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Soil phosphorus availability and transformation rates in relictic pinsapo fir forests from southern Spain

BENJAMÍN VIÑEGLA*, ROBERTO GARCÍA-RUIZ, JOSÉ LIÉTOR, VICTORIA OCHOA and JOSÉ ANTONIO CARREIRA
*Área de Ecología, Universidad de Jaén, Paraje Las Lagunillas s/n, Edif. B3, E-23071 Jaén, Spain; *Author for correspondence (e-mail: bvinegla@ujaen.es; phone: +34-(9)53-212791; fax: +34-(9)53-211873)*

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Abstract. *Abies pinsapo* fir forests are remnant of temperate-like coniferous forests currently subjected to the typical seasonal constraints of Mediterranean-type climates. We have examined for the first time, P availability and cycling in *A. pinsapo* forests from southern Spain by using both, measurements of concentrations of soil P fractions (resin extractable P, bicarbonate extractable P and NaOH extractable P) and rates of P supply to (P solubilization, P mineralization and phosphomonoesterases (PMEase activity) and P immobilization from the soil solution. Soils from *A. pinsapo* stands differing in lithology (serpentinitic and calcareous substrates) and successional status (from young-agradative to old-growth stages) were chosen for this study. Labile organic P fractions, PMEase activity and rates of P gross mineralization and immobilization were significantly higher in agradative stands on serpentines than in successionally comparable calcareous stands. This suggests an important role of the organic P subcycle in the *A. pinsapo* stand on serpentine. On calcareous lithology, PMEase activity and all soil P transformation rates significantly increased throughout the successional series. Similarly, all organic and inorganic P fractions measured in old-growth forests showed the maximum values of the series. These trends fit the predictions of standard patterns of P cycling changes along with succession, in which P supply to plants greatly depends on solubilization from mineral forms at early-to-mid successional stages, whereas the importance of processes related to the organic P subcycle increases as succession progresses.

Introduction

Abies pinsapo Boiss. is an endemic and relictic species, now restricted to small patches in north-facing slopes of coastal mountain-ranges in southern Spain. These stands are remnants of temperate-like *Abies* forests that covered a much broader area during the last ice-age periods (Carrión et al. 2001). Previous studies have demonstrated that the relictic *A. pinsapo* forests, despite they are currently subjected to mediterranen-type climate, still maintain some canopy-structural and stand-productivity patterns resembling those in typical temperate coniferous forests (Arista 1995; Liétor 2002). The enormous scientific significance of *Abies pinsapo* fir-forests relies on the fact that they can be used

as an experimental model of temperate-like conifer forests that are currently subjected to a warmer and drier (summer drought) climate. Hence, pinsapo forests can be used for testing the robustness of the theory on forest P biogeochemistry that have been almost exclusively developed from studies in temperate regions of North America and Europe.

Soil nutrient availability, as an essential component of biogeochemical cycles, is of primary importance since it determines short and long term forest dynamics and conservation (Brozek 1990). Phosphorus, under non-limiting nitrogen conditions, is the most important soil nutrient element controlling forest productivity, as it has been reported in tropical (Richter and Babbar 1991), temperate (Mohren et al. 1986) and Mediterranean regions (Carreira et al. 1997).

The availability of P for plant growth depends on complex geochemical (weathering release, pH, and soil chemistry dependent sorption and desorption reactions) and biochemical processes (microbial and plant mediated immobilization and mineralization) (Frossard et al. 1993). Moreover, geochemical processes are highly dependent on lithology (Tiessen et al. 1984), whereas forest succession appears to be a critical factor for biochemical processes. Vitousek and Farrington (1997) have hypothesized that potential phosphorus limitation is a general property of old-growth forests due to progressive immobilization of P into resistant organic compounds and occlusion into secondary minerals as forest matures. Binkley et al. (1999) have also shown that P fertilization may enhance available inorganic P in the soil solution, but few years after fertilization, most of the soil P remains as a poorly-available P pool.

As a result of the complexity of soil P cycling, a suitable universal estimator of P availability has not been found, since none of them gathers the four factors governing it: intensity, capacity, replenishment and diffusion (Harrison and Helliwell 1979). Studies on P cycling in terrestrial ecosystems have, mainly, focused on the concentration of soluble and particulate P fractions, often using variations of the P fractionation scheme developed by Hedley et al. (1982) (e.g. Lajtha and Schlesinger 1988; Walbridge et al. 1991). However, the interpretation of ecosystem P availability, based solely on soil P pools, may be difficult since it depends on an equilibrium between both P supply from soil and uptake by organisms.

Laboratory incubation experiments have been extensively used to model kinetics and fluxes of soil N (Bonde and Lindberg 1988). However, few studies have attempted to measure P fluxes and to separate mineralization from immobilization and solubilization processes (Grierson et al. 1998; Carreira et al. 2000), mainly because of the lack of a suitable analytical procedure. Until the nineties, ^{32}P was used to estimate net P mineralization rates (Walbridge and Vitousek 1987). Only recently, a simple, non-radioisotopic and rapid method for estimating P fluxes in forest soils, covering gross P mineralization rates and net P solubilization and immobilization rates, has been made available (Zou et al. 1992).

On the other hand, soil phosphomonoesterase activity (PMEase, alkaline: EC 3.1.3.1, and acid: EC 3.1.3.2) plays an important role in the supply of P in terrestrial ecosystems when inorganic P availability is low and demand is high (Firsching and Claassen 1996). Pang and Kolenko (1986) found that, in forest soils, organic P mineralised by PMEases accounted for a significant proportion of the P uptake by organisms. In this sense, PMEase activity has shown to be a more sensitive and earlier indicator of ecosystem P limitation than the traditionally used physico-chemical P fractionation schemes (Arévalo et al. 1994; Aon et al. 2001).

The objective of this study was to assess the effects of lithology and successional status on the patterns of P availability and cycling in pinsapo fir forests which is a temperate-like conifer forests but subjected to a warmer and drier climate. To reach this objective we combined measurements of soil P fractions pools, as well as soil P transformation rates by means of a non-radioisotopic method and PMEase activity, in a set of pinsapo stands varying either in successional status or in lithology.

Material and methods

Study area and experimental design

Relictic *A. pinsapo* forests are distributed in three main patches (less than 2500 ha in total) in Southern Spain. They are always located in moderate to steep north-facing slopes between ca. 1100–1800 m a.s.l. Despite the fact that the physiognomy and stand-dynamic of pinsapo-fir forests resembles that of typical temperate forests (Liétor 2002), they are currently subjected to Mediterranean-type climate; typical mean annual air temperature is around 15 °C, with hot and dry summers (mean summer monthly temperature higher than 22 °C) and mild winters (mean monthly temperature higher than 5 °C). However, annual precipitation is usually higher than 1000 mm, most of it being concentrated between October and May (Liétor 2002).

