Ann. For. Sci. 58 (2001) 89–98

Original article

^{31}P-NMR characterization of phosphorus fractions in natural and fertilized forest soils

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(Received 13 December 1999; accepted 3 April 2000)

Abstract – The amount, quality, and turnover of soil P is influenced by climate and changes in soil management. The objectives of this study were to evaluate the influence of edaphic properties, mean annual precipitation, and P-fertilization on soil organic P. ^{31}P-NMR spectroscopy was applied to investigate P forms of forest soils of the Central Western Spain. The concentrations of NaOH-extractable inorganic-P were significantly higher in fertilized than in natural soils. Monoester-P was the dominant organic-P species in both natural and fertilized soils, representing between 19 and 54% of NaOH-extractable P. The highest concentrations of monoester-P were observed in the soil with higher content of Fe oxides. The high charge density of monoester-P allows rapid adsorption on soil minerals and extensive interaction with sesquioxides that protect inositols from degradation. Diester-P represented between 3 and 17% of alkali-extractable P, reflecting a relatively low microbial activity in the soils on schists with a high content of Al and Fe oxides.

organic P / P-fertilization / forest soil / diester-monoester-P / ^{31}P-NMR

Résumé – **Caractérisation du phosphore et de ses fractions par la technique du NMR dans des sols forestiers fertilisés et non-fertilisés.** La teneur, la qualité et le turnover du P du sol sont influencés par le climat et les changements dans la gestion du sol. L'objectif de cette étude a été d'évaluer l'influence des propriétés édaphiques, de la pluviométrie moyenne annuelle et de la fertilisation phosphatée sur le P organique du sol. La spectroscopie NMR a été appliquée pour rechercher les formes du P dans des sols forestiers du Centre Ouest de l'Espagne. La concentration en P inorganique extrait avec NaOH a été significativement plus haute dans le sol fertilisé que dans le sol naturel. Le monoester phosphorique (représentant entre 19 et 54 % du P extractable avec NaOH) est la forme dominante de P organique, aussi bien dans le sol naturel que dans le sol fertilisé. La plus haute concentration de monoester phosphorique a été observée dans le sol avec la plus haute teneur en oxydes ferriques. La haute densité de charge du monoester phosphorique permet sa rapide adsorption sur les composés mineraux du sol et son interation marquée avec les sesquioxydes, protégeant ainsi de la dégradation les inositols. Les diesters phosphoriques représentent entre 3 et 17 % du P extractable avec NaOH, montrant par là une relativement basse activité microbienne dans les sols sur schistes avec une haute teneur en oxydes aluminiques.

P organique / fertilisation phosphorique / sols forestiers / esters phosphoriques / NMR du ^{31}P

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1. INTRODUCTION

In natural ecosystems, amounts and chemical nature of soil organic P are mainly determined by a combination of the major soil-forming factors (time, parent material, climate, topography, and organisms) [24]. The distribution of P among different organic and inorganic forms reflects the history, present structure, and function of an ecosystem [15, 32].

The organic P (P_o) content of soil may vary from traces in arid regions to several hundred mg kg^{-1} in thick, humic forest soils. Often, nearly half of the total P in soil occurs in organic forms, most of which is derived from plant residues and, in part, synthesized by soil organisms from inorganic sources [21]. Brannon and Sommers [3] observed that more than 40% of the P_o in soils is typically associated with the fulvic and humic fractions and also reported the especial association of P_o with high-molecular-weight humic fractions.

Several sequential extraction schemes have been developed to fractionate both inorganic and organic P into fractions based on the solubility of soil P forms in different chemical extractants [4, 12, 31]. These schemes do not identify the chemical forms of the P compounds extracted, but they drive to an easy way of quantifying organic P according to its susceptibility or resistance to certain chemical treatments. It is known that NaOH with sonification does not extract all the soil P and various studies have shown that this extraction with NaOH and sonification does not extract every class of chemical P-species to the same extent [9, 10, 19], then the results cannot be used to calculate the amounts of different P-species in the soil. However, the NaOH with sonification extracts the P_o, except the more chemically recalcitrant forms [12, 26].

