Characterization of soil phosphorus in a fire-affected forest *Cambisol* by chemical extractions and ^{31}P-NMR spectroscopy analysis

María-Belén Turrion [a,b,*], Francisco Lafuente [a], María-José Aroca [a], Olga López [a], Rafael Mulas [a], Cesar Ruipérez [a,b]

[a] *Area of Soil Science and Soil Chemistry, E. T. S. II. AA., University of Valladolid, Avda. de Madrid 54, Palencia 34004, Spain*
[b] *Sustainable Forest Management Institute University of Valladolid-INIA, ETS Ingenierías Agrarias, University of Valladolid, Avda. Madrid 44, 34071, Palencia, Spain*

ARTICLE INFO

ABSTRACT

This study was conducted to investigate the long-term effects of fire on soil phosphorus (P) and to determine the efficiency of different procedures in extracting soil P forms. Different P forms were determined: labile forms (Olsen-P, Bray-P, and P extracted by anion exchange membranes: AEM-P); moderately labile inorganic and organic P, obtained by NaOH-EDTA extraction after removing the AEM-P fraction; and total organic and inorganic soil P. ^{31}P-NMR spectroscopy was used to characterize the structure of alkali-soluble P forms (orthophosphate, monoester, pyrophosphate, and DNA). The studied area was a *Pinus pinaster* forest located at Arenas de San Pedro (southern Avila, Spain). The soils were *Dystric Cambisols* over granites. Soil samples were collected at 0–2 cm, 2–5 cm, and 10–15 cm depths, two years after a fire in the burned area and in an adjacent unburned forest area. Fire increased the total N, organic C, total P, and organic and inorganic P content in the surface soil layer. In burned soil, the P extracted by the sequential procedure (AEM and NaOH + EDTA) was about 95% of the total P. Bray extraction revealed a fire-induced increase in the sorption surfaces. Analysis by chemical methods overestimated the organic P fraction in the EDTA–NaOH extract in comparison with the determination by ignition procedure. This overestimation was more important in the burned than unburned soil samples, probably due to humification promoted by burning, which increased P sorption by soil particles. The fire-induced changes on the structure of alkali-soluble P were an increase in orthophosphate-P and a decrease in monoester-P and DNA-P.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Forest wild fires currently constitute one of the most serious environmental problems affecting semiarid areas. Forest fires can be extremely destructive of vegetation cover and soil, thereby interfering with forest ecosystem functions, such as water supply maintenance, protection against soil erosion, and nutrient accumulation. During a forest fire, significant rearrangement of soil organic matter (SOM) occurs and refractory and oxidation-resistant SOM of considerable residence time is formed (Schulze et al., 2000). In Mediterranean regions, where extreme climatic conditions alternate throughout the year, the relative importance of abiotic constraints (such as fire and irreversible dehydration favored by intense solar radiation and extreme drying cycles) is an important factor affecting the formation of stable SOM (González-Pérez et al., 2004).

Phosphorus is commonly a limiting nutrient for plant growth in many soils (McDowell and Stewart, 2006), but the effects of fire on the P cycle are poorly known (Debano and Klopatek, 1988; Saá et al., 1993). Phosphorus losses in soluble and particulate forms can increase from organic matter mineralization, mineral fraction weathering, and fire-induced soil erosion (Saá et al., 1998). The available P in surface horizons can rise immediately after fire (Debano and Klopatek, 1988), but these are short-term increases that produce long-term losses, thus reducing forest productivity (Saá et al., 1993; Romanyá et al., 1994; Cade-Menun et al., 2000). Most studies on burning have investigated changes in available P. Little is known about the effect of fire on other P forms and the relative changes in P levels after burning (Cade-Menun et al., 2000).

Organic P (P_o) is an important P source for plants in both natural and managed environments (Turrión et al., 2007). Despite the importance of soil P_o, its chemical nature and dynamics remain poorly understood; this is partly due to analytical limitations, which are mostly related to the fact that P_o must be extracted from soil before being quantified and identified. The use of ^{31}P nuclear magnetic resonance (^{31}P-NMR) spectroscopy has allowed the influence of cultivation and fertilization on structural soil P_o composition to be determined, screening P_o forms in different soil types and ecosystems (Zech et al., 1985, 1987), studying transformations due to land use changes in different environments (Condron et al., 1990;

* Corresponding author. Area of Soil Science and Soil Chemistry, E. T. S. II. AA., University of Valladolid, Avda. de Madrid 54, Palencia 34004, Spain. Tel.: +34 979 108331; fax: +34 979 108301.

E-mail address: bturrion@agro.uva.es (M.-B. Turrión).

Guggenberger et al., 1996; Turrión et al., 2000; Solomon et al., 2002; Cardoso et al., 2003), and studying the effect of temperature and precipitation gradients on soil P_o (Turrión et al., 2001; Makarov et al., 2002; McDowell and Stewart 2006; Turrión et al., 2008). Although ^{31}P-NMR spectroscopy is a powerful tool for assessing the structural composition of soil P_o, there are few published studies showing ^{31}P-NMR spectra of forest soils following burning (Cade-Menun et al., 2000).

The objectives of this study were to characterize the soil P in a fire-affected forest Cambisol and to determine the capacity of different procedures to extract various soil P forms from burned and unburned soils.