Our study was conducted at Sierra de las Nieves Natural Park (Yunquera, 4°58′0″ W, 36°43′30″ N) and Los Reales de Sierra Bermeja Natural Site (5°12′9″ W, 36°29′20″ N), both in the province of Málaga, southern Spain, and approximately 30 km apart. Both locations differ in lithological properties since limestones and dolomites dominate the parent material in Sierra de las Nieves Natural Park, while the dominant substrates in Sierra Bermeja are ultrabasic serpentines and peridotites. We selected stands of varying successional stage in both locations although comparable in terms of topography (40% average north-facing slope, between 1200 and 1500 m of elevation). At Sierra de las Nieves we selected calcareous stands at three successional stages (*sensu* Oliver 1981): agradative stands in the stem-exclusion phase, agradative stands in trasition to the understory reinitiation

phase, and old-growth stands (Liétor 2002). Our previous survey showed that old-growth stands of enough extension to be included in the study were lacking at Sierra Bermeja (at the most only small patches with old trees were found). Thus, this study only considers the two agradative successional phases in the case of serpintinic pinsapo stands. The general experimental design, and the codes for each combination of 'lithology' and 'successional stage' treatments, are shown in Table 1. Although the design was incomplete for the 'serpentine' vs. 'old-growth' treatment, we can test for differences between agradative stands either in serpentinic or calcareous substrates, and for differences among calcareous pinsapo stands of widely varying successional stages. Basic properties of the soil at each stand are shown in Table 2.

Soil phosphorus fractionation

Five composite samples, each made up of 3 soil cores (6 cm internal diameter) were collected from the top 10-cm mineral soil in all experimental plots on February 1999 and transported, on ice, to the lab within 48 h. Soil P fractionation was carried out on freshly sieved (<2 mm) soil samples, following the method described by Hedley et al. (1982). The HCl-extractable fraction was not determined; thus, the residual fraction data presented in this study includes apatite-like P minerals and also might contain organic P originating from alkali-insoluble organic matter that might be bioavailable. The fractionation scheme involved the sequential extraction of 1 g field moist sample. The P forms analysed in sequence were: (a) Resin extractable P (res-Pi hereafter). This form of extractable P is the most biologically labile form of inorganic P (Sibbesen 1978); (b) Bicarbonate extractable P: labile inorganic P (bic-Pi) and organic P (bic-Po) forms sorbed onto soil surfaces (Bowman and Cole 1978); (c) NaOH extractable P: non-occluded inorganic P (NaOH–Pi) and organic P (NaOH–Po) from surfaces of Fe and Al particulates (Ryden et al. 1977). Supernatants were analysed for inorganic phosphate concentration using the ascorbic acid–molybdenum blue method as described by John (1970). Organic P fractions, in bicarbonate and NaOH extraction steps, were estimated by subtracting inorganic P from the total P determined in each extract after sulphuric acid plus potassium persulfate digestion (D'Elia et al. 1977).

Microbial P (fum-P) was estimated as the difference, in sodium-bicarbonate extractable P, between previously chloroform-fumigated and non-fumigated pairs of replicated samples (Brookes et al. 1982; Hedley and Stewart 1982). A factor of 0.4 was used to calculate total microbial P (mic-P) as follows: mic-P = fum-P/0.4 (Brookes et al. 1982; Hedley and Stewart 1982).

Total soil P was determined on separate soil subsamples (0.5 g DW) after acid digestion (D'Elia et al. 1977).

Table 1. Experimental design of the study. '*Pn*' denotes the different successional phases of the study plots on the basis of structural analysis (Liétor 2002). C and S denote stands on calcareous and serpentinitic parent materials, respectively.

Successional stage		Lithology	
		Calcareous	Serpentinitic
Agradative ($\leq$ 60 years)	P1: Stands in stem exclusion phase (*sensu* Oliver 1981)	C1	S1
	P2: Stands in understory reinitiating phase (*sensu* Oliver 1981)	C2	S2
Old-growth (*sensu* Oliver 1981)	P3: Small seedlings and saplings bank	C3b	Nonexistent combination
($>$ 60 years)	P3: Large seedlings and saplings bank	C3a	

Table 2. General surface (0–10 cm) soil properties of the studied pinsapo stands differing in lithology and successional stage (ND: not determined).

Lithology	Successional stage	Plots	pH (H_2O) soil/litter	% Sand	% Silt	% Clay	Soil classification (*)	Bulk density (g cm^{-3})	Organic matter (%)	Total N (%)	C/N
Serpentinitic	P1	S1	6.3/5.8	ND	ND	ND	Oxiacuic Hapludoll	1.33	18.1	0.48	16.2
	P2	S2	6.3/5.9	39.8	35.5	23.9	Oxiacuic Hapludoll	1.01	25.0	0.70	17.9
Calcareous	P1	C1	7.9/7.4	26.9	49.6	22.9	Calcic Haploxerept	1.52	11.7	0.29	16.7
	P2	C2	8.0/7.6	24.5	49.9	24.9	Typic Haploxerept	1.52	13.8	0.35	19.0
	P3	C3a	7.6/6.8	20.2	35.0	44.8	Typic Calcixeroll	0.83	36.6	0.98	19.2
		C3b	7.8/7.3	63.8	14.5	19.7	Lytic Haploxeroll	0.73	32.1	0.98	20.3

*Soil Survey Staff (1999).

Phosphomonoesterase activity

Phosphomonoesterase (PMEase) activity was measured on litter and soil samples collected on February 1999 following the method developed by Tabatabai and Bremner (1969), using *p*-nitrophenyl phosphate (*p*NPP) at 8.7 mM final concentration as enzyme substrate. Litter (five samples per plot) was sampled using a 40×40 cm square frame, and soil (top 10 cm) as indicated above. Since PMEase activity is strongly affected by pH, standard assays are usually carried out at pH 6.5 and/or pH 11, the average optimal pH for acid and alkaline PMEase in a wide group of soils. However, we were interested in differences induced by the parent material (serpentinic vs. calcareous) which were related to differences in soil pH in more than one unit (Table 2). On this basis, assays were performed at sample field pH (pH 6 for litter and soil in serpentinitic plots, and pH 8 and 7 for soil and litter, respectively, in calcareous plots) in a buffered solution, incubated at 37 °C for 1 h.

In order to assess correspondence between the optimal pH for PMEase activity and the soil pH, we additionally analysed soil PMEase activity, at nine pH values within a range of pH 3–13, in soil samples from plots S1, S2, C2 and C3a, in order to reduce the number of samples but still covering the two main comparison factors (lithology and successional stage).

Kinetics parameters of PMEase activity were determined on fresh soil samples from plots S1, S2, C2 and C3a stored at 4 °C. Soil samples were incubated at a final *p*NPP concentration ranging from 0.22 to 20.0 mM (except for samples from plot C2 in which maximum *p*NPP concentration was 8.7 mM, since activity reached full maximum at lower concentrations). We calculated the amount of *p*NPP adsorbed onto soil particles, and thus, not available for enzymatic hydrolysis, as *p*NPP adsorbed = *p*NPP added – *p*NPP hydrolysed – *p*NPP remaining in solution, following the procedure of Irving and Cosgrove (1976). Kinetic parameters were corrected for the amount of *p*NPP adsorbed onto soil. The kinetics parameters apparent affinity (K_m) and maximum velocity (V_{max}) were calculated by means of linear regression using the Lineweaver–Burk transformation of the Michaelis–Menten equation.