The ^{31}P-NMR spectroscopy is a useful tool for characterizing the structural composition of P in alkali extracts of soils [4, 19, 21]. Different types of P compounds can be distinguished on the basis of the electronic environment around the various P nuclei. The number of ester linkages and the presence or absence of direct C-P bonds, e.g., in phosphonates, are the major factors affecting resonances frequencies of P [2] in soil organic matter (SOM). Thus, the quantitative detection and differentiation of monoester-P, diester-P, phosphonate, pyrophosphate, polyphosphate, and inorganic orthophosphate by ^{31}P-NMR is well established [27]. The group of monoester-P comprises mainly inositol phosphates [1]. Their high stability against microbial and enzymatic attack is caused by their strong interactions with soil minerals, because of their high charge density and by precipitation as Al, Fe, and Ca salts of low solubility [1]. The diester-P fraction, which contains nucleic acids, phospholipids, and other compounds, characterizes a labile soil P_o fraction. The occurrence of phosphonates in soils was explained with the absence of bacteria containing the phosphonatase enzyme [11].

Studies using ^{31}P-NMR spectroscopy have focused on the influence of cultivation and fertilization of agricultural soils on structural soil P_o composition or on screening of P_o forms in different ecosystems [9, 10, 33, 34]. Although ^{31}P-NMR spectroscopy is a powerful tool to assess the structural composition of soil P_o, little data is available on forms of P_o in natural and P-fertilized Mediterranean forest soils and few researchers have addressed the role of precipitation and temperature on soil P_o dynamics [29].

The objective of the present study was to evaluate the influence of edaphic properties (texture, pH, C, Fe, Al and C/N ratio), mean annual precipitation (MAP), and the effects of P-fertilization with superphosphate on the structural composition of P_o from forest humic soil horizons of the Central Western Spain.

2. MATERIALS AND METHODS

2.1. Site description

The soils under study were forest soils located in the "Sierra de Gata" mountains (40°2' N; 3°0' W, province of Salamanca, Central Western Spain). Four experimental plots, situated close to one another, were selected following a rainfall gradient (*table I*). Three of these stands were *Quercus pyrenaica* Willd oak coppices, and the other was a *Castanea sativa* Miller chestnut coppice. These tree species are representative of the subhumid Mediterranean climate. These four forest plots were fertilized superficially with triple superphosphate at 100 kg P ha^{-1} in April 1992. Some climatic and edaphic data are given in *tables I* and *II*.

The climate of the area is characterized by rainy autumn and spring, and hot, dry summers; some winters are also dry and dryness is intense during summers, principally in Fuenteguinaldo. The climate is classified as temperate Mediterranean, with a MAP of 720 l m^{-2} y^{-1} and a mean annual temperature (MAT) of 13.3 ºC at Fuenteguinaldo, and 1 570 l m^{-2} y^{-1} and 11.3 °C at Navasfrías. The MAT of the sites under study is similar, being precipitation the differentiating factor of the climate [30, 31]. The dominant soils are *Humic Cambisols* [7] over acid bedrock (granite or schist). Hereinafter the following symbols will be used: FG for Fuenteguinaldo stand; NF for Navasfrías; VR for Villasrubias; and SM for the San Martín.

Table I. Characteristics of the forest plots studied.

	Fuenteguinaldo (FG)	Villasrubias (VR)	Navasfrías (NF)	San Martín (SM)
Vegetation	*Quercus pyrenaica*			*Castanea sativa*
Soil	*Humic Cambisol*			
Altitude (m a.s.l.)[a]	870	900	960	940
Bedrock	Granite	Schist	Schist	Granite
Mean rainfall (l m^{-2} y^{-1})	720	872	1570	1150
Mean T[b] (°C)	13.3	N.d.[c]	11.3	14.2
Density (trees ha^{-1})	738	1 043	820	3 970
DBH[d] (cm)	15.2	25.4	16.5	10.0
Mean tree height (m)	12.0	8.5	13.0	13.0
Basal area (m^2 ha^{-1})	21.2	13.5	15.6	30.0
LAI[e] (m^2 m^{-2})	2.6	2.0	1.8	3.7
Aboveground production (mg ha^{-1} y^{-1})	4.09	2.83	2.60	5.25

Notes: [a] meters above sea level; [b] mean annual temperature; [c] no data available; [d] mean trunk diameter; [e] leaf area index.

Table II. Chemical and physicochemical properties of the forest soils.