2. Material and methods

2.1. Experimental site

This study was performed in a valley region located on the southern face of the Gredos Mountain foothills (Sistema Central Mountain Range, southern Avila province, central Spain), at the spot known as "El Berrocal" (Arenas de San Pedro). The mean altitude is 750 m, slope is about 5–20%, and exposure is mainly NW. According to its position and altitude, this sampling area is expected to have a dry, cold continental climate. Nevertheless, its proximity to the Gredos Mountains modifies weather conditions. The mountain range acts as a barrier against cold, dry northern winds, and it generates a humid microclimate with mild temperatures. Therefore, mean annual temperature and rainfall are 14.2 °C and 1510 mm, respectively. Geological parent materials are granites, schist and quartzite. Soils are Dystric Cambisols characterized by poorly developed profiles. Prevailing texture is sandy. Initial SOM classification is mor–moder.

The study area was located in the "El Berrocal" forest, which was burned in September 1999. The fire was active for 5 h, affecting 20 ha. Vegetation was originally an old growth stand of maritime pine (Pinus pinaster Ait.). The fire severity can be classified as moderate (Pausas et al., 2003). The fire consumed litter layer, but did not alter the surface of the mineral soil visibly. After the fire, pine regeneration and crimson spot rockrose (Cistus ladanifer L.) brushes settled on the area.

2.2. Soil sampling

Soil was sampled two years after fire. Three depth levels were considered in the experimental design (0–2 cm, 2–5 cm, and 10–15 cm) and sampled at four sampling points randomly selected along the experimental area in the burned area and the adjacent unburned one. Every sample was made up of 10 sub-samples taken randomly around each sampling point.

2.3. Soil analysis

Soils were air-dried, crushed, and sieved (<2 mm). Soil pH was measured in a 1:2.5 soil:water suspension using a glass electrode. Organic C (C_o) and total N were determined using a LECO CHN 2000 Analyzer.

Total phosphorus (P_T) was determined after digestion with perchloric acid (Kuo, 1996). Total organic P (P_{oT}) was determined by the Saunders and Williams (1955) procedure. Both Olsen and Bray procedures were those described by Kuo (1996), the latter using Bray 1 reagent (1:7 w:v; extraction time, 1 min). Phosphorus in extracts was determined by the ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962).

2.4. ^{31}P-NMR measurements

Phosphorus-31NMR was used to characterize the structural composition of alkali-soluble P. We used a modified version of the extraction

procedure of Cade-Menun and Preston (1996). In our modification two strips (9 × 62 mm) of no. 55164 BDH anion exchange membrane (AEM) in HCO_3^- form were shaken for 16 h in centrifuge tubes containing 3 g of soil and 30 ml of deionized water. Three replicates of each soil were extracted simultaneously. The membrane extraction removed readily extractable inorganic P (AEM-P). The resin procedure was repeated with one strip, after which the samples were centrifuged (11,850 × g) and the supernatant discarded. The AEM were desorbed in 0.3 M HCl (Turrión et al., 1997), and the P concentration (AEM-P) was measured colorimetrically. The remaining pellets were treated with 30 ml of 0.1 M EDTA + 0.5 M NaOH (henceforth, EDTA–NaOH) and shaken for 16 h. Subsequently, the samples were centrifuged (11,850 × g). A 25-ml supernatant aliquot was transferred to a plastic snap-cap bottle with 3 cm^3 of Chelex-X100, shaken for 6 h and filtered (Whatman no. 42). The filtrates from the three replicates were mixed, and an aliquot (45 ml) was freeze-dried. The remaining solution was analyzed for total and inorganic EDTA–NaOH-P. Total P in the EDTA–NaOH extract was determined after digestion with sodium persulfate in an acid medium; EDTA–NaOH-P_i was measured directly in the extracts after removing organic matter by acidification and centrifugation (Tiessen and Moir, 1993). Finally, P was analyzed in all the extracts by the method of Murphy and Riley (1962). P_o was estimated as the difference between EDTA–NaOH-P_T and EDTA–NaOH-P_i. Each freeze-dried extract was dissolved into ice in 2 ml D_2O. The ^{31}P-NMR spectra were recorded with a Bruker DPX-300 spectrometer operating at 121.49 MHz using a 90-degree pulse length, an acquisition time of 0.96 s and a total accumulation time of 2 h and 3800 scans. Spectra were recorded with a line-broadening of 20 Hz.

In order to obtain the P to paramagnetic elements (Fe and Mn) quotient in the resuspended solution, Fe and Mn were analysed by atomic absorption spectroscopy after partial neutralization and adequate dilution and total P was determined after digestion with sodium persulfate in an acid medium. The mean value for this quotient was 3.5. The relaxation delay time used was 2 s, which was higher than the spin-lattice relaxation times obtained from the linear regressions of McDowell et al. (2006) for this quotient (0.9 s for monoesters, diesters and pyrophosphate and 1.3 s for orthophosphate). This delay time value was considered a reasonable compromise solution between excessively long spectrophotometer time and the better precision of the data.

Quantification was achieved by integrating peaks, using spectral deconvolution software. Spectrum interpretations (Fig. 1) were based on literature assignments (Turner et al., 2003) and standards (orthophosphate, glucose-6-phosphate and pyrophosphate).

2.5. Statistical analysis

A two fixed-factor factorial experimental design was considered; the first factor was the fire effect on soil properties (burned and unburned), while the second factor was the soil depth effect, with three samplings along the profile: 0–2 cm, 2–5 cm and 10–15 cm. The analysis of variance (ANOVA) model was: $X_{ijk} = \mu + F_i + D_j + FD_{ij} + e_{ijz}$, where X_{ijk} was the analyzed soil property, μ was the overall mean, F_i was the main effect of Fire, D_j was the main effect of Soil Depth, FD_{ij} was the interaction between Fire and Soil Depth, and e_{ijz} was the experimental error. When the main depth effect turned out to be significant, differences among soil depth levels were evaluated by the Bonferroni Test. SPSS 14.0 software was used to implement statistical analysis.