Phosphorus transformation rates

The effects of lithology and successional status of pinsapo stands on potential soil P transformation rates were evaluated using a modification of the Γ-radiation–autoclaving–incubation procedure of Zou et al. (1992). Five composite samples per plot were taken in October 2000; 3 air-dried subsets (20 g each) per sample were separated. Then, a subset of samples, used as a control, was brought to 80% WHC with deionised water. The other two subsets of soil were sterilised using $HgCl_2$ addition at a concentration of 1500 mg kg^{-1} dry soil (Wolf and Skipper 1994), instead of using Γ-radiation (Zou et al. 1992). To

apply the $HgCl_2$ treatment, the corresponding samples were evenly sprinkled with the suitable volume of a concentrated $HgCl_2$ solution to bring soils to 80% WHC. To check for the degree of microbial sterilisation achieved by the $HgCl_2$, dehydrogenase activity was measured in the samples after 7 days of incubation. Dehydrogenase activity on samples treated with $HgCl_2$ was, at the most, less than 9% of that on the corresponding non-sterilised sample (1.9 vs. 21.7 μg TPF g^{-1} h^{-1}). After that, one of the two $HgCl_2$-treated subsets of samples was autoclaved (120 °C, 30 min.) to denature soil enzymes. Thus, three independent treatments were applied to soils: (i) control (C samples hereafter); (ii) $HgCl_2$-addition (S samples); and (iii) $HgCl_2$-addition plus autoclaving (SD samples).

Immediately after treatment, all the soil samples (2 g), together with an anion exchange membrane 204-U-386 saturated with Cl^- (12.5 cm^2 in surface or 1 g of oven-dry equivalent), were incubated for 48 h, with 30 ml deionised water, in 50-ml centrifuge tubes in a reciprocal shaker (200 strokes min^{-1}). To ensure complete sterilisation of the S- and SD-samples, and to maintain sterile conditions during incubation with the membranes, additional $HgCl_2$ (2500 mg kg^{-1} of dry soil) was added with the deionised water to the corresponding centrifuge tubes. After incubation, resins were extracted with 30 ml 0.5 M HCl on a reciprocal shaker for 1 h. Phosphate concentrations in these solutions were analysed using the ascorbic acid–molybdenum blue method according to John (1970).

Phosphorus extracted by resines from the control soils (C) accounts for the net balance among solubilization of inorganic P, mineralization of organic P, and immobilization of solution P. Resin-extractable P from the $HgCl_2$-treated samples (S) results from the sum of solubilized P and mineralized P ($HgCl_2$-sterilization avoids microbial immobilization of solution P). Phosphorus coming from the $HgCl_2$-treated plus autoclaved soils (SD) comes from the solubilization of inorganic P only (autoclaving of $HgCl_2$-treated soils additionally avoids P mineralization by phosphatase enzymes) and gives an estimation of net P solubilization rate. The difference in resin-extractable P between the $HgCl_2$-addition and the $HgCl_2$-addition plus autoclaved treatments is an estimate of gross mineralization rate, whereas the difference between control and $HgCl_2$-addition treatments is an estimate of P immobilization rate.

To check for the direct effect of $HgCl_2$-addition and autoclaving on resin-extractable P, three subsamples of 2.5 g moist soil from each treatment (C, S and SD) were extracted with anion exchange membranes as above, except for that extraction was performed for only 1 h. If there were differences in res-Pi concentrations between controls and $HgCl_2$-addition or $HgCl_2$-addition plus autoclaving treatments, correction factors should be applied in the calculation of P transformation rates to allow for direct treatment effects (Zou et al. 1992). In our case, correction was applied for autoclaving treatment in soils from plots C3a and C3b, which had the highest organic matter contents.

Statistical analysis

To test for differences between lithologies, only data from agradative stands were used. For such comparison, data from both, S1 and S2 plots (agradative serpentine stands), and for C1 and C2 plots (agradative calcareous stands), were averaged. To look for differences among successional stages, only data from the calcareous stands were used. The following planned comparisons following analysis of variance (ANOVA) were used: (i) 'serpentines' (S1 and S2) vs. 'calcareous substrates' (C1 and C2) to test for differences due to lithology; (ii) 'stem-exclusion phase' (C1) vs. 'understory reinitiating phase' (C2) and 'old-growth phase' (C3a and C3b), to test for differences between successional stages. The significance was tested at the $\alpha = 0.05$ level. ANOVA requirements of normality (Kolmogorov–Smirnov test) and homogeneity (Bartlett F test) were also checked at $\alpha = 0.05$. For variables that did not meet ANOVA requirements, $\log(x + 1)$ transformations were applied. When this transformation turned out ineffective, a non-parametric Kruskall–Wallis test followed by a HSD Tukey's test adapted to non-parametric analysis was applied.

Results

Phosphorus fractionation

Results from the sequential P fractionation of soil samples in all the pinsapo stands are shown in Table 3. Total phosphorus concentration was not significantly different in agradative *A. pinsapo* stands differing in lithology. However, remarkable differences were found for the different P fractions. The concentration of the most easily, available inorganic P (resin-extractable P), was three times higher in serpentine stands compared with stands of similar successional stage on calcareous parent material. NaOH–Pi concentration was almost three times higher in serpentinitic stands accounting for the 9.2 and 3.6% of the total P in serpentinitic and calcareous stands, respectively. No significant differences between serpentinitic and calcareous stands were found for bic-Pi. Labile (bic-Po), and more resistant and associated with humic compounds (NaOH–Po) organic phosphorus concentrations were two times higher in serpentinitic than in calcareous stands. No single organic P fraction comprised more than 10% of the total phosphorus. No differences were found for microbial P concentration (4.3 and 4.9% of total P, for the serpentinitic and calcareous plots, respectively). The residual P fraction (including apatite-like, plus occluded inorganic and organic phosphorus), was significantly higher in agradative calcareous (81.9% of total P) than in serpentine stands of a similar successional stage (65.8% of total P).

Table 3. Phosphorus fractions (μg P g^{-1} DW) in top (10 cm) mineral soil of study plots. ΣPi and ΣPo represent the addition of the corresponding Pi and Po labile fractions extractable with sodium bicarbonate and sodium hydroxide, respectively. Residual P corresponds to apatitic P plus occluded Pi and Po and was calculated as follows: Res-P = P total − (mic-P + ΣPi + ΣPo). Data are means (SD) (n = 5 samples per plot, 10 samples per plot for res-P). Values with different letters within each column (a, b for 'lithology' and a′, b′ for 'successional stage') indicate significant differences, $p < 0.05$ (planned comparisons between lithologies and successional stages following ANOVA).

Plots	res-Pi	bic-Pi	NaOH–Pi	ΣPi	bic-Po	NaOH–Po	ΣPo	mic-P	Residual P	Total P
Comparison factor: 'lithology'										
S1 & S2	7.31(4.97)^{a}	12.4(7.4)^{a}	41.4(10.4)^{a}	53.9(13.4)^{a}	34.5(11.2)^{a}	38.6(13.2)^{a}	73.1(21.5)^{a}	19.4(12.7)^{a}	295.7(48.5)^{a}	449.2(102.2)^{a}
C1 & C2	2.52(2.03)^{b}	11.8(7.3)^{a}	16.3(3.9)^{b}	28.1(8.5)^{b}	15.0(16.9)^{b}	20.4(7.7)^{b}	33.9(15.7)^{b}	22.1(11.3)^{a}	371.6(20.6)^{b}	453.6(30.3)^{a}
Comparison factor: 'successional stage'										
C1	2.04(1.22)$^{a'}$	14.6(8.9)$^{a'}$	16.0(3.8)$^{a'}$	30.6(11.5)$^{a'}$	18.6(23.5)$^{a'}$	18.1(6.5)$^{a'}$	33.0(20.7)$^{a'}$	23.5(19.5)$^{a'}$	379.9(24.0)$^{a'}$	459.7(35.2)$^{a'}$
C2	3.01(2.59)$^{a'}$	9.0(4.5)$^{a'}$	16.5(4.4)$^{a'}$	25.6(3.8)$^{a'}$	11.4(12.6)$^{a'}$	22.7(8.8)$^{a'}$	35.0(11.0)$^{a'}$	20.6(5.3)$^{a'}$	363.4(14.6)$^{a'}$	447.6(28.4)$^{a'}$
C3a & C3b	9.75(3.08)$^{b'}$	33.4(9.3)$^{b'}$	42.0(7.8)$^{b'}$	75.5(12.3)$^{b'}$	40.9(17.7)$^{b'}$	37.0(17.3)$^{b'}$	79.3(18.8)$^{b'}$	38.7(19.0)$^{b'}$	427.6(41.2)$^{a'}$	674.6(86.6)$^{b'}$

The mean organic to inorganic P ratios for the different P fractions were close to 1 in calcareous soils, but always exceeded 1.5 in serpentinitic soils (up to 3 for the bic-Po/bic-Pi ratio).