Plots[a]	Depth (cm)	pH (H_2O)	Corg[b] (g kg^{-1})	Nt[c] (g kg^{-1})	C/N	Sand (%)	Silt (%)	Clay (%)	Fe_d[d] (g kg^{-1})	Al_d[e] (g kg^{-1})	P_{Tsoil}[f] (mg kg^{-1})
FG	0–10	5.4	41.5	3.22	13	60	26	14	7.3	6.5	767
	10–20	5.1	25.0	2.08	12	57	29	14	5.6	8.1	645
	20–40	5.3	12.3	1.35	9	62	26	12	5.5	7.2	499
VR	0–10	4.6	67.3	3.99	17	24	62	14	23.8	25.2	965
	10–20	5.1	12.0	1.25	10	14	69	17	21.3	26.0	869
	20–40	5.2	6.1	0.91	7	10	74	16	19.4	26.9	794
NF	0–10	4.9	105.0	4.98	21	35	48	17	14.4	33.5	720
	10–20	4.8	58.0	3.37	17	33	53	14	15.6	39.4	677
	20–40	5	5.0	0.47	11	16	77	7	13.7	36.0	648
SM	0–10	5.1	45.0	2.53	18	68	19	13	7.8	13.5	1 206
	10–20	4.7	34.0	1.85	21	64	21	15	8.5	18.5	1 005
	20–40	4.9	18.0	1.08	17	64	26	10	9.6	19.1	903

Notes: [a] FG, Fuenteguinaldo, VR, Villasrubias, NF, Navasfrías, SM, San Martín; [b] total organic carbon, [c] total nitrogen, [d] free iron, [e] free aluminum, [f] total phosphorus in soil.

2.2. Methods

2.2.1. Soil sampling

Samples were collected at 0–10 cm, 10–20 cm, and 20–30 cm depth (inside the A_h horizon) in both P-fertilized and not fertilized forest soils. Results were calculated on a 105 °C soil dry base. All analyses were done in duplicate.

2.2.2. General analyses

The pH was measured in H_2O at a soil: solution ratio of 1:2.5 using a glass electrode. Total organic carbon (C_{org}) was determined by dry combustion on a Carmhograph 12 Wösthoff analyser; total nitrogen (Nt) was measured by Dumas oxidative digestion on a Macro N Heraeus apparatus. The particle-size distribution was determined by the International pipette method [25].

Free Fe (Fe_d) and Al (Al_d) contents were extracted with a mixed complexing and reducing buffer solution of Na citrate and Na dithionite as described by Holmgren [14].

The most important physical and chemical properties of the soils are shown in *table II*.

2.2.3. ^{31}P-NMR spectroscopy

^{31}P-NMR analysis was used to get information on the structural composition of alkali-soluble P. Extracts for ^{31}P-NMR analysis were obtained using the method of Newman and Tate [19]. For this purpose, 6.7 g of finely ground samples were dispersed ultrasonically with 90 ml of 0.5 M NaOH and the suspension was centrifuged at $12\,500 \times g$ for 120 min at 0 °C. The resulting supernatant was concentrated to about 2 ml at 40 °C in a rotary evaporator, then 1 ml of D_2O was added. ^{31}P-NMR spectra were recorded on a Bruker AM 500 NMR spectrometer (11.7 T; 202.5 MHz) without proton decoupling at a temperature of 280 °K. An acquisition time of 0.1 s, a 90° pulse and a relaxation delay of 0.2 s were used. The spectra were recorded with a line broadening factor of 20 Hz. Chemical shifts were measured relative to 85% H_3PO_4 in a 5 mm tube inserted into the 10 mm sample tube before the measurement of each sample. Peak assignments were according to Newman and Tate [19] and Condron et al. [5]. Intensities of signals were determined by integration. The mean signal-to-noise ratio was 66 for the most intensive peak and 10 to 14 for the less intensive peaks.

Total P in soil (P_{Tsoil}) and total P in the 0.5 M NaOH extract (P_{TNaOH}) were determined after ignition (550 °C, 17 h) and dissolution of the residue in 0.1 M H_2SO_4. Inorganic P in the NaOH extract (P_i) was measured directly in the extracts, and organic P in the NaOH extract (P_o) was calculated as difference [22]. In all cases, P was analysed by a modified molybdenum-blue method [18].

3. RESULTS AND DISCUSSION

3.1. Effects of rainfall gradient and edaphic properties on the P forms characterized by ^{31}P-NMR

^{31}P-NMR analysis was applied to investigate the structure of alkali-soluble P forms (*figure 1*). δ is the rate between the chemical displacement of the resonance, in Hertz, and the total frequency used. Due to the low value of this rate, it is multiplied by 10^6 to transform in an easier to use number, for this reason δ have ppm units.

