonical Dimensions for the CCA based on P fractions and physical fertility-related parameters. Dimensions with *P* value < 0.05 in bold case.

Dimension	Canonical correlation	F-value	df1	df2	<i>P</i> value
1	0.89	2.52	32	75.3	0.0005
2	0.75	1.38	21	60.8	0.167
3	0.40	0.61	12	44.0	0.825
4	0.35	0.64	5	23.0	0.669

TABLE S9.1.6. | Test of Canonical Dimensions for the CCA based on P fractions and chemical fertility-related parameters. Dimensions with *P* value < 0.05 in bold case.

Dimension	Canonical correlation	F-value	df1	df2	<i>P</i> value
1	0.95	2.38	72	98.8	0.00003
2	0.92	1.71	56	91.5	0.011
3	0.75	1.07	42	83.2	0.387
4	0.59	0.86	30	74.0	0.674
5	0.53	0.84	20	63.9	0.663
6	0.45	0.81	12	53.2	0.635
7	0.41	0.83	6	42.0	0.552
8	0.21	0.49	2	22.0	0.619

TABLE S9.1.7. | Test of Canonical Dimensions for the CCA based on P fractions and organic matter-related parameters. Dimensions with *P* value < 0.05 in bold case.

Dimension	Canonical correlation	F-value	df1	df2	<i>P</i> value
1	0.92	2.26	56	96.9	0.0002
2	0.81	1.56	42	87.9	0.041
3	0.75	1.24	30	78.0	0.224
4	0.67	0.83	20	67.3	0.669
5	0.27	0.23	12	55.8	0.996
6	0.20	0.20	6	44.0	0.975
7	0.12	0.16	2	23.0	0.854

TABLE S9.1.8. | Test of Canonical Dimensions for the CCA based on P fractions and soil enzymatic activity-related parameters. Dimensions with *P* value < 0.05 in bold case.

Dimension	Canonical correlation	F-value	df1	df2	<i>P</i> value
1	0.93	2.08	56	96.9	0.0008
2	0.77	1.30	42	87.9	0.154
3	0.67	1.06	30	78.0	0.412
4	0.53	0.88	20	67.3	0.608
5	0.50	0.84	12	55.9	0.613
6	0.36	0.55	6	44.0	0.764
7	0.10	0.12	2	23.0	0.886

TABLE S9.1.9. | Variance explained by each component and accumulated variance of the matrix of independent variables (X) and the vector of dependent variable (Y). Selected components in bold case.

	% variance explained		% cumulative variance	
	X	Y	X	Y
Component 1	14.38	21.19	14.38	21.19
Component 2	11.38	26.5	25.76	47.69
Component 3	23.16	10.89	48.92	58.58
Component 4	8.25	13.5	57.17	72.08
Component 5	4.79	6.19	61.96	78.27
Component 6	4.13	5.66	66.09	83.93
Component 7	4.51	3.36	70.6	87.29
Component 8	4.93	2.04	75.53	89.33
Component 9	2.62	2.33	78.15	91.66
Component 10	2.18	1.62	80.33	93.28
Component 11	1.77	1.55	82.1	94.83
Component 12	1.5	1.86	83.6	96.69
Component 13	1.23	1.39	84.83	98.08
Component 14	2.7	0.33	87.53	98.41
Component 15	2.22	0.33	89.75	98.74
Component 16	1.24	0.32	90.99	99.06
Component 17	1.01	0.23	92	99.29
Component 18	1.67	0.18	93.67	99.47
Component 19	1.03	0.21	94.7	99.68
Component 20	1.17	0.09	95.87	99.77
Component 21	0.65	0.12	96.52	99.89
Component 22	0.68	0.06	97.2	99.95
Component 23	0.48	0.04	97.68	99.99
Component 24	0.43	0.01	98.11	100
Component 25	0.27	0	98.38	100
Component 26	0.48	0	98.86	100
Component 27	0.28	0	99.14	100

TABLE S9.1.10. | Weight of each variable within each component of the PLS model. Variables with weight > 10% in bold case.