Total phosphorus concentration in the top-10 cm soil of calcareous plots increased significantly along with forest succession, from 459.7 μg P g^{-1} in the stem-exclusion phase (C1), to 674.6 μg P g^{-1}, in the old-growth phase (C3a and C3b) (Table 3). All inorganic (res-Pi, bic-Pi, NaOH–Pi) and organic (bic-Po and NaOH–Po) phosphorus fractions were typically from two to three-fold higher in old-growth than in the agradative (C1 and C2) calcareous plots (Table 3). Microbial P showed also values two times higher in old-growth plots.

Phosphorus supply rates

PMEase activity

Mean soil PMEase activity was 1337 μg *p*NP $g^{-1}h^{-1}$ on agradative serpentinitic stands. In contrast, soil PMEase activity in successionally-comparable calcareous stands, averaged 436 μg *p*NP $g^{-1}h^{-1}$(Figure 1a). As expected, PMEase activity was much higher in litter than in soil samples. As for soil, averaged litter PMEase activity in agradative serpentinitic pinsapo stands (4360 μg *p*NP $g^{-1}h^{-1)}$ was significantly higher ($p < 0.05$) than that of calcareous stands (1922 μg *p*NP $g^{-1}h^{-1}$) (Figure 1a).

Litter PMEase activity did not show any significant increase ($p = 0.058$) along the succesional stages. On the other hand, soil PMEase activity increased significantly ($p < 0.05$) throughout the calcareous successional series, from an average value of 436 μg *p*NP g^{-1} h^{-1} in agradative stands (C1 and C2) to 584 μg *p*NP $g^{-1}h^{-1}$ in old-growth stands (C3a and C3b) (Figure 1b).

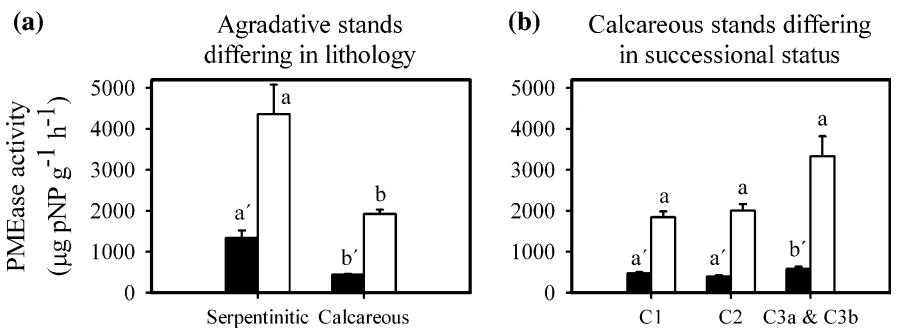

Figure 1. Phosphomonoesterase activity at the sample pH in surface soil (dark bars) and litter (light bars) in *A. pinsapo* stands differing in lithology (a) and successional status (b). Data are means (±standard error). Letters on bars indicate significant differences for lithology or successional stage ($p < 0.05$, planned comparisons between lithologies or successional stages following ANOVA).

Effect of pH on PMEase activity

Soil PMEase activity was clearly higher under acid pH values in serpentinitic soils, showing a maximum between pH values 4 and 6, and decreasing progressively at higher pH values (Plots S1 and S2, Figures 2a and b, respectively). In agradative calcareous soils (C2), soil PMEase activities were maximum at acid and alkaline pH (3–5 and 10–11, respectively) (Figure 2c). In old-growth stands (C3b) two maximum were also achieved but at higher pH ranges (pH 6–8 and 12–13) (Figure 2d).

In all soil plots tested, except for plot C2, *in situ* pH assay and pH value for maximum PMEase activity were coincident (indicated by arrows in Figure 2).

Kinetic parameters of PMEase activity

Values of *p*NPP adsorbed onto soil particles at different substrate concentrations, on selected stands, are shown in Figure 3a and 3b. An asymptotical response in adsorbed *p*NPP was found when increasing substrate concentration. The mean percentage of adsorbed *p*NPP for all soils tested was ca. 35% from a concentration of 5.2 mM of added *p*NPP (Figure 3c). No increases were found in this percentage for higher concentrations of added *p*NPP.

Soil PMEase activity showed a typical Michaelis–Menten kinetic curve for samples collected in *A. pinsapo* stands differing in lithology and succesional stage (Figure 4). Soils from serpentinitic stands showed much higher V_{max}

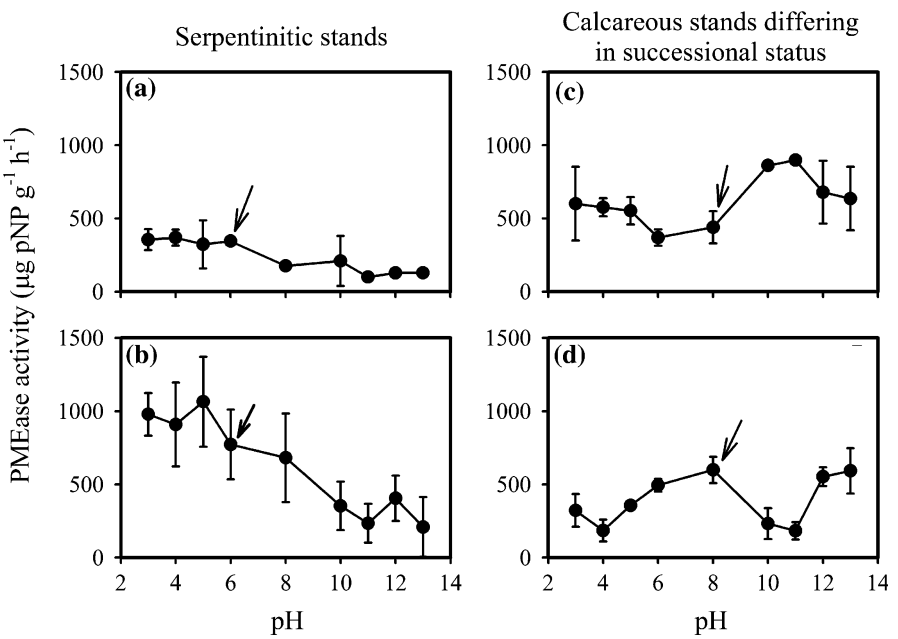

Figure 2. Effect of pH on soil PMEase activity in S1 (a), S2 (b), C2 (c) and C3a (d) plots. Bars stand for the standard error ($n = 3$). Arrows indicate the *in situ* pH at which PMEase activity was assayed.