Signals at δ = 3.0 to 6.2 ppm were due to orthophosphate monoesters, a group comprising inositol phosphates, sugar phosphates, and mononucleotides [5]. Range δ = –0.3 to 3.0 ppm correspond to diester signals, including teichoic acids because diester signals at δ = 1.0 to 3.0 ppm were assigned to these teichoic acids (which consist of chains of glycerol or sugar molecules linked by phosphate and attached to the mureine of Gram-positive bacteria; Guggenberger et al. [10]). These authors followed this assignment and found substantial amounts of teichoic acids in the fulvic acid fractions of the tropical savanna soils. Diesters, e.g., phospholipids and DNA, peaked at δ = –0.3 to 1.0 ppm [19]. The resonance at –1.0 to –3.0 ppm was referred to as unknown P. Signals accounting for P_i resonated at δ = 6.5 ppm (orthophosphate-P), δ = –4.4 ppm (pyrophosphate-P), and δ = –20 ppm (polyphosphate-P). The relative proportions of P_i in the NaOH extract (the sum of orthophosphate-P, pyrophosphate-P, and polyphosphate-P) and P_o in the NaOH extract (the sum of monoester-P, diester-P, and teichoic acid P) determined by ^{31}P-NMR and chemical analysis were similar (*table III*).

The relative proportions of P_o in the NaOH extracts were higher in VR and NF soils (*table III*), both over schist and with a higher content of free Fe and Al (*table II*) and greater amounts of fine fractions (silt + clay) than the soils over granite (*table II*).

Table III. Relative proportions of Pi (sum of orthophosphate-P, pyrophosphate-P and polyphosphate-P) and P_o (sum of monoester-P, diester-P, teichoic acid P, and unknown-P) determined by ^{31}P-NMR and chemical analysis.

Plots[a]	Depth (cm)	Chemical Analysis % Pi	Chemical Analysis % Po	^{31}P-NMR % Pi	^{31}P-NMR % Po
FG	0–10	41	59	63	37
	10–20	50	50	59	41
	20–40	55	45	58	42
VR	0–10	30	70	34	66
	10–20	29	71	29	71
	20–40	25	75	40	60
NF	0–10	25	75	29	71
	10–20	29	71	25	75
	20–40	27	73	26	74
SM	0–10	68	32	69	31
	10–20	61	39	63	37
	20–40	63	37	60	40

Notes: [a] FG, Fuenteguinaldo, VR, Villasrubias, NF, Navasfrías, SM, San Martín.