3. Results and discussion

3.1. Fire effect on pH, total organic C, N, and C/N ratio

No significant effects of fire and depth were observed in soil pH, but the interaction of fire and depth effects was significant (Table 1). Fire increased the pH of the surface samples due to the so-called "ash
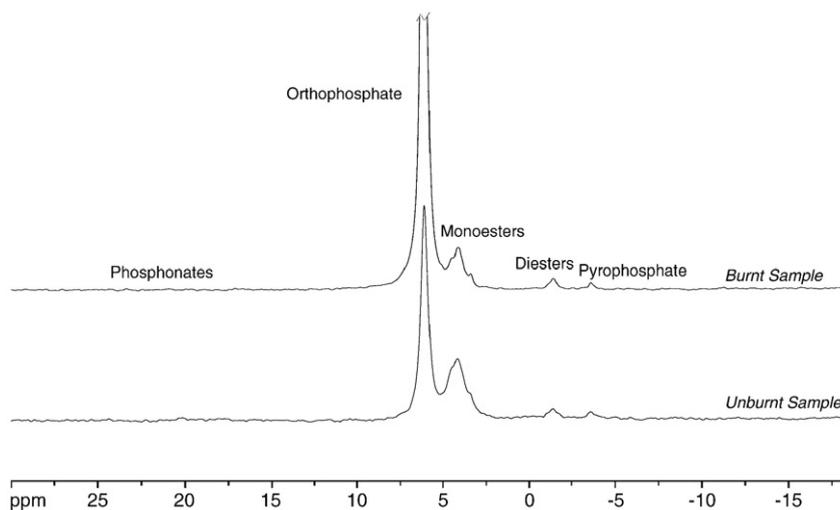
Fig. 1. ^{31}P-NMR spectra of EDTA–NaOH soil extract from the burned and the unburned soils.

bed effect" of fire on soils (Cade-Menun et al., 2000); however, this effect was not observed in the subsurface layers.

Total organic carbon concentration (C_o) in soil decreased significantly with depth (Tables 1 and 2). However, although C_o concentrations were lower in burned than unburned soils (Table 1), the fire effect and the fire-depth interaction were not significant (Table 1). Macadam (1987) and Cade-Menun et al. (2000) reported a decrease in total soil carbon after fire.

Total nitrogen concentrations (N_t) were lower in burned than unburned soils at all the depths studied, but only the depth effect was significant (Table 1). Contrasting reports are present in the literature, as both decreases (Macadam, 1987; Cade-Menun et al., 2000) and increases (Mangas et al., 1992) in N_t contents after fire can be found. Nitrogen can be volatilized during fire and the amount of N lost depends on fire intensity.

The burned soil showed significant higher C/N ratio values than the unburned soil, except in the surface layer, with significant interaction between fire and depth effects (Table 1). The depth effect was also significant, this ratio being lower in surface than deeper soil layers, in the burned soil. The C/N ratios of soil after burning are usually lower than in the unburned soil (Romanyá et al., 1994; Cade-Menun et al., 2000).

3.2. Fire effect on total P and organic and inorganic P

Total P concentrations of control soil ranged from 423 to 516 mg kg^{-1}, whereas they ranged from 566 to 632 mg kg^{-1} in burned soil (Table 1). No significant differences were observed among the depths studied (Table 1). Higher total P concentrations were found in the burned soil than in unburned at all the depths studied. It is probable that no P volatilization occurred during the fire and that litter P was incorporated into the profile because of the sandy soil texture and high rainfall (1510 mm). Debano and Klopatek (1988) have indicated that ash P translocation in soil profiles depends on soil texture.

In the unburned soil, total inorganic P (P_{iT}) represented around 51% of total P, increasing in the burned soil to 78%. The concentration of P_{iT} was significantly higher in the burned soil than in unburned, probably due to the fire effect on the residual P_i solubilization, on P_o mineralization, and on ash input.

Total organic P (P_{oT}) ranged from 175 to 284 mg kg^{-1} in the unburned soil and from 117 to 131 mg kg^{-1} in the burned soil

Table 1

Contents of pH, total organic C, total N, C/N ratio, total P, total organic P, and total inorganic P, and pH values of the burned and unburned soils. Relative contribution [%] of inorganic and organic P to total P in brackets. ANOVA significance levels of the effects of fire, depth, and fire and depth interaction.

	pH	C_o	N	C/N	P_T	P_{iT}	P_{oT}
		[mg g^{-1}]			[mg kg^{-1}]		
Unburned							
0–2 cm	5.1	114.6	4.17	27.5	439	155 (35)	284 (65)
2–5 cm	5.5	57.7	2.03	28.4	423	219 (52)	204 (48)
10–15 cm	5.4	34.9	1.20	29.1	516	341 (66)	175 (34)
Burned							
0–2 cm	5.5	64.9	2.11	30.8	566	437 (75)	129 (25)
2–5 cm	5.3	43.7	1.25	35.0	599	468 (78)	131 (22)
10–15 cm	5.2	25.0	0.64	39.1	632	515 (81)	117 (19)
ANOVA							
Fire	ns	ns	ns	**	*	**	**
Depth	ns	*	*	*	ns	*	ns
Fire × depth	*	ns	ns	*	ns	ns	ns

P_{iT} and P_{oT}, represent the total inorganic P and total organic P of soil, respectively, measured by Saunders and Williams (1955) procedure; P_T is the total P measured by digestion with perchloric acid. Significance levels: * ($p<0.05$), ** ($p<0.01$), ns: not significant.