X matrix variables	Component 1	Component 2
BD	6.35	13.30
Porosity	2.43	6.24
FC	9.53	16.16
PWP	11.82	13.27
pH	12.63	0.61
1/CEC		12.22
1/Fe	5.98	1.03
log(Cu)	0.21	1.17
Mn	2.81	
log(Zn)	0.30	
1/K	0.67	15.01
Mg	3.50	13.45
Na	1.88	7.41
1/EOC	1.57	4.99
log(TN)	1.46	7.95
TOC		1.02
log(Cmic)	0.93	8.88
Cmin	0.36	0.72
Nmic	2.53	3.61
Pmic	1.57	
Clay	3.74	
SiltUS	21.80	4.06
√SandUS	16.70	6.53
Carb²	18.74	3.61
ActiveC	17.85	1.53
Gypsum	2.73	1.23
FDA	0.37	
log(DHA)	1.39	0.53
log(Ure)	4.61	7.54
1/Cat	0.68	5.95
AlkP		6.38
AcP		3.55
√WSP	7.72	2.59
log(PAEM)		7.57
√P_iNaHCO₃	0.45	0.34
√P_oNaHCO₃	0.18	2.63
log(P _i NaOH)	4.93	5.97
log(P_oNaOH)	0.13	11.12
√PHCl_{1M}	14.08	6.03
log(PHCl _{conc})	2.12	2.52
PHClO ₄	6.46	0.29

9.2. Molybdenum-blue colorimetric procedure

Here, we detail the methodology followed to perform the molybdenum-blue colorimetric method described by Murphy & Riley (1962) and modified by Watanabe & Olsen (1965). This method was used for the final determination of P concentrations in the extracts obtained in the P sequential fractionation. This method is based on the formation of a complex between ammonium molybdate and the phosphate ion that presents a blue coloration when reduced with ascorbic acid. For a specific extract, the amount of phosphorus present is what determines the intensity of the color, since the rest of the reagents are always added in excess. Therefore, the intensity of the color developed is directly proportional to the amount of phosphorus present in the extracts (Lambert-Beer Law), which facilitates its measurement through the absorbance measured by spectrophotometry. To know the existing phosphorus concentrations in the samples, calibration curves are made using the same matrix like the one presented in the extracts to be analyzed and the least-squares adjustment equation is determined. The range of this series of standard concentrations should encompass the absorbance data set of the analyzed samples. In addition, both in the samples to be analyzed and in the standards, the pH must always be adjusted so that it is suitable for color development (see Supplementary Information 9.3.). Before proceeding with the measurement of absorbance at 882 nm, allow 30 minutes to elapse to allow color development.

The advantages of this method over other colorimetric methods are the stability of the blue color of the complex and the tolerance to high concentrations of Fe^{3+} (Kuo, 1996). Watanabe & Olsen (1965) modified the method of Murphy & Riley (1962) using reagent A (which can be stored for long periods of time) and reagent B which is prepared daily by dissolving ascorbic acid in reagent A.

– MATERIAL –

- Volumetric flasks 25 ml
- Wash bottle
- Automatic pipette
- Spectrophotometer

– REAGENTS –

Colorimetric reagent A: Weigh 12 g of ammonium molybdate and dissolve in 250 ml of distilled water; Weigh 0.2908 g of antimony potassium tartrate and dissolve in 100 ml of

distilled water. Pour 138.1 ml of concentrated H₂SO₄ (97%) into distilled water and then gradually add more water to make up to 1 L. Mix the first two solutions in a 2 L beaker and add 1 L of 5 N H₂SO₄ solution. Store in a dark glass bottle in the refrigerator.

Colorimetric reagent B: Weigh 0.528 g of ascorbic acid and dissolve in 100 ml of reagent A. It should be prepared daily.

Standard solution of 1000 ppm of P: Dissolve 4.394 g of monopotassium phosphate (previously oven-dried) in 1 L of distilled water.

Standard solution of 100 ppm of P: Take 100 ml of the 1000 ppm standard solution and dissolve in 1 L of distilled water.