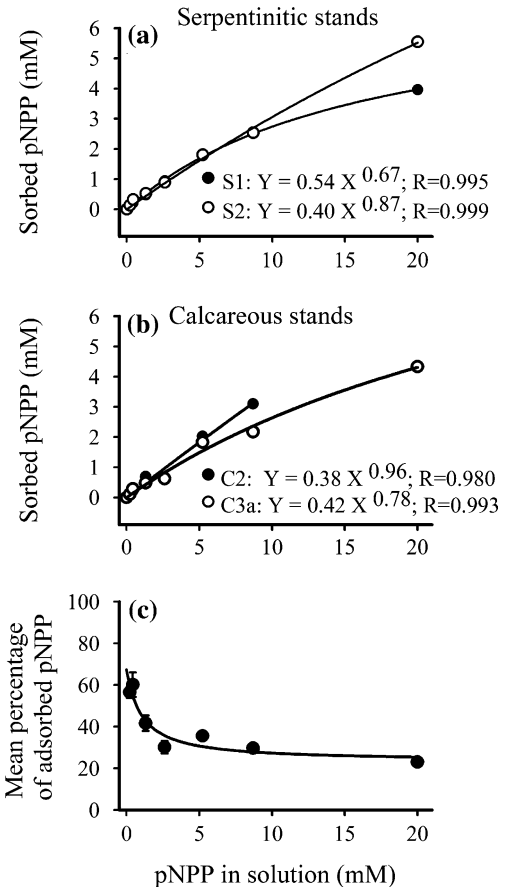

Figure 3. *p*-Nitrophenil phosphate (*p*NPP) adsorption isotherms in soils from serpentinitic (a) and calcareous (b) *A. pinsapo* stands. (c) Mean percentage of *p*NPP adsorbed at different *p*NPP concentrations in the assay solution. Buffer pH was 6 for serpentinitic and 8 for calcareous soils. Bars are ±SE ($n = 4$).

values than those from calcareous parent material (Table 4). For the S2 plot, saturation of PMEase activity at the maximum assayed *p*NPP concentration was not achieved. In the same way, K_m values were also higher (one order of magnitude) in serpentinitic than in calcareous soils.

On the other hand, no significant differences in kinetic parameters of PMEase activity from calcareous stands differing in successional stages were found (Table 4).

Phosphorus transformation rates

Figures 5a and b show rates of gross mineralization, and net solubilization and immobilization of P in soils from *A. pinsapo* stands differing in lithology and successional stage.

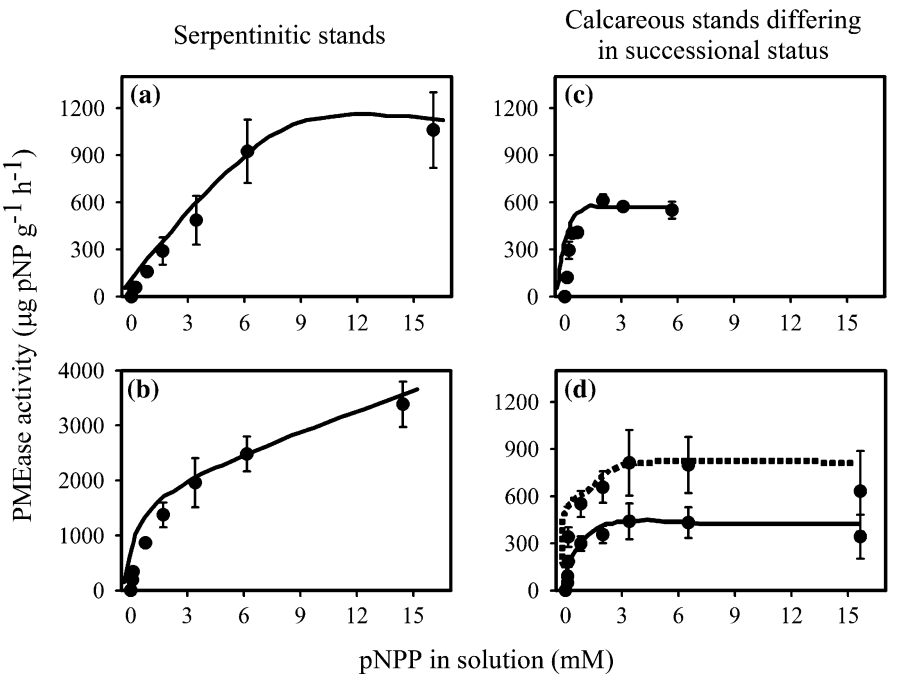

Figure 4. Effects of initial *p*NPP concentration in the reaction mixture on soil PMEase activity (at field pH) in the S1 (a), S2 (b), C2 (c) and C3a (d) stands. Note the different *Y* axis scale for plot S2. Bars are ±SE ($n = 3$).

Table 4. Kinetic parameters of PMEase activity (V_{max} and K_m), in soils from selected study plots. Data are means (SD) ($n = 3$, except for S1 & S2, $n\times 2$). Different letters within each column (a, b for 'lithology' and a′, b′ for 'successional stage') indicate significant differences, $p < 0.05$ (planned comparisons between lithologies and successional stages following ANOVA).

Plots	V_{max} (µg *p*NP g^{-1} h^{-1})	K_m (mM)
Comparison factor: 'lithology'		
S1 & S2	2813.8(1205.2)[a]	4.85(2.00)[a]
C2	593.0(67.5)[b]	0.27(0.09)[b]
Comparison factor: 'successional stage'		
C2	593.0(67.5)[a′]	0.27(0.09)[a′]
C3a	753.9(187.1)[a′]	0.37(0.31)[a′]

Total soil P fluxes in agradative serpentinitic pinsapo stands were almost one order of magnitude higher (10.56 µg P g^{-1} 2 d^{-1}) than in successionally comparable calcareous stands (1.48 µg P g^{-1} 2 d^{-1}). Differences in lithology did not affect ($p < 0.05$) net P solubilization rates. However, P solubilization in calcareous soils accounted for ca. 50% of total P transformation fluxes, compared to only a 4.5% in serpentinitic soils (Figure 5a). Significantly higher