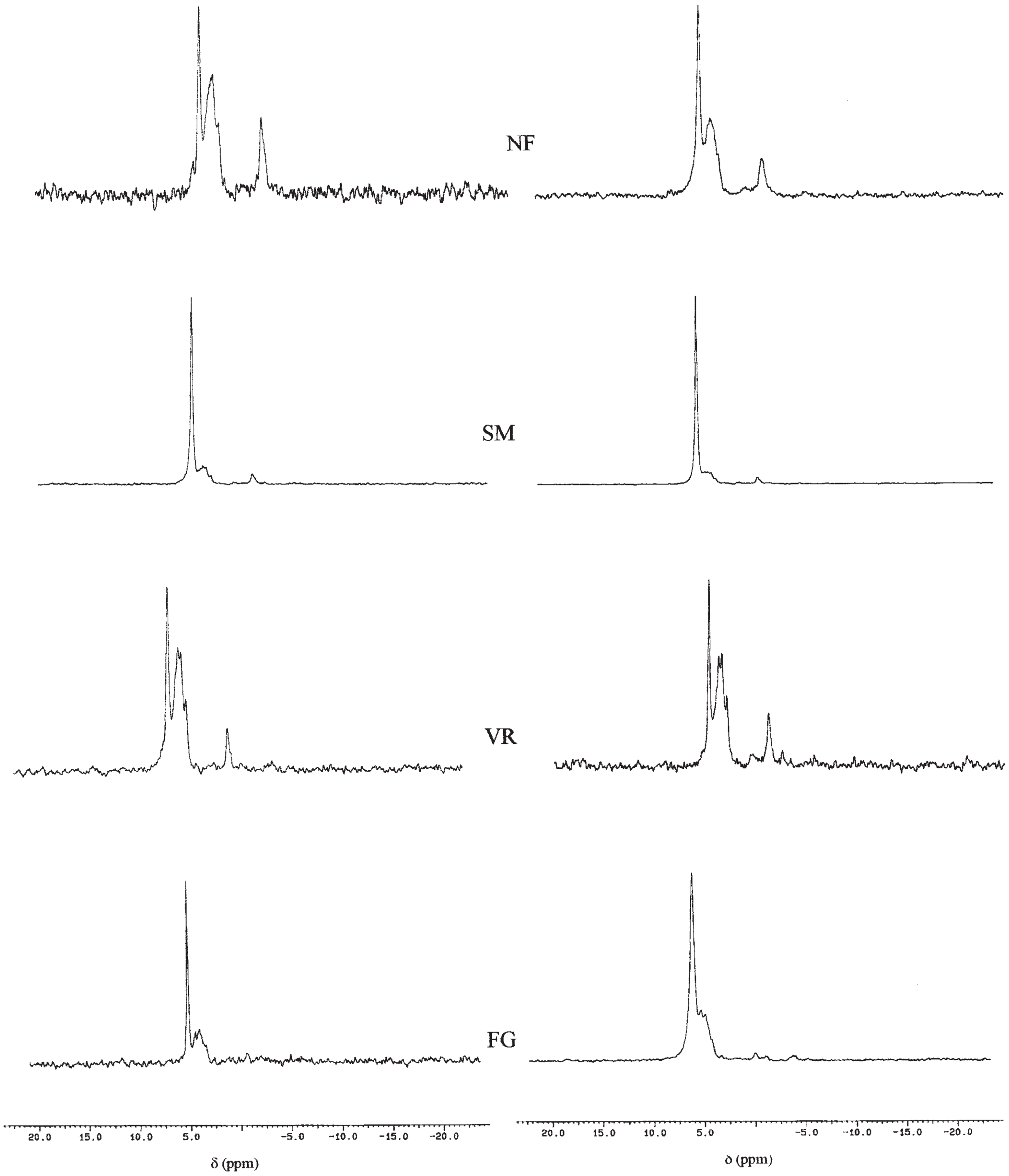

Figure 1. Phosphorus-31 nuclear magnetic resonance (^{31}P-NMR) spectra of the alkaline extracts of the natural (left) and P-fertilized (right) forest soils studied, at 0–10 cm.

To facilitate comparisons between soils, percentage peak areas (indicating the proportion of total spectral area allocated to the different P forms) were converted to amounts of alkali-extractable P on the basis of total P contents of the extracts as measured by chemical analysis (*table IV*).

In all forest soils studied (*table IV*), monoester-P was the dominant P_0 species in the NaOH extract, representing between 22 and 61% of NaOH-extractable P.

The highest concentrations of monoester-P in the NaOH extract were observed in VR. The concentrations of monoester-P showed positive relationship to the free

Table IV. P forms in the alkaline extracts of the unfertilized forest soils (mg kg^{-1}). Relative contribution (%) of each P species to the total NaOH-extractable P in brackets.

Plots[a]	Depth (cm)	Orthoph[b]	Monoester	Teichoic[c]	Diester	Pyroph[d]	Unknown	Mono/Dies[e]
FG	0–10	221 (61)	117 (32)	7 (2)	9 (2)	6 (2)	3 (1)	13
	10–20	246 (58)	147 (35)	8 (2)	9 (2)	3 (1)	9 (2)	16
	20–40	191 (56)	120 (35)	7 (2)	12 (3)	4 (1)	7 (2)	10
VR	0–10	164 (32)	277 (54)	13 (3)	38 (7)	12 (2)	10 (2)	7
	10–20	138 (28)	278 (57)	17 (4)	39 (8)	5 (1)	12 (2)	7
	20–40	161 (36)	215 (48)	13 (3)	30 (7)	11 (3)	9 (2)	7
NF	0–10	85 (28)	150 (49)	11 (4)	48 (16)	4 (1)	7 (2)	3
	10–20	73 (22)	176 (53)	19 (6)	46 (14)	tr[f]	7 (2)	4
	20–40	92 (26)	214 (61)	5 (2)	33 (9)	1 (0)	5 (1)	6
SM	0–10	440 (68)	142 (22)	12 (2)	39 (6)	8 (1)	6 (1)	4
	10–20	326 (64)	129 (25)	11 (2)	26 (5)	7 (2)	8 (2)	5
	20–40	231 (60)	126 (33)	6 (2)	18 (5)	1 (1)	5 (1)	7

Notes: [a] FG, Fuenteguinaldo; VR, Villasrubias; NF, Navasfrías; SM, San Martín; [b] orthophosphate-P; [c] teichoic acid-P; [d] pyrophosphate-P; [e] monoester to diester-P ratio, [f] traces.