Standard solution of 5 ppm of P: Take 5 ml of the 100 ppm solution and dissolve in 100 ml of distilled water. Required only for fractionation step 6 (PHCl_{1M}). It should be prepared daily.

Standard solution of 2.5 ppm of P: Take 2.5 ml of the 100 ppm standard solution and dissolve in 100 ml of distilled water. It should be prepared daily.

TABLE S9.2.1. | Relationship between the volume of the known P concentration standard and the concentration obtained after making up to 25 ml and after adjusting the pH. The standard solutions in gray color were only needed in step 6 of the sequential fractionation of P (PHCl_{1M}).

Standard	2.5 ppm standard volume	Extractant volume	p-nitrophenol	5 M NaOH	1 M HCl	Reagent B volume	Total volume
P0	-	Different in each step*	1 drop	Dropwise [#]	Dropwise ⁺	4 ml	25 ml
P0.05	0.5 ml [0.05 ppm]	*	1 drop	#	+	4 ml	25 ml
P0.1	1 ml [0.1 ppm]	*	1 drop	#	+	4 ml	25 ml
P0.15	1.5 ml [0.2 ppm]	*	1 drop	#	+	4 ml	25 ml
P0.2	2 ml [0.2 ppm]	*	1 drop	#	+	4 ml	25 ml
P0.4	4 ml [0.4 ppm]	*	1 drop	#	+	4 ml	25 ml
P0.6	3 ml [0.6 ppm]**	*	1 drop	#	+	4 ml	25 ml
P0.8	4 ml [0.8 ppm]**	*	1 drop	#	+	4 ml	25 ml
P0.9	4.5 ml [0.9 ppm]**	*	1 drop	#	+	4 ml	25 ml

**Standard solution of 5 ppm of P. [#]Dropwise until the solution turns yellow. ⁺Dropwise until the solution turns colorless.

9.3. Procedure for extracts pH adjustment

To determine the concentration of P by colorimetry, it is necessary to adjust the pH, both of the extracts to be measured and of the standards, so that the color development is adequate. For this, 1 drop of p-nitrophenol indicator is added, 5 M NaOH is added dropwise until the solution turns yellow (Figure S9.3.1. (a)) and finally, 1 M HCl dropwise until a new turn to colorless (Figure S9.3.1. (b)). Each flask of the calibration curve in addition to the matrix must have the corresponding volume of the standard of known concentration and once the pH is adequate, the colorimetric reagent B is added, made up to 25 ml, shaken and allowed to settle 30 minutes (see Supplementary Information 9.2., Table S9.2.1.).



FIGURE S9.3.1. | Adjustment of pH. (a) Turn from colorless to yellow by adding 5 M NaOH. (b) Turn from yellow to colorless by adding 1 M HCl. Photos by ©R.C. Martín-Sanz.

– MATERIAL –

- Volumetric flasks 25 ml
- Automatic pipette
- Beakers
- Dropper

– REAGENTS –

p-nitrophenol: Dissolve 1 g of 4-nitrophenyl in distilled water and make it to one liter.

1 M sulfuric acid: Take 165.61 ml of 37 % HCl and level it to 2 liters with distilled water.

5 M sodium hydroxide: Weigh 50 g of NaOH and add distilled water until 250 ml.

9.4. Procedure to remove P from active carbon

Below, the procedure followed to remove phosphorus from the active carbon is shown.

Although commercial active carbon for analysis is assumed not to contain phosphorus, various tests have indicated that it contains enough amount of this element to cause contamination in phosphorous analyses. For this reason, it was necessary to purify the active carbon to eliminate the phosphorus.

– MATERIAL –

- 1 L beaker
- Magnetic stirrer and rod
- Büschner vacuum funnel
- Filter paper
- Spatula
- Tweezers
- Wash bottle
- Porcelain capsule
- Stove
- Distilled water

– REAGENTS –

0.5 M sodium bicarbonate at pH 8.5: Dissolve 42 g of NaHCO_3 in one liter of distilled water. Adjust the pH to 8.5 with 40% NaOH.