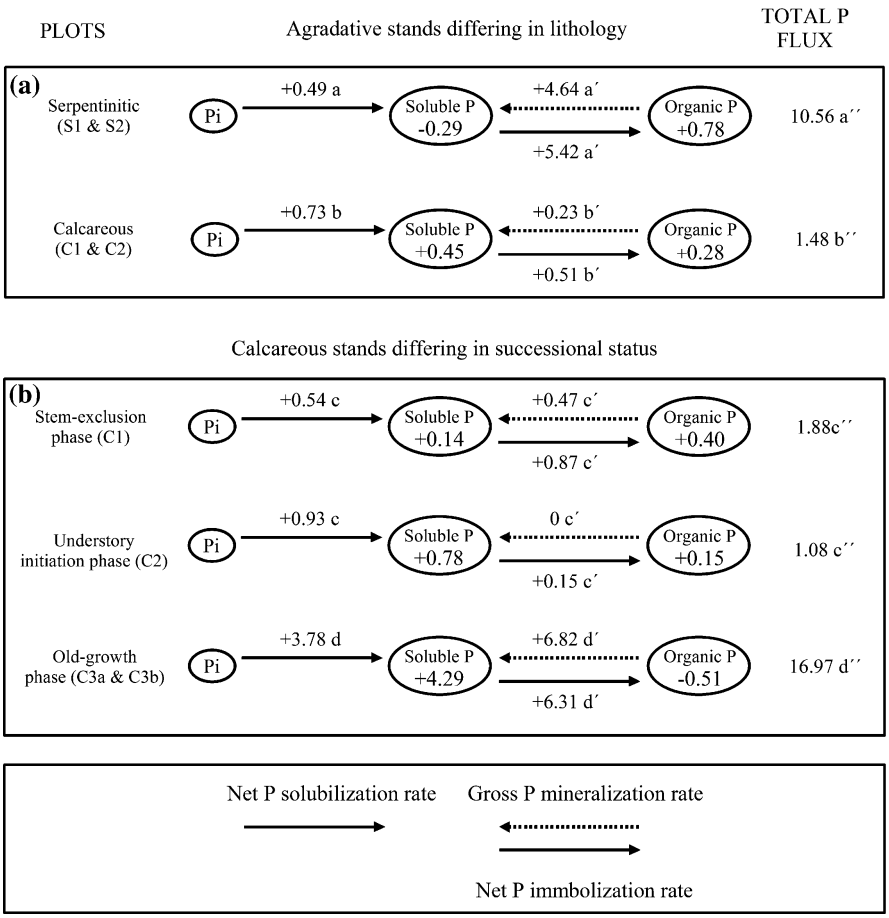

Figure 5. Gross mineralization, net solubilization and immobilization rates of P in stands differing in lithology (a) and successional stage (b). Circles stand for pools and arrows indicate processes rates. Numbers in circles and numbers associated to arrows represent the average ($n = 3$; $n \times 2$ for S1&S2, C1&C2 and C3a&C3b) of net changes in P pool sizes (μg P g^{-1} soil) and P transformation rates (μg P g^{-1} soil 2 $days^{-1}$), respectively. Total P fluxes are calculated adding all P transformation rates. Different letters for the same process (a, b, a′, b′, a″ and b″ for 'lithology' and c, d, c′, d′, c″ and d″ for 'successional stage') indicate significant differences, $p < 0.05$ (non-parametric Tukey's HSD following Kruskall–Wallis test).

gross P mineralization and net P immobilization rates were found in soils from serpentinitic stands compared to those from calcareous stands. These rates compromised 44.0 and 51.5% of total P fluxes in serpentinitic soils, while only 12.5 and 30% for calcareous soils. Net change in soil solution P was negative in serpentinitic stands due to high rates of P immobilization, while this trend was inverted in successionally comparable calcareous stands. Net changes in organic P pools were positive both in serpentinitic and calcareous soils (Figure 5a).

Total P fluxes increased significantly ($p < 0.05$) throughout the successional series on calcareous stands, from 1.88 μg P g^{-1} 2 d^{-1} in agradative stands in stem-exclusion phase to 16.97 μg P g^{-1} 2 d^{-1} in old-growth stands (Figure 5b). Net solubilization, gross mineralization, and net immobilization rates of P were significantly higher ($p < 0.05$) in old-growth stands. Net changes in soil P solution were positive in all calcareous stands, although increased significantly along the successional stages, from 0.14 to 4.29 μg P g^{-1}. Net changes in organic P pools were positive in agradative stands in stem-exclusion and in understory reinitiating phases but negative in the old-growth stands.

Discussion

Effects of lithology on phosphorus availability and transformation rates in A. pinsapo *stands*

Successionally comparable *A. pinsapo* stands that differ in lithology showed marked differences in soil phosphorus fraction pools, PMEase activity and P transformation rates. Labile organic P fractions, PMEase activity and rates of gross mineralization and immobilization were significantly higher in the serpentinitic than in the calcareous soils, despite successional stage was similar. Moreover, the most easily available Pi fraction (resin extractable P) was also significantly higher in the serpentinic than in the calcareous soils. These differences can be hardly attributed only to lithological differences, since P availability is generally low in serpentinitic soils (e.g. Kruckeberg 1992; Proctor 1999). The main primary minerals of peridotitic bedrocks at Sierra Bermeja are P-poor talc, chrysolite and chlorite, and secondary minerals in the soils only show, at the most, the first steps of neo-formation (saponites) (Aguilar-Ruiz et al. 1997). In addition, Fe and Al sesquioxides usually have higher P-fixing capacity on acid soils than in calcareous soils (Stevenson 1986). This is consistent with the high NaOH–Pi concentration (41.4 μg g^{-1}) found at the slightly acid (pH 5.8–6.3) serpentinic soil at Sierra Bermeja.

Soil nutrient availability ratios (e.g., soil available N/P) and litter production have already been analyzed in the study plots. Liétor (2002) found that agradative serpentinic and calcareous stands differed in stand productivity, although not in the expected way. According to the generally accepted phytotoxic character of serpentine soils (Proctor 1999) it can be hypothesized that serpentinitic stands will be less productive than calcareous ones. However, litter production and litter decomposition rate (estimated as the quotient between litter production and litter mass), were significantly higher in serpentinitic (6.1 Mg ha^{-1} yr^{-1} and 0.57 yr^{-1}, respectively) that in calcareous stands (3.5 Mg ha^{-1} yr^{-1} and 0.15 yr^{-1}, respectively) of comparable successional stage (Liétor 2002). This might explain the higher soil labile organic P content on serpentines compared to that found in calcareous parent materials reported here, despite similar successional status. Other studies have demonstrated a

direct relationship between litter production and pools of soil organic matter and soil organic P in Douglas-fir stands (Verburg et al. 2001). Further studies are under way to get insights on why, contrarily to what we expected, serpentinic stands show higher productivity than calcareous stands. In this sense, differences in annual atmospheric N wet deposition between the two stands (17.0 kg ha^{-1} yr^{-1} at Sierra Bermeja vs. 2.7 kg ha^{-1} yr^{-1} at Sierra de las Nieves; Liétor 2002) may explain such differences in productivity.

Optimum pH for PMEase activity was in the acid range for serpentinitic soils, whereas it was in the alkaline range for calcareous soils. These pH optima were, in general, coincident with soil pH values (except for C2 plot) (Table 2), which suggests a selective process on soil PMEase enzymes to optimise Po mineralization. The lower soil PMEase activity found in plot C2 (agradative calcareous *A. pinsapo* stand) might be partially explained by the uncoupling between the optimum pH of PMEase from this soil and the actual fresh soil pH.

Soil PMEase activity on serpentinitic plots was greater than 1000 μg *p*NP g^{-1} h^{-1} (even more than 4000 μg *p*NP g^{-1} h^{-1} for litter), higher than those on calcareous samples and only comparable with data reported for soils from forests showing high P deficiencies (Giardina et al. 1995; Saá et al. 1998). PMEase activity did not even show saturation in serpentinitic soils at the highest substrate concentration assayed. On the other hand, activity on serpentinitic soils exhibited low substrate affinity, which is normally an inherent property for acid PMEases (Dick and Tabatabai 1984), even lower than those reported for acid free soil (Dick and Tabatabai 1984; Juma and Tabatabai 1988) and acid root PMEases (Firsching and Claassen 1996). Arévalo et al. (1994) found similar results (unsaturated kinetic and high K_m values for acid PMEases) on soils of a shrubland community which showed high P deficiency following frequent fire events. The competitive disadvantage of a poor substrate affinity would be compensated by a greater enzyme concentration in the soil, since V_{max} is considered as indicative of the amount of enzyme (Tabatabai 1994). Thus, in serpentinitic soils, the supply of P to meet organism demand appears to be highly dependent on the activity of PMEase enzymes.