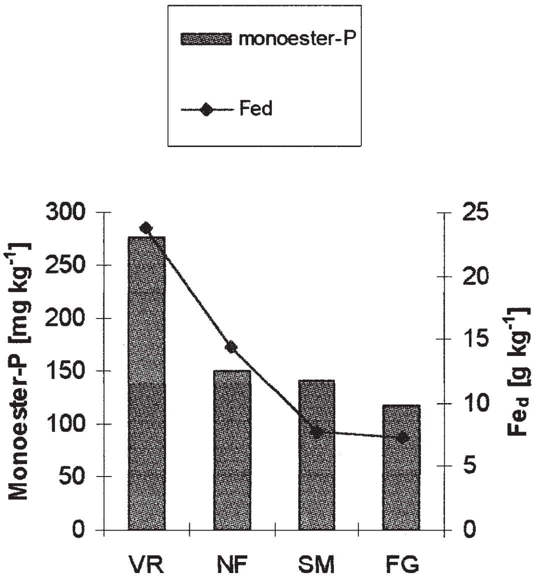

Figure 2. Monoester-P and free Fe contents (Fe$_d$) in the unfertilized forest soils studied, at 0–10 cm depth. (VR, Villasrubias; NF, Navasfrías; SM, San Martín; FG, Fuenteguinaldo).

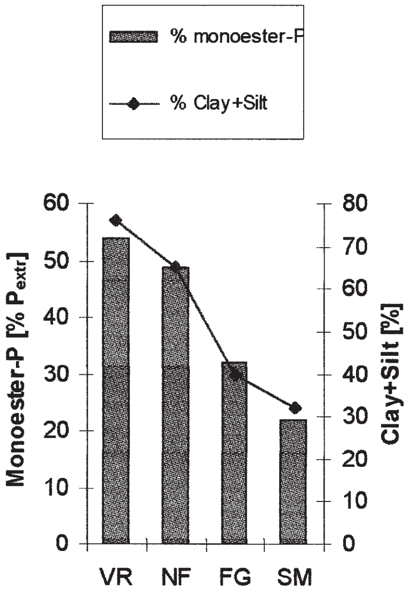

Figure 3. Relative proportions of monoester-P respect to alkaline P extracted and the percentage of silt plus clay in the unfertilized forest soils studied, at 0–10 cm depth. (VR, Villasrubias; NF, Navasfrías; FG, Fuenteguinaldo; SM, San Martín).

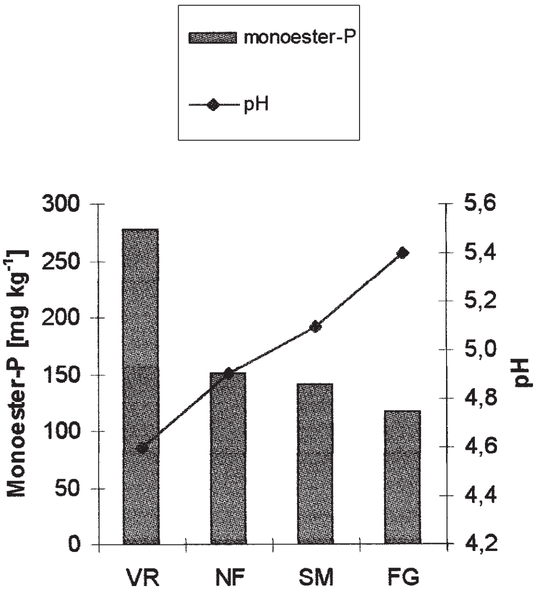

Figure 4. Monoester-P contents and soil pH in the unfertilized forest soils studied, at 0–10 cm depth. (VR, Villasrubias; NF, Navasfrías; SM, San Martín; FG, Fuenteguinaldo).

forms of Fe (Fe$_d$, *figure 2*), with the percentage of silt plus clay (*figure 3*), and negative with the pH (*figure 4*), indicating a stabilization of monoester-P with fine fractions of soil and sesquioxides. The high charge density of monoesters (e.g., inositol phosphates) allows rapid adsorption on soil minerals, and extensive interaction with sesquioxides that protect free inositols from degradation [28].

Diester-P comprising sugar-diester P, phospholipids, and DNA represented between 2 and 17% of NaOH-extractable P (*table IV*).