– PROCESS –

1. Weigh 25 g of active carbon.
2. Dissolve in 500 ml of 0.5 M NaHCO_3 at pH 8.5 in a 1 L beaker.
3. Shake on a magnetic stirrer for 30 minutes (Figure S9.4.1.(a)).
4. Filter the mixture in a Büschner vacuum funnel (Figure S9.4.1.(b)). An activated carbon cake is obtained. Remove the whole cake with the filter paper.
5. Place the cake in a 1 L beaker.
6. Separate the filter paper with tweezers and wash it with distilled water.
7. Add 500 ml of distilled water.
8. Shake on a magnetic stirrer for 30 minutes.
9. Filter again in a Büschner vacuum funnel.
10. Separate the cake and place it in a porcelain capsule (Figure S9.4.1.(c)).
11. Dry in a stove at 80°C for 24 hours.

12. When the active carbon is dry, separate the filter paper with tweezers and store in a bottle previously washed with HCl (to avoid contamination by phosphorus).

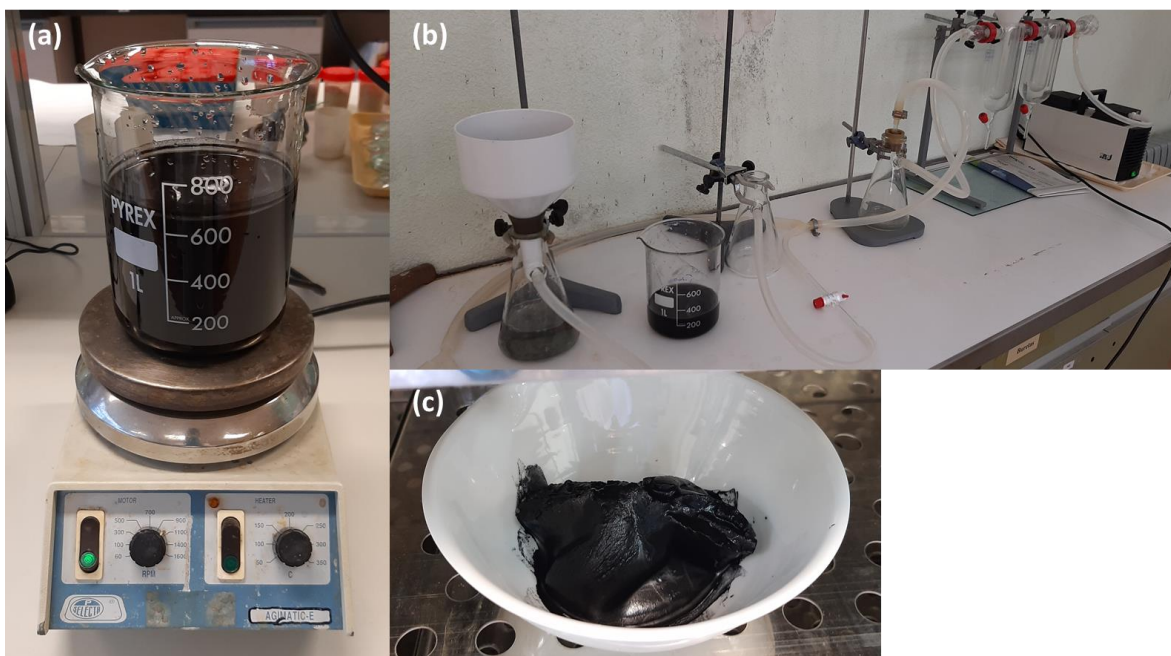


FIGURE S9.4.1. | Purification of active carbon to remove P content. (a) Shaking. (b) Filtering in a Büchner vacuum funnel. (c) Active carbon cake in a porcelain capsule for drying. Photos by ©R.C. Martín-Sanz.