The supply of P to the soil solution comes from the net balance between geochemical and biological controls over P transformation processes in the solid phase of soil. Soil gross P mineralization rates were 20-times higher on early-to-mid successional serpentinitic stands compared to successionally comparable calcareous stands. This agrees with the higher PMEase activity found in the serpentinic soil. However, these high mineralization rates were compensated by also high P immobilization rates. Moreover, net P solubilization rates were very low, and accounted for only 5% of total P fluxes, in the serpentinic soils. This is consistent with the lower soil pH on these plots, and the resulting higher fixing capacity (McDowell and Condron 2001). Thus, there was a net change of only -0.29 μg P l^{-1} in the soluble P pool during incubation of soils from the serpentinic plots.

Overall, these results indicate that available P is in short supply at the serpentinic stands and that it strongly relies on the organic P subcycle. Again, these results are unexpected, since it is generally accepted that P supply in early-to-mid successional forests mainly relies on geochemical processes, whereas biologically-mediated processes only gain predominance at later stages in succession. Conversely, P cycling features in soils at the young successional calcareous stands fit with the general model, net P solubilization rates being the highest single P flux, and more than three times higher than P mineralization rates. As indicated earlier, P cycling anomalies in serpentinic stands may be related to disfunctions in the N cycle at Sierra Bermeja pinsapo stands due to high atmospheric N inputs (Liétor 2002).

Successional patterns of P availability and transformation rates on calcareous pinsapo *stands*

Total P and different P fractions were significantly higher in the old-growth stands. This is in agreement with higher litter production rates (3.5 and 5.3 Mg $ha^{-1}yr^{-1}$ in agradative and old-growth stands, respectively; Liétor 2002) and soil organic matter content (almost three times higher in the old-growth stands).

Total soil P concentrations in calcareous pinsapo-fir forests were relatively high. These levels, in old-growth stands, were close to those reported by Saá et al. (1998) for P rich soils, while in agradative stands were significantly higher than those reported by Carreira et al. (1997) for P poor shrublands of the same area. Despite this, P availability was relatively low in agradative stands, where relatively low resin Pi and labile organic P concentrations and a large residual P fraction (more than 80% of the total P) were found, compared to old-growth stands, where residual P fraction was reduced to ca. 60% of total P. The low soil P availability seemed to be alleviated with succession progress, since all available P (Pi and Po) fractions were, typically, three times higher in old-growth stands. In a review of nine soil orders, Cross and Schlesinger (1995) found, as we did in the successional series of calcareous pinsapo stands, that organically-bound P increased and percentage of apatite P decreased along a weathering gradient from Entisols to Oxisols. These trends on soil P availability throughout successional series agree with the general biogeochemical principle stated by Walker and Syers (1976): at the beggining of forest succession, Pi supply is provided by weathering processes (of apatite-P) and rapidly immobilised both in biomass and secondary minerals.

P supply to the soil solution in these agradative stands appeared to mainly rely on the P geochemical subcycle (Walbridge and Vitousek 1991). Net P solubilization rates in agradative calcareous stands were significantly higher than gross P mineralization rates, and high enough to overcome microbial immobilization of P from the soil solution. P mineralization rates in these soils from agradative stands are low even though their PMEase enzymes showed the

maximum substrate affinities (lowest K_m). In general, K_m values of alkaline PMEases of calcareous pinsapo stands were under those reported in the literature for alkaline PMEases (0.4–2.6 mM; Dick and Tabatabai 1984; Juma and Tabatabai 1988). However, it must be considered that K_m values calculated throughout kinetics corrected for *p*NPP amounts adsorbed to the soil are usually slightly lower than those obtained from non-corrected data (Irving and Cosgrove 1976).

A significant positive correlation was found between gross P mineralization rate and total soil C content ($r = 0.93$, $p < 0.01$, data not shown). This suggests that the changes in soil P pools and transformation rates we have observed throughout the pinsapo-fir forests successional series might be controlled by the successional increases of C inputs to the soil.

In old-growth stands, all P transformation rates were one order of magnitude higher than in agradative stands, suggesting that as succession progresses P supply will increasingly rely on processes involved in the organic subcycle of P (Walbridge et al. 1991). In this sense, increasing PMEase activity along successional series on calcareous substrates will determine P availability for plants and microorganisms with increasing dependence on P release by enzymatic mineralization, once nutrient return in litterfall is fixed at a constant rate (Arévalo et al. 1994).

The results shown in this work indicate that the patterns of P availability and supply in the soil of relictic pinsapo-fir forests from Southern Spain are in agreement with the general principles proposed by the Biogeochemical Theory, where, as succession progresses, both Pi and Po concentrations increase at a similar rate (balanced P cycle) and there is a shift in the factors controlling P availability, changing from a geochemical to a biochemical control, with increasing relevance of PMEase activity for P supply. However, as forest succession progressed, the net balance of soluble P was more positive in old-growth stands than in agradative stands, with increasing P mineralization rates compensated by increasing immobilization rates, suggesting a less important P limitation process in the old-growth relative to the agradative stands than could be expected according to the hypothesis of Vitousek and Farrington (1997).

In the particular case of pinsapo-fir forests on serpentinitic parent materials, the imbalance between the availabilities of N and P in the soil, and the increasing demand of P by trees, is presumably inducing an acceleration of the supply process integrated in the organic subcycle of P (PMEase activity and mineralization of organic P compounds).

According to the results shown in this work, P is in short supply and it strongly relies on the organic P subcycle in agradative serpentinitic *A. pinsapo* stands. However, P cycling features in soils from successionally comparable calcareous stands seem to rely on geochemical processes, according to the general theory on changes of governing P processes throughout forest succession. Those differences does not seem to be related to differences in lithology but on dysfunctions in N cycle in the serpentinic stands. Finally, in the calcareous stands differing in successional stage, the general biochemical principles stated

by Walker and Syers (1970) are confirmed since geochemical P subcyle dominate in early-to-mid successional stage and organic subcycle in the old-growth stands.

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References

Aguilar-Ruiz J., Calvo De Anta R., Fernández-Ondoño E. and Macías-Vázquez F. 1997. Geoquímica de la alteración y edafogénesis de rocas serpentinizadas de la Sierra Bermeja (Málaga). Boletín de la Sociedad Española de la Ciencia del Suelo 5: 135–151.

Aon M.A., Cabello M.N., Sarena D.E., Colaneri A.C., Franco M.G., Burgos J.L. and Cortassa S. 2001. Spatio-temporal patterns of soil microbial and enzymatic activities in an agricultural soil. Appl. Soil Ecol. 18: 239–254.

Arévalo J.R., Carreira J.A. and Niell F.X. 1994. Kinetic parameters of phosphatase activity along a xeric dolomitic soil chronosequence related to wildfire. Geomicrobiol. J. 11: 235–245.

Arista M. 1995. The structure and dynamics of an *Abies pinsapo* forest in Southern Spain. For. Ecol. Manag. 74: 81–89.

Binkley D., Giardina C. and Bashkin M.A. 1999. Soil phosphorus pools and supply under the influence of *Eucalyptus saligna* and nitrogen-fixing *Albizia facaltaria*. For. Ecol. Manag. 128(3): 241–247.