Table 2

Concentrations of different P forms in burned and unburned soils [mg P kg^{-1}]. Relative contribution [%] of AEM-P and EDTA–NaOH-P_T to total P, and of EDTA–NaOH-P_o to total P_o, are shown in brackets after their concentrations. ANOVA significance levels of the fire effects, depth, and fire and depth interaction.

	AEM-P	Olsen-P	Bray-P	EDTA–NaOH-P_T	EDTA–NaOH-P_i	EDTA–NaOH-P_o
	[mg kg^{-1}]					
Unburned						
0–2 cm	27.3 (6.2)	22.6	27.7	304 (69)	116	188 (66)
2–5 cm	23.4 (5.5)	22.5	47.7	302 (71)	147	155 (76)
10–15 cm	28.2 (5.5)	43.6	84.0	453 (88)	305	148 (85)
Burned						
0–2 cm	39.8 (7.0)	59.3	150.8	456 (81)	295	161 (125)
2–5 cm	39.1 (6.5)	83.8	151.2	521 (87)	328	193 (147)
10–15 cm	18.7 (3.0)	70.5	132.0	556 (88)	364	192 (164)
ANOVA						
Fire	*	**	**	**	**	ns
Depth	*	*	*	**	*	ns
Fire × depth	*	*	**	*	**	ns

Significance levels: * ($p<0.05$), ** ($p<0.01$), ns: not significant.

(Table 1). This decrease probably depended on fire-induced P_o mineralization in surface soil samples.

3.3. Extractable P

The percentage of sequential extractable P using anion exchange membranes (AEM-P) and EDTA–NaOH extraction (EDTA–NaOH-P) was significantly higher in the burned soil than in the unburned, mainly at 0–2 cm and 2–5 cm (incremented from 76.5% in the unburned soil to 93.5% in the burned soil, at 2–5 cm). These results indicate that only a small amount of P remains in soil after this sequential extraction, particularly in the surface of the burned soil, probably as the result of a solubilization of recalcitrant P_i forms by fire. Our results confirm what Cade-Menun et al. (2000) and Turner et al. (2005) suggested, that most of total P is recovered by EDTA–NaOH extraction for soils.

The burned soil showed significantly higher values of inorganic EDTA–NaOH-P than the unburned soil (Table 2). This was probably due to the mineralization effect of the fire and to P_i translocation in the 0–5 cm soil layer from the litter layer.

In the unburned soil, the P_o extracted by EDTA–NaOH accounted for 66, 76 and 85% of P_{oT} in the 0–2, 2–5, and 10–15 cm layers, respectively. In the burned soil, EDTA–NaOH extraction gave more P_o than the ignition method (Tables 1 and 2); this anomalous behavior in burned soil may depend on underestimation of P_{oT} by the ignition method or overestimation of P_o during EDTA–NaOH extraction. As there are no direct methods to determine P_{oT} in soil, it is difficult to verify which hypothesis was right. In the ignition procedure, P_{oT} is calculated as the difference in acid-extractable P between ignited and unignited samples. This procedure may overestimate P_{oT} in most soils by increasing the solubility of P_i minerals by ignition and these minerals may be incompletely extracted by acid extraction in unignited samples (Dormaar and Webster, 1964). However, underestimation by the ignition method can occur in soils high in SOM because P_{oT} may be hydrolyzed to phosphate during acid extraction of unignited samples (Saunders and Williams, 1955). Because our soil contained less than 65 mg C g^{-1} soil, we can exclude P_{oT} underestimation determined by the ignition method, as has been suggested for non-organic soils (Turner et al., 2005, 2006). On the other hand, when P_o is determined in an alkaline extract, the most serious source of errors stems from the interaction of P_i with humic substances (Guggenberger et al., 1996; Turner et al., 2005), which prevents the detection of phosphate by the molybdate colorimetric method. The quantitative importance of this error is unclear, and depends on soil type (Turner et al., 2005). This overestimation of alkaline extracted P_o may have occurred in both burned and unburned soils; however, it did occur only in the burned soil and we are unable to explain such an observation.

During a forest fire, organic forms become rearranged considerably, and resistant organic forms are formed (Schulze et al., 2000). The concentrations of labile P forms (AEM-P, Olsen-P and Bray-P) were significantly higher in the burned soil than in the unburned (Table 2), with the greatest effects of fire in the surface soil layers (0–2 and 2–5 cm). Giardina and Rhoades (2001) found a significant increase in NaHCO$_3$ extractable P and NaOH extractable P one year after burning, but no significant effect on anion exchange resin P. The labile P forms in the depths studied behaved differently in the burned soil than in the unburned (Table 2). In the unburned soil, both Olsen-P and Bray-P increased with depth, but this did not occur for AEM-P; in the burned soil, Bray-P did not vary with depth, whereas Olsen-P decreased in the surface layer and the AEM-P decreased in the deeper layers (Fig. 2). In the burned soil, an increase in the labile P at the surface may be expected due to the oxidation of organic forms by the fire, as suggested by Giardina and Rhoades (2001); this new labile P was probably translocated.