9.5. Detailed procedure for P sequential fractionation

In this last section of Supplementary Information, we detail the different steps of the phosphorus sequential fractionation method followed in this study, developed by Hedley et al. (1982) and modified by Tiessen & Moir (1993). This method aims to break down the total P found in the soil into a series of fractions. The difference between these fractions is established by the availability degree to be absorbed by the roots of the plants. Thus, the first fraction extracted corresponds to the reservoir more loosely linked to the soil components and the last to the reservoir that is more strongly retained by them and so, the one less available to plants. This method also permits to differentiate between organic and inorganic P fractions. The sum of the quantities extracted in each fraction is not exactly equivalent to the total phosphorus in the soil, since there is a residual quantity very strongly bound to the edaphic components that cannot be separated from the soil with this procedure.

9.5.1. STEP 1: Determination of labile P by anion exchange membranes (PAEM)

Anion exchange membranes (AEM) act as a surface to which soil P ions adhere, simulating the action of the roots of vegetation (Zamuner, 1999).

– MATERIAL –

- Precision scale
- 50 ml centrifuge tubes
- 30 ml tubes
- Tweezers
- Ion exchange membranes (AEM)*
- Mechanical rotator shaker
- Parafilm
- Adjustable dispenser
- Dropper
- Wash bottle
- Automatic pipette
- Volumetric flasks 25 ml
- Beakers
- pH-meter
- Distilled water

*Resin membranes of 10 cm² surface. They should be stored in a 0.5 M NaHCO₃ solution at pH 8.5. Before being used, they must be conditioned by stirring in the same solution for at least one hour (Figure S9.5.1.).

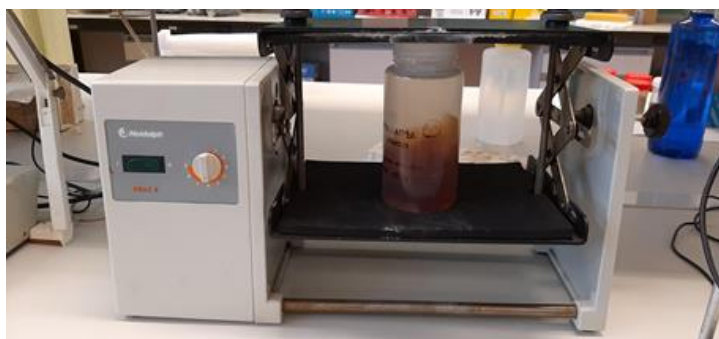


FIGURE S9.5.1. | Conditioning of the ion exchange membranes. Photo by ©R.C. Martín-Sanz.

– REAGENTS –

0.5 M sodium bicarbonate at pH 8.5: Dissolve 42 g of NaHCO₃ in one liter of distilled water. Adjust the pH to 8.5 with 40% NaOH.

0.3 M HCl solution (EXTRACTANT): Dissolve 49.68 ml of 37% HCl in two liters of distilled water.

Phosphorus standard solutions for the calibration curve (see Supplementary Information 9.2.).

Colorimetric reagents A and B for colorimetry (see Supplementary Information 9.2.).

Reagents and indicator for pH adjustment (see Supplementary Information 9.3.).

– PROCESS –

1. Weigh 2 g of soil into a 50 ml centrifuge tube, noting the exact weight of the soil.
2. Add 30 ml of distilled water.
3. Insert with sterile tweezers (or lined in sterile Parafilm) an AEM pre-washed with distilled water.
4. Shake by rotation for 16 hours.
5. Remove the AEM with sterile tweezers. Reserve the soil with water.
6. Place the AEM in a 30 ml centrifuge tube.
7. Add 20 ml of 0.3 M HCl with an automatic pipet.
8. Shake for 2 hours.
9. Remove the AEM with sterile tweezers and store them in 0.5 M NaHCO₃.
10. Take **15 ml of ALIQUOT 1** to 25 ml flask.

Prepare patterns with the same matrix.

Adjust the pH of samples and patterns.

Colorimetric procedure in samples and patterns.

Measure absorbance.