Diester-P have low charge densities and their phosphate groups are shielded from ionic interactions. This leaves them accessible to microbial or enzymatic attack in the soil environment, and explains the small proportions present in the investigated soils [28], although higher in NF and VR soils than in FG and SM. Guggenberger et al. [9] showed that alkali-extractable diester-P represents a pool of organic phosphorus that is readily available for microbial mineralization. Persistence and accumulation of diester-P has been shown to occur in soils where microbial activity is restrained due to acidity, waterlogging, or low temperature [16]. So, literature data show that in acid or wet soils diester-P represents between 10% and 36% of alkali-extractable P [16], while in neutral, well-drained soils the percentage of phospho-diesters is frequently <10% [4]. The higher percentages of alkali-extractable diester-P (including sugar-diester P) obtained in the soils over schists than those over granites reflect relatively lower soil microbial activity probably due to a lower drainage, lower soil pH, and higher free Al concentrations (*table II*). Stabilization of microbial metabolites by association with clay minerals and oxides and hydroxides [21] may protect the diester-P in the clay and silt fractions from mineralization and explain the higher diester proportion observed in VR and NF soils than in SM and FG (*table IV*) with higher amounts of these fine fractions (*table II*, *figure 3*) and, furthermore, VR soil is characterized by waterlogging in winter. Condron et al. [5] applied ^{31}P-NMR to cultivated and uncultivated soils under different environmental conditions and concluded that decomposition of diester-P was limited by waterlogging in subhumid climate. Tate and Newman [29] studied ^{31}P-NMR spectra of alkaline extracts from soils of a tussock grassland climosequence in New Zealand and also found a strong positive correlation between MAP and diester-P.

The different P species identified by ^{31}P-NMR spectroscopy can be characterized with regard to microbial degradability [13]. An index of mineralization capability is the ratio of monoester to diester-P, which ranged between 3 and 17 (*table IV*) in the studied soils. Ratios given in the literature vary between 1 and 20 [5, 10, 11]. The major P$_0$ structure in microorganisms is diester-P, whereas plants contribute primarily to monoester-P in soil [20, 27]. In the present study the plots with lowest plant production (NF, VR, *table I*), but with the highest SOM content, showed a consequently higher monoester-P concentration in soils than the other plots (FG, SM, *table IV*).

A decrease of the ratio of monoester to diester P with the increase of MAP was observed (*figure 5*). Several authors have also found that microbial activity increases with increasing MAP and soil moisture content, if drainage is good enough [6, 8, 17]; Sumann et al. [27] have reported that MAP also affects soil P$_0$ composition by increasing the proportion of microbial derived compounds.

A decrease of the ratio of monoester to diester-P (increase of microbial activity) was observed with increasing C/N ratio (*figure 6*). It is known that the quality of SOM decreases when the C/N ratio increases. Schneider [23] found for these forest soils that an increase of the rainfall causes a decrease of the quality of SOM as a consequence of decreasing the pH and increasing of free Al. Then, at least two factors, MAP and SOM quality affecting the microbial activity and the ratio monoester to diester-P.

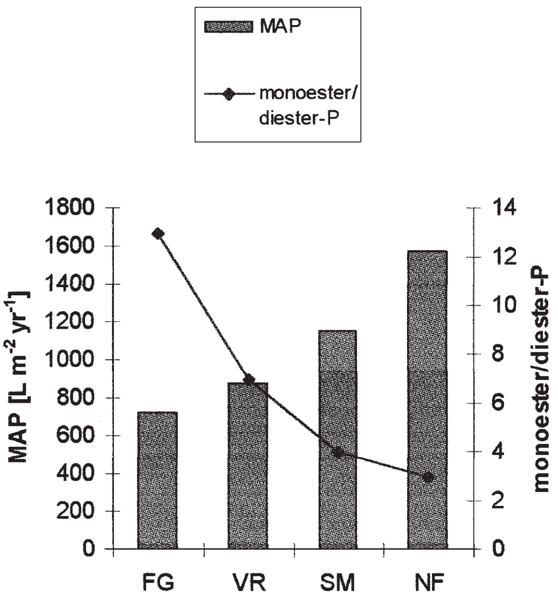

Figure 5. Monoester to diester-P ratio and mean annual precipitation (MAP) in the unfertilized forest soils studied, at 0–10 cm depth. (FG, Fuenteguinaldo; VR, Villasrubias; SM, San Martín; NF, Navasfrías).

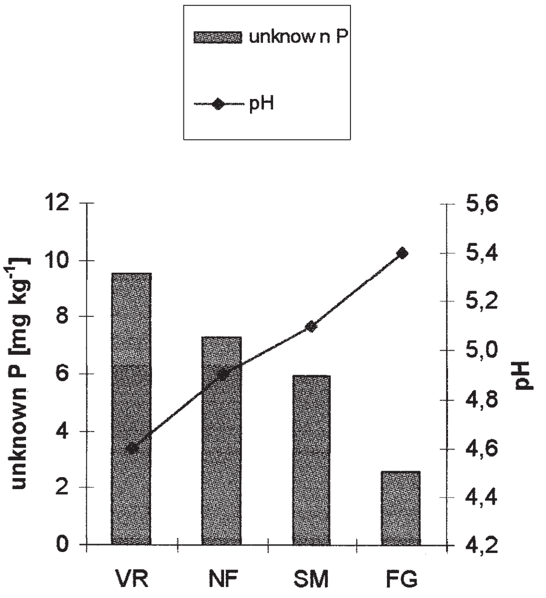

Figure 7. Unknown P and pH in the unfertilized forest soils studied, at 0–10 cm depth. (FG, Fuenteguinaldo; VR, Villasrubias; SM, San Martín; NF, Navasfrías).