The Bray-P concentrations in the 0–2 cm layer were 5 times higher in the burned soil than in the unburned, whereas the Olsen-P were only 2.5 times higher. Both procedures supply labile P forms and both are adequate to the pH range of the soils studied. This difference can be due to the solubilization of ash residues and the liberation of P adsorbed by Fe and Al by the fluoride present in the Bray 1 extractant. In fact, P adsorption can increase with burning (Romanyá et al., 1994).

3.4. Liquid-state ^{31}P-NMR spectroscopy determination

Percentages of P_i and P_o in EDTA–NaOH extracts measured by chemical procedure and by ^{31}P-NMR spectroscopy are shown in Table 3. The proportion of P_o determined chemically was always higher than that determined by ^{31}P-NMR spectroscopy, which seems to support the overestimation. This overestimation was higher in the burned soil (average of 25%) than in unburned (8% on average). Turner et al. (2005) stated that problems in P_o detection can be overcome by solution ^{31}P-NMR spectroscopy, which avoids the formation of complexes between phosphate and humic substances and allows quantifying P_o from condensed inorganic phosphates separately. However, P_o may be hydrolyzed to P_i during re-dissolution of freeze-dried extracts prior to NMR spectroscopy (Guggenberger et al., 1996; Cade-Menun et al., 2006). In order to reduce this hydrolysis, we carried out re-dissolution into ice. The colorimetric method used in the chemical determination does not detect condensed inorganic phosphates, so the quantity of P_i determined by colorimetric method is underestimated by the proportion of P as pyrophosphate and polyphosphate, although this amount in our samples was small (mean of 2% of total EDTA–NaOH-P, Table 3).

The chemical shift of the inorganic orthophosphate signal in ^{31}P-NMR spectra occurred between 5.97 and 6.18 ppm (Fig. 1), which is consistent with the peak assignment indicated by Turner et al. (2003) in soil EDTA–NaOH extracts. Inorganic orthophosphate represented between 53% (at 2–5 cm) and 74% (at 10–15 cm) of total P extracted by NaOH-EDTA from the unburned soil, and between 76% (at 10–15 cm) and 84% (at 0–2 cm) for the burned soil. Orthophosphate concentrations were significantly higher in the burned soil than in the unburned (Table 4) due to fire-induced P_o mineralization. However, the behavior of the orthophosphate concentrations throughout the depths was significantly different in the burned and the unburned soils (Table 4). In the unburned soil, orthophosphate concentration increased significantly with depth, while in the burned soil the increase was only significant in the first soil layer. Zech et al. (1987) stated that cutting and burning Pinus mugo Turra and establishing pasture did not significantly influence the patterns of P forms in ^{31}P-NMR spectra of surface soil horizons. Adams and Byrne (1989) reported higher concentrations of P_i forms in surface soils of recently burned Eucalyptus diversicolor F. Muell forests than in unburned soil, the increase depending on the time after burning; however, they did not examine P forms along the soil profile. Cade-Menun et al. (2000) compared P forms of forest floor and soils from clear-cut cedar-hemlock burned forests with those from an undisturbed old growth forest. Both clearcutting and burning reduced the content of organic matter, and mineralized organic P to inorganic P, with a short-lived increase in plant available P.

Phosphate monoesters were the main functional class of P_o in unburned and burned soils, representing 83% and 91% of the P_o in the surface samples, respectively. Inositol phosphates, phosphoric esters of hexahydroxycyclohexane (inositol), are usually the dominant compounds in this fraction (Turner et al., 2003). Other phosphate monoesters present in small amounts in soil include sugar phosphates, phosphoproteins and mononucleotides (Turner et al., 2005). However, the complex signals from myo-inositol hexakisphosphate make it difficult to quantify these compounds by solution ^{31}P-NMR spectroscopy; for this reason, although spectral deconvolution
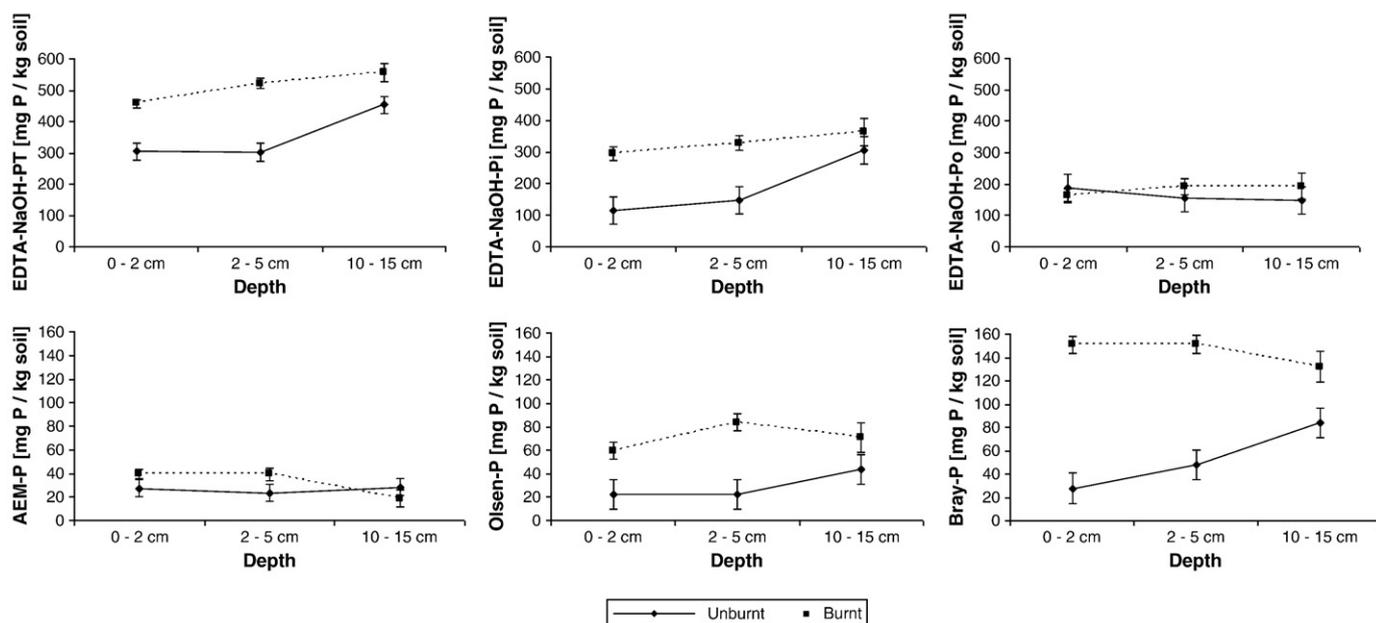
Fig. 2. Interaction plots and confidence Bonferroni intervals ($p < 0.05$) for EDTA–NaOH extractable total, organic and inorganic P and available P extracted by anion exchange membranes (AEM) and Olsen and Bray procedures.