9.5.2. STEP 2: Determination of highly labile P using bicarbonate (PNaHCO₃)

– MATERIAL –

- 50 ml centrifuge tubes
- 30 ml tubes
- Mechanical rotator shaker
- Parafilm
- Adjustable dispensers
- Dropper
- Wash bottle
- Automatic pipette
- Volumetric flasks 25 ml
- Beakers
- Centrifuge with temperature control
- Funnels
- Filter paper

- Support for filtering
- Capped test tubes
- Freezer
- Autoclave
- Active carbon
- Distilled water
- pH strips

– REAGENTS –

0.5 M sodium bicarbonate at pH 8.5 (EXTRACTANT): Dissolve 42 g of NaHCO_3 in one liter of distilled water. Adjust the pH to 8.5 with 40% NaOH.

Concentrated sulfuric acid to precipitate organic matter (96% H_2SO_4).

Active carbon previously purified to remove organic matter (see Supplementary Information S9.4.).

Potassium persulfate for digestion.

0.5 M sulfuric acid for digestion: Take 55.50 ml of 96% H_2SO_4 and gradually make up to 2 l with distilled water.

Phosphorus standard solutions for the calibration curve (see Supplementary Information 9.2.).

Colorimetric reagents A and B for colorimetry (see Supplementary Information 9.2.).

Reagents and indicator for pH adjustment (see Supplementary Information 9.3.).

– PROCESS –

1. Water + soil in centrifuge tubes from the previous step.
2. Centrifuge at 10,000 rpm for 10 minutes at 0°C.
3. Remove water by decantation.
4. Add 30 ml of 0.5 M NaHCO_3 to the soil.
5. Separate the soil from the bottom of the tube and shake for 16 hours.
6. Centrifuge at 10,000 rpm for 10 minutes at 0°C.
7. Separate the extract by decantation ("EXTRACT NaHCO_3 "). Reserve the soil in the centrifuge tubes.

At this point, the method makes it possible to differentiate the amount of P extracted into two fractions, that corresponding to total P (previous digestion of the sample) and that

corresponding to inorganic P (previous precipitation of organic matter at pH 1.5 and addition of active C); organic P is determined by difference.

Inorganic P extraction:

1. Pour 15 ml of the "EXTRACT NaHCO₃" into a centrifuge tube.
2. Add concentrated H₂SO₄ dropwise to pH 1.5 (measure with pH strips).
3. Add 0.4 g of previously purified active carbon (see Supplementary Information 9.4.).
4. Shake to homogenize.
5. Freeze for 10 minutes.
6. Centrifuge at 10,000 rpm for 10 minutes at 0°C.
7. Filter with filter paper (the extract should be transparent).
8. Discard the precipitate with organic matter and active C.
9. Take **10 ml of ALIQUOT 2.1.** to a 25 ml volumetric flask.

Prepare patterns with the same matrix.

Adjust the pH of samples and patterns.

Colorimetric procedure in samples and patterns.

Measure absorbance.

Organic P extraction:

1. Pour 10 ml of the "EXTRACT NaHCO₃" into a stoppered test tube.
2. Add 5 ml of 0.5 M H₂SO₄ (carefully, strong reaction occurs).
3. Homogenize.
4. Add 0.5 g of potassium persulfate.
5. Homogenize.
6. Autoclave at 120°C for 45 minutes (the extract should be clear and without precipitates).
7. Take **7 ml of ALIQUOT 2.2.** to a 25 ml volumetric flask.

Prepare patterns with the same matrix.

Adjust the pH of samples and patterns.

Colorimetric procedure in samples and patterns.

Measure absorbance.

9.5.3. STEP 3: Determination of moderately labile P using sodium hydroxide (PNaOH)

– MATERIAL –

- 50 ml centrifuge tubes
- 30 ml tubes
- Mechanical rotator shaker
- Parafilm
- Adjustable dispensers
- Dropper
- Wash bottle
- Automatic pipette
- Volumetric flasks 25 ml
- Beakers
- Centrifuge with temperature control
- Funnels
- Filter paper
- Support for filtering
- Capped test tubes
- Distilled water
- pH strips

– REAGENTS –

0.1 M sodium hydroxide (EXTRACTANT): Dissolve 8 g of NaOH in two liters of distilled water.

Concentrated sulfuric acid to precipitate organic matter (96% H₂SO₄).