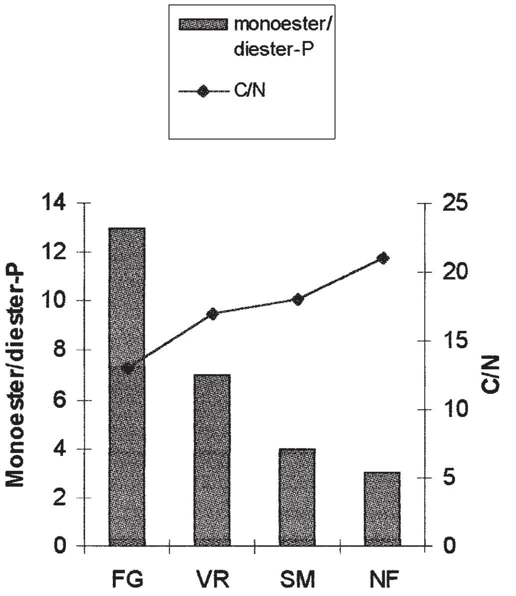

Figure 6. Monoester to diester-P ratio and carbon-to-nitrogen (C/N) ratios in the unfertilized forest soils studied, at 0–10 cm depth. (FG, Fuenteguinaldo VR, Villasrubias; SM, San Martín; NF, Navasfrías).

The concentration of the unknown P structures increased with decreasing soil pH (*figure 7*). Pyrophosphate was found only in small amounts in all investigated samples, comprising a maximum of 3% of NaOH-extractable P (*table IV*). According to Condron et al. [6], pyrophosphate can originate from hydrolysed organic esters. Polyphosphates were either not detected or formed in traces only.

The relative contribution of orthophosphate P ranged from 22 to 68% of NaOH-extractable P (*table IV*). The soils over granite showed percentages of orthophosphate P higher than 60% and the soils over schist only about 30% in relation to P_{TNaOH}.

3.2. Effects of fertilization on the phosphorus forms

The ^{31}P-NMR spectra revealed the same forms of P in non-fertilized and P-fertilized soils (*figure 1*).

As can be seen in *table IV* and *table V*, in FG and VR soils, the concentrations of P_i forms in fertilized soils were lower than in non-fertilized soils. However, in NF and SM soils the concentrations of P_i forms increased with P-fertilization.

Table V. P forms in the alkaline extracts of the P-fertilized forest soils (mg kg^{-1}) at 0–10 cm depth. Relative contribution (%) of each P species to the total NaOH-extractable P in brackets.

Plots[a]	Orthoph[b]	Monoester	Teichoic[c]	Diester	Pyroph[d]	Unknown	Mono/Dies[e]
FG	180 (44)	159 (39)	19 (5)	23 (6)	6 (1)	23 (6)	7
VR	151 (25)	318 (51)	29 (5)	75 (12)	18 (3)	21 (3)	4
NF	154 (41)	156 (41)	16 (4)	49 (13)	2 (1)	Tr[f]	3
SM	538 (71)	147 (19)	18 (2)	35 (5)	7 (1)	10 (1)	4

Notes: [a] FG, Fuenteguinaldo; VR, Villasrubias; NF, Navasfrías; SM, San Martín; [b] orthophosphate-P; [c] teichoic acid-P; [d] pyrophosphate-P; [e] monoester to diester-P ratio, [f] traces.

4. CONCLUSIONS

Edaphic properties and MAP influenced the composition of soil P_o in A_h horizons of forest soils of the Western Central Spain. ^{31}P-NMR spectroscopy showed high proportions of monoester-P and relatively high proportions of diester-P, reflecting a limited microbial activity in the soil studied due to the acid soil pH and the high content of free Al in these forest soils.

Acknowledgements: The authors wish to thank the “Junta de Castilla y León” for allowing them the use of the forest stands and to the European Union (MEDCOP/AIR and CAST/ENVIRONMENT Programs) and the C.I.C.Y.T. Spanish Fund for financial support. A fellowship of the Spanish Government enabled Dr. M.B. Turrión to participate in the Project. English editing was done by B.C. Knowles.

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