software was used, it was not possible to discriminate among the various compounds in the orthophosphate monoester region. Recently, Doolette (2009) have developed a method of spiking to identify the peaks other than phytate in the monoester region. In the present study monoester-P concentration was significantly smaller in the burned soil than in the unburned, but the depth had no significant effect on their concentrations. Fire, as a strong mineralizing agent, probably converted the predominantly P_o forms of the forest soils studied into orthophosphate.

Phosphate diesters include nucleic acids (DNA and RNA), phospholipids, and teichoic acids. Phosphate diesters are the main input of P_o to soils, but typically constitute only a small fraction of soil P_o. This fraction in the EDTA–NaOH extract represented 6% of total P extracted from the superficial layer of the unburned soil and was not present in surface samples of the burned soil. Phosphate diesters usually constitute less than 10% of soil P_o, but higher proportions have been detected in some forest soils (Cade-Menun et al., 2000). Orthophosphate diesters dominate P_o inputs to soil from plants and microbes, but are quickly degraded and usually represent only a small proportion of soil P_o (Turner et al., 2005). NMR signals from the orthophosphate diesters are commonly grouped together as a single functional class of P_o, but various compounds can be discriminated (Turner et al., 2003). In our spectra, only signals from DNA

(-0.37 ppm) were clearly distinguished, whereas alkali stable phospholipids (1.5 to 1.8 ppm) were not observed. Signals between 0 and 1 ppm were previously assigned to teichoic acids (Condron et al., 1990; Turrión et al., 2001), present in the cell walls of gram-positive bacteria and also produced by some actinomycetes. However, teichoic acids were recently measured at 1.9 ppm in delipidized extracts of gram-positive bacteria (Makarov et al., 2002), suggesting that they did not contribute greatly to signals observed in the orthophosphate diester region of alkaline soil extracts (Turner et al., 2003). The fast degradation of some orthophosphate diesters in alkaline solutions is an unavoidable problem that affects the quantification and characterization of soil P_o (Turner et al., 2003), because alkaline solutions are needed to extract P_o from most soils. Some compounds such as RNA and phosphatidylcholine can become degraded, which represents an artifact of soil P_o analysis by current procedures (Turner et al., 2003; Cade-Menun et al., 2006). However, the fact that orthophosphate diesters in soil may be complexed with humic material limits their degradation in alkaline solutions; nevertheless, this seems unlikely, at least for RNA (Makarov et al., 2005).

The DNA-P concentrations were similar in all the depths studied in the unburned soil and there were no significant differences between burned and unburned soils except in the surface layer. It is widely accepted that an increase or decrease in the proportion of diester P in soils is associated with corresponding changes in microbial P compounds (Guggenberger et al., 1996). In contrast, easily mineralizable phosphate diesters or phosphonates in soils can accumulate when conditions for microbial decomposition become limited by climate, as suggested by Makarov et al. (2002), who hypothesized that most of soil DNA is probably present as part of extracellular DNA, adsorbed by clays, and protected against nuclease degradation (Pietramellara et al., 2009). Our results showed a decrease of DNA-P in the surface sample of the burned soil compared with the unburned soil, probably resulting from the decrease of microbial biomass by burning or from a decrease of plant residue. Microbial orthophosphate diesters can represent a considerable proportion of soil P_o (Turner et al., 2003), but Makarov et al. (2005) have suggested that care is needed when interpreting the results on P_o origins in soils of different climate, vegetation, land use, and so on, given that the proportion of diesters in microbial and plant material can change.

Table 3
Percentages of total P present as inorganic and organic P in EDTA–NaOH extracts determined by chemical analysis and ^{31}P NMR spectroscopy.

	Inorganic P		Organic P	
	Chem.	NMR	Chem.	NMR
Unburned				
0–2 cm	38	48	62	52
2–5 cm	49	56	51	44
10–15 cm	67	75	33	25
Burned				
0–2 cm	65	86	35	14
2–5 cm	63	81	37	19
10–15 cm	47	83	53	17

Table 4
Concentrations [mg P kg^{-1}] of different P forms measured as ^{31}P-NMR in burned and unburned soils. Relative percentages of total P present in each P species in brackets. ANOVA significance levels of the effects of fire, depth, and fire and depth interaction.