Active carbon previously purified to remove organic matter (see Supplementary Information S9.4.).

Potassium persulfate for digestion.

0.5 M sulfuric acid for digestion: Take 55.50 ml of 96% H₂SO₄ and gradually make up to 2 L with distilled water.

Phosphorus standard solutions for the calibration curve (see Supplementary Information 9.2.).

Colorimetric reagents A and B for colorimetry (see Supplementary Information 9.2.).

Reagents and indicator for pH adjustment (see Supplementary Information 9.3.).

– PROCESS –

1. Soil in centrifuge tubes from the previous step.
2. Add 30 ml of 0.1 M NaOH to the soil.
3. Separate the soil from the bottom of the tube and shake for 16 hours.
4. Centrifuge at 10,000 rpm for 10 minutes at 0°C.
5. Separate the extract by decantation ("EXTRACT NaOH"). Reserve the soil in the centrifuge tubes.

At this point, we can also differentiate the amount of P extracted into two fractions, the corresponding to total P (previous digestion of the sample) and that corresponding to inorganic P (previous precipitation of organic matter at pH 1.5 and addition of active C); organic P is determined by difference.

Inorganic P extraction:

1. Pour 15 ml of the "EXTRACT NaOH" into a centrifuge tube.
2. Add concentrated H₂SO₄ dropwise to pH 1.5 (measure with pH strips).
3. Add 0.4 g of previously purified active carbon (see Supplementary Information 9.4.).
4. Shake to homogenize.
5. Freeze for 10 minutes.
6. Centrifuge at 10,000 rpm for 10 minutes at 0°C.
7. Filter with filter paper (the extract should be transparent).
8. Discard the precipitate with organic matter and active C.
9. Take **5 ml of ALIQUOT 3.1.** to a 25 ml volumetric flask.

Prepare patterns with the same matrix.

Adjust the pH of samples and patterns.

Colorimetric procedure in samples and patterns.

Measure absorbance.

Organic P extraction:

1. Pour 5 ml of the "EXTRACT NaOH" into a stoppered test tube.
2. Add 10 ml of 0.5 M H₂SO₄ (carefully, strong reaction occurs).
3. Homogenize.
4. Add 1 g of potassium persulfate.

5. Homogenize.
6. Autoclave at 120°C for 45 minutes (the extract should be clear and without precipitates).
7. Take **5 ml of ALIQUOT 3.2.** to a 25 ml volumetric flask.

Prepare patterns with the same matrix.

Adjust the pH of samples and patterns.

Colorimetric procedure in samples and patterns.

Measure absorbance.

9.5.4. STEP 4: Determination of primary P using dilute hydrochloric acid (PHCl_{1M})

– MATERIAL –

- 50 ml centrifuge tubes
- 30 ml tubes
- Mechanical rotator shaker
- Parafilm
- Adjustable dispensers
- Dropper
- Wash bottle
- Automatic pipette
- Volumetric flasks 25 ml
- Beakers
- Centrifuge with temperature control
- Distilled water

– REAGENTS –

1 M hydrochloric acid (EXTRACTANT): Take 165.61 ml of 37 % HCl and level it to 2 liters with distilled water.

Phosphorus standard solutions for the calibration curve (see Supplementary Information 9.2.).

Colorimetric reagents A and B for colorimetry (see Supplementary Information 9.2.).

Reagents and indicator for pH adjustment (see Supplementary Information 9.3.).

– PROCESS –

1. Soil in centrifuge tubes from the previous step.
2. Add 30 ml of 1 M HCl to the soil. **IMPORTANT NOTE:** Add it little by little, very carefully, and let it rest as it produces a very strong reaction due to the presence of carbonates in the soil. We should

stop the process at least two days in this step to let the reaction finish completely, thus avoiding loss of sample when continuing the procedure.

3. After two days, when the reaction is finished, separate the soil from the bottom of the tube and shake for 16 hours.
4. Centrifuge at 10,000 rpm for 10 minutes at 0°C.
5. Separate the extract by decantation. Reserve the soil in the centrifuge tubes.
6. Take **1.5 ml of ALIQUOT 4** to a 25 ml volumetric flask.

Prepare patterns with the same matrix. NOTE: in this step, we need standards with a higher concentration of phosphorus than in the rest of the fractionation steps (see Supplementary Information 9.2.).

Adjust the pH of samples and patterns.

Colorimetric procedure in samples and patterns.

Measure absorbance.

9.5.5. STEP 5: Determination of stable P using concentrated hydrochloric acid ($\text{PHCl}_{\text{conc}}$)

– MATERIAL –

- 50 ml centrifuge tubes
- 30 ml tubes
- Mechanical rotator shaker
- Parafilm
- Adjustable dispensers
- Dropper
- Wash bottle
- Automatic pipette
- Volumetric flasks 25 ml
- Beakers
- Centrifuge with temperature control
- Water bath at 90°C
- Laminar flow hood
- Distilled water

– REAGENTS –

Concentrated hydrochloric acid (*EXTRACTANT*): 37% HCl for analysis.

Phosphorus standard solutions for the calibration curve (see Supplementary Information 9.2.).

Colorimetric reagents A and B for colorimetry (see Supplementary Information 9.2.).

Reagents and indicator for pH adjustment (see Supplementary Information 9.3.).

– PROCESS –

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1. Soil in centrifuge tubes from the previous step.
2. Add 30 ml of concentrated HCl to the soil.
3. Separate the soil from the bottom of the tube.
4. Heat in a water bath at 90°C for 15 minutes inside the laminar flow hood and without covering the tubes.
5. Shake for 1 hour.
6. Centrifuge at 10,000 rpm for 10 minutes at 0°C.
7. Separate the extract by decantation. Reserve the soil in the centrifuge tubes.
8. Take **1 ml of ALIQUOT 5** to a 25 ml volumetric flask.

Prepare patterns with the same matrix.

Adjust the pH of samples and patterns.

Colorimetric procedure in samples and patterns.

Measure absorbance.

9.5.6. STEP 6: Determination of residual P using perchloric acid (PHClO₄)

– MATERIAL –

- 50 ml centrifuge tubes
- Parafilm
- Adjustable dispensers
- Dropper
- Wash bottle
- Automatic pipette
- Volumetric flasks 25 ml
- Beakers
- Funnels
- Filter paper
- Support for filtering
- Digestion block and controller
- Digestion block tubes
- Volumetric flasks 100 ml
- Laminar flow hood
- Distilled water

– REAGENTS –

Concentrated perchloric acid (EXTRACTANT): 70% HClO₄ for analysis.

Phosphorus standard solutions for the calibration curve (see Supplementary Information 9.2.).

Colorimetric reagents A and B for colorimetry (see Supplementary Information 9.2.).

Reagents and indicator for pH adjustment (see Supplementary Information 9.3.).

– PROCESS –

1. Soil in centrifuge tubes from the previous step.
2. Add 15 ml of concentrated HClO₄ to the soil.
3. Separate the soil from the bottom of the tube and pour to a digestion block tube.
4. Add another 15 ml of HClO₄ to the digestion block tube.
5. Heat in the digestion block at 130°C during 55 minutes (until the dark color disappears).
6. Raise the temperature to 230°C for 35 minutes (until the color turns whitish).
7. Allow cooling overnight inside the laminar flow hood.
8. Add 50 ml of distilled water and homogenize.
9. Filter with a funnel and filter paper directly into a 100 ml flask.
10. Make up to 100 ml with distilled water and homogenize.
11. Take **3 ml of ALIQUOT 6** to a 25 ml volumetric flask.

Prepare patterns with the same matrix.

Adjust the pH of samples and patterns.

Colorimetric procedure in samples and patterns.

Measure absorbance.

9.6. References for Supplementary Information

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