	Orthophosphate-P	Monoesters-P	Diesters (DNA)-P	Pyrophosphate-P	NMR-P$_o$	Diester/monoester
	[mg kg^{-1}]					
Unburned						
0–2 cm	161 (53)	118 (39)	16 (6)	9 (3)	134	0.13
2–5 cm	160 (53)	117 (39)	16 (5)	9 (3)	133	0.13
10–15 cm	373 (74)	100 (22)	13 (3)	6 (1)	112	0.13
Burned						
0–2 cm	366 (84)	62 (14)	0 (0)	6 (1)	62	0
2–5 cm	434 (81)	86 (17)	12 (2)	1 (1)	98	0.14
10–15 cm	458 (76)	73 (17)	20 (4)	5 (3)	93	0.28
ANOVA						
Fire	**	**	ns	*	*	ns
Depth	**	ns	ns	ns	ns	ns
Fire × effect	**	ns	ns	ns	ns	ns

Note: Significance levels: * ($p<0.05$), ** ($p<0.01$), ns: not significant.

Pyrophosphate signals occur between -3 and -6 ppm (Cade-Menun and Preston, 1996; Turner et al., 2003). In this study, the signal was observed around -3.5 ppm, and the contribution of pyrophosphate was demonstrated by adding a pyrophosphate standard. Pyrophosphate-P was significantly higher in the unburned soil than in the burned, but no significant differences in pyrophosphate concentrations were observed with depth. According to Makarov et al. (2005), high proportions of pyrophosphate in NaOH extracts from soils can indicate significant contributions by fungal phosphorus compounds. McDowell and Stewart (2006) obtained greater concentrations of pyrophosphate in pasture or native soils than in forest soils, and they suggested that pyrophosphate may undergo enzymatic hydrolysis to produce orthophosphate. Fire can denaturalize enzymes (Saá et al., 1998) and thus pyrophosphate hydrolysis cannot occur in burned soil, increasing its concentration. However, we observed a fire-induced decrease in pyrophosphate, which may depend on the recovery of soil microbial activity after fire.

Phosphonates were not found in the samples studied. The proportion of diester P, or the ratio of diester to monoester, is used to assess the relative lability of soil P$_o$ or the P$_o$ turnover rate. However, Makarov et al. (2005) indicated that this index should be used with caution for interpreting the origins of soil P$_o$. In our study, the diester to monoester ratio was constant along the unburned soil profile studied, whereas it increased with depth in the burned soil. No significant differences in the ratio between burned and unburned soils were observed.

4. Conclusions

Our results indicate that caution should be exercised when Po of EDTA–NaOH extracts is determined by the difference between extract P$_T$ and P$_i$, because this determination can be overestimated. This overestimation can be more important in the burned soil than in unburned; however, further research is necessary to verify it. The ^{31}P-NMR spectra revealed the same forms of P in the burned and unburned soils, but the P form concentrations were different: orthophosphate-P increased and monoester-P and DNA-P decreased. The unburned soil showed a higher proportion of pyrophosphate P, indicating a significant contribution by fungal compounds. The diester to monoester ratio was not sensitive to the fire effect.

References

Adams MA, Byrne LT. ^{31}P-NMR analysis of phosphorus compounds in extracts of surface soils from selected karri (*Eucalyptus diversicolor* F Muell) forest. Soil Biol Biochem 1989;21:523–8.
Cade-Menun BJ, Berch SM, Preston CM, Lavkulich LM. Phosphorus forms and related soil chemistry of podzolic soils on Northern Vancouver Island II. The effects of clear-cutting and burning. Can J For Res 2000;30:1726–41.
Cade-Menun BJ, Preston CM. A comparison of soil extraction procedures for ^{31}PNMR spectroscopy. Soil Sci 1996;161:770–85.
Cade-Menun BJ, Navaratnam JA, Waldbridge MR. Characterizing dissolved and particulate phosphorus in water with ^{31}P nuclear magnetic resonance spectroscopy. Environ Sci Technol 2006;40:7874–80.
Cardoso IM, Van der Meer P, Oenema O, Janssen BH, Kuyper TW. Analysis of phosphorus by ^{31}PNMR in oxisols under agroforestry and conventional coffee systems in Brazil. Geoderma 2003;112:51–70.
Condron LM, Frossard E, Tiessen H, Stewart JWB. Chemical nature of organic phosphorus in cultivated and uncultivated soils under different environmental conditions. J Soil Sci 1990;41:41–50.
Debano LF, Klopatek JM. Phosphorus dynamics of pinyon-juniper soil following simulated burning. Soil Sci Soc Am J 1988;52:271–7.
Doolette AL, Smernik RJ, Dougherty WJ. Spiking improved solution phosphorus-31 Nuclear Magnetic Resonance Identification of soil phosphorus compounds. Soil Sci Soc Am J 2009;73:919–27.
Dormaar JF, Webster GR. Losses inherent in ignition procedures for determining total organic phosphorus. Can J Soil Sci 1964;44:1–6.
Giardina CP, Rhoades CC. Clear cutting and burning affect nitrogen supply, phosphorus fractions and seedling growth in soil from Wyoming lodgepole pine forest. For Ecol Manage 2001;140:19–28.
González-Pérez JA, González-Vila FJ, Almendros G, Knicker H. The effect of fire on soil organic matter — a review. Environ Int 2004;30:855–70.
Guggenberger G, Christensen BT, Rubaek G, Zech W. Land-use and fertilization effects on P forms in two European soils: resin extraction and ^{31}P-NMR analysis. Eur J Soil Sci 1996;47:605–14.
Kuo S. Phosphorus. In: Sparks DL, editor. Methods of soil analysis part 3: chemical methods. Madison, WI: SSSA ASA; 1996. p. 869–920.
Macadam AM. Effects of broadcast slash burning on fuels and soil chemical properties in the sub-boreal spruce zone of central British Columbia. Can J For Res 1987;17:1577–84.
Makarov MI, Haumaier L, Zech W. Nature of soil organic phosphorus: an assessment of peak assignments in the diester region of ^{31}P NMR spectra. Soil Biol Biochem 2002;34:1467–77.
Makarov MI, Haumaier L, Zech W, Marfenina OE, Lysak LV. Can ^{31}P NMR spectroscopy be used to indicate the origins of soil organic phosphates? Soil Biol Biochem 2005;37:15–25.
Mangas VJ, Sánchez JR, Ortiz C. Effects of fire on runoff and erosion on Mediterranean forest soils in SE-Spain. Pirineos 1992;140:7-51.
McDowell RW, Stewart I. The phosphorus composition of contrasting soils in pastoral, native and forest management in Otago, New Zealand: sequential extraction and ^{31}PNMR. Geoderma 2006;130:176–89.

McDowell RW, Stewart I, Cade-Menun BJ. An examination of spin lattice relaxation times for analysis of soil and manure extracts by phophorus-31 nuclear magnetic resonance spectroscopy. J Env Quality 2006;35:293–302.

Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural water. Anal Chem Acta 1962;27:31–6.

Pausas JG, Ouadah N, Ferran A, Gimeno T, Vallejo R. Fire severity and seedling establishment in *Pinus halepensis* woodlands, Eastern Iberian Peninsula. Plant Ecol 2003;169:205–13.

Pietramellara G, Ascher J, Borgogni F, Ceccherini MT, Guerri G, Nannipieri P. Extracellular DNA in soil and sediment: fate and ecological relevance. Biol Fertil Soils 2009;45:219–35.

Romanyá J, Khanna PK, Raison RJ. Effects of slash burning on soil phosphorus fractions and sorption and desorption of phosphorus. For Ecol Manage 1994;65:89-103.

Saá A, Trasar-Cepeda MC, Carballas T. Soil P status and phosphomonoesterase activity of recently burnt and unburnt soil following laboratory incubation. Soil Biol Biochem 1998;30:419–28.

Saá A, Trasar-Cepeda MC, Gil-Sotres F, Carballas T. Changes in soil phosphorus and acid phosphatase activity immediately following forest fires. Soil Biol Biochem 1993;25: 1223–30.

Saunders WH, Williams EG. Observations on the determination of organic phosphorus in soils. J Soil Sci 1955;6:254–67.

Schulze ED, Wirth C, Heimann M. Managing forests after Kyoto. Science 2000;289: 2058–9.

Solomon D, Lehman J, Mamo T, Fritzsche F, Zech W. Phosphorus forms and dynamics as influenced by land use changes in the sub-humid Ethiopian highlands. Geoderma 2002;105:21–48.

Tiessen H, Moir JO. Characterization of available P by sequential extraction. In: Carter MR, editor. Soil sampling and methods of analysis. Can Soc Soil Sci.Boca Raton: Lewis Publishers; 1993. p. 75–86.

Turner BL, Mahieu N, Condron LM. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH–EDTA extracts. Soil Sci Soc Am J 2003;67:497–510.

Turner BL, Cade-Menun BJ, Condron LM, Newman S. Extraction of soil organic phosphorus. Talanta 2005;66:294–306.

Turner BL, Newman S, Reddy KR. Overestimation of organic phosphorus in wetland soils by alkaline extraction and molybdate colorimetry. Environ Sci Technol 2006;40:3349–54.

Turrión MB, Gallardo JF, González MI. Nutrient availability in forest soils as measured with anion exchange membranes. Geomicrobiol J 1997;14:51–64.

Turrión MB, Glaser B, Solomon D, Ni A, Zech W. Effects of deforestation on phosphorus pools in mountain soils of the Alay Range, Khyrgyzia. Biol Fertil Soil 2000;31: 134–42.

Turrión MB, Gallardo JF, Haumaier L, González MI, Zech W. ^{31}P NMR characterization of phosphorus fractions in natural and fertilized forest soils. Ann For Sci 2001;58: 89–98.

Turrión MB, López O, Lafuente F, Mulas R, Ruipérez C, Puyo A. Soil phosphorus forms as quality indicators of soils under different vegetation covers. Sci Total Environ 2007;378:195–8.

Turrión MB, Schneider K, Gallardo JF. Soil P availability along a catena located at the Sierra de Gata Mountains, Western Central Spain. For Ecol Manage 2008;255:3254–62.

Zech W, Alt HG, Zucker A, Kögel I. ^{31}P-NMR-spectroscopic investigations of NaOH extracts from soils with different land use in Yucatan (Mexico). Z Pflanzenernaehr Bodenkd 1985;148:626–31.

Zech W, Alt HG, Blasek R. Characterization of phosphorus fractions in mountain soils of the Bavarian Alps by ^{31}P NMR spectroscopy. Z Pflanzenernaehr Bodenkd 1987;150: 119–23.