Bonde T.A. and Lindberg T. 1988. Nitrogen mineralization kinetics in soil during long-term aerobic incubations: a case study. J. Environ. Qual. 17: 414–417.

Bowman R.A. and Cole C.V. 1978. Transformations of organic phosphorus substances in soil as evaluated by sodium bicarbonate extraction. Soil Sci. 125: 49–54.

Brookes P.C., Powlson D.S. and Jenkinson D.S. 1982. Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem. 14: 319–329.

Brozek S. 1990. Effect of soil changes caused by red alder (*Alnus rubra*) on biomass and nutrient status of Douglas-fir (*Pseudotsuga menziesii*) seedlings. Can. J. For. Res. 20: 1320–1325.

Carreira J.A., Lajtha K. and Niell F.X. 1997. Phosphorus transformations along a soil/vegetation series of fire-prone, dolomitic, semiarid shrublands of southern Spain. Biogeochemistry 39: 87–120.

Carreira J.A., García-Ruíz R., Liétor J. and Harrison A.F. 2000. Changes in soil phosphatase activity and P transformation rates induced by application of N-and S-containing acid-mist to a forest canopy. Soil Biol. Biochem. 32: 1857–1865.

Carrión J.S., Riquelme J.A., Navarro C. and Munuera C.M. 2001. Pollen in hyaena cropolites reflects late glacial landscape in Southern Spain. Palaeogeogr. Palaeoclimatol. Palaeoecol. 176: 193–205.

Cross A. and Schlesinger W. 1995. A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma 64: 197–214.

D'Elia C.F., Steudler P.A. and Corwin N. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnol. Oceanogr. 22(4): 2–6.

Dick W.A. and Tabatabai M.A.l 1984. Kinetic parameters of phosphatases in soils and organic waste materials. Soil Sci. 137(1): 7–15.

Firsching B.M. and Claassen N. 1996. Root phosphatase activity and soil organic phosphorus utilization by Norway spruce (*Picea abies* (L.) Karst.). Soil Biol. Biochem. 28(10/11): 1417–1424.

Frossard E., Feller C. and Tiessen H. 1993. Can an isotopic dilution method allow for the determination of the phosphate fixing capacity on soils? Commun. Soil Sci. Plant Anal. 24: 367–378.

Giardina C.P., Huffman S., Binkley D. and Caldwell B.A. 1995. Alders increase phosphorus availability in a Douglas-fir plantation. Can. J. For. Res. 25: 1652–1657.

Grierson P.F., Comerford N.B. and Jokela E.J. 1998. Phosphorus mineralization kinetics and response of microbial phosphorus to drying and rewetting in a Florida spodosol. Soil Biol. Biochem. 30(10/11): 1323–1331.

Harrison A.F. and Helliwell D.R. 1979. A bioassay for comparing phosphorus availability in soils. J. Appl. Ecol. 16: 497–505.

Hedley M.J. and Stewart J.W.B. 1982. A method to measure microbial phosphorus in soils. Soil Biol. Biochem. 14: 377–385.

Hedley M.J., Stewart J.W.B. and Chauhan B.S. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46: 970–976.

Irving G.C.J. and Cosgrove D.J. 1976. The kinetics of soil acid phosphatase. Soil Biol. Biochem. 8: 335–340.

John M.K. 1970. Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. Soil Sci. 109(4): 214–220.

Juma N.G. and Tabatabai M.A. 1988. Comparison of kinetic and thermodynamic parameters of phosphomonoesterases of soils and of corn and soybean roots. Soil Biol. Biochem. 20(4): 533–539.

Kruckeberg A.R. 1992. Plant life of western North American ultramafics. In: Roberts B.A. and Proctor J. (eds), The Ecology of Areas with Serpentinized Rocks. A World View. Kluwer Academic Publishers, Dordrecht, pp. 31–74.

Lajtha K. and Schlesinger W.H. 1988. The effect of $CaCO_3$ on the uptake of phosphorus by 2 desert shrub species, *Larrea-tridentata* (dc) cov and *Parthenium-incanum* hbk. Bot. Gaz. 149(3): 328–334.

Liétor J. 2002. Patrones de disponibilidad y limitación por nutrientes como indicadores de estado en masas de pinsapar (*Abies pinsapo* Boiss.). PhD Dissertation, University of Jaén.

McDowell R. and Condron L. 2001. Influence of soil constituents on soil phosphorus sorption and desorption. Commun. Soil. Sci. Plant Anal. 32: 2531–2547.

Mohren G.M., Burg J.Van Der and Burger F.G. 1986. Phosphorus deficiency induced by nitrogen input in Douglas fir in the Netherlands. Plant Soil 95: 191–200.

Oliver C.D. 1981. Forest development in North America following major disturbances. For. Ecol. Manag. 3: 153–168.

Pang P.C.K. and Kolenko H. 1986. Phosphomonoesterase activity in forest soils. Soil Biol. Biochem. 18: 35–40.

Proctor J. 1999. Toxins, nutrient shortages and droughts: the serpentine challenge. Tree 14(9): 334–335.

Richter D.D. and Babbar L.I. 1991. Soil diversity in the tropics. Adv. Ecol. Res. 21: 315–389.

Ryden J.C., McLaughlin J.R. and Syers J.K. 1977. Mechanisms of phosphate sorption of soils and hydrous ferric oxide gel. J. Soil Sci. 28: 72–92.

Saá A., Trasar-Cepeda M.C. and Carballas T. 1998. Soil P status and phosphomonoesterase activity of recently burnt and unburnt soil following laboratory incubation. Soil Biol. Biochem. 30(3): 419–428.

Sibbesen E. 1978. An investigation of the anion-exchange resin method for soil phosphate extraction. Plant Soil 50: 305–321.

Soil Survey Staff 1999. Keys to Soil Taxonomy, 8th ed. USDA, Lincoln.

Stevenson F.J. 1986. Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, and Micronutrients. John Wiley & Sons, New York.

Tabatabai M.A. 1994. Soil enzymes. In: Weaver R.W. (ed.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. Book series no. 5. Soil Science Society of America, Madison, pp. 775–883.

Tabatabai M.A. and Bremner J.M. 1969. Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1: 301–307.

Tiessen M., Stewart J.W.B. and Cole C.V. 1984. Pathways of phosphorus transformations in soils of differing pedogenesis. Soil Sci. Soc. Am. J. 48: 853–858.

Verburg P.S.J., Johnson D.W. and Harrison R. 2001. Long-term nutrient cycling in Douglas-fir and red alder stands: a simulation study. For. Ecol. Manag. 145: 203–217.

Vitousek P.M. and Farrington H. 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. Biogeochemistry 37: 63–75.

Walbridge M.R., Richardson C.J. and Swank W.T. 1991. Vertical distribution of biological and geochemical phosphorus subcycles in two southern Appalachian forest soils. Biogeochemistry 13: 61–85.

Walbridge M.R. and Vitousek P.M. 1987. Phosphorus mineralization potential in acid organic soils: processes affecting $^{32}PO_4^{3-}$ isotope dilution measurements. Soil Biol. Biochem. 19: 709–717.

Walker T.W. and Syers J.K. 1976. The fate of phosphorus during pedogenesis. Geoderma 15: 1–19.

Wolf D.C. and Skipper H.D. 1994. Soil sterilization. In: Weaver R.W. (ed.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical properties. Book series no. 5. Soil Science Society of America, Madison, pp. 41–49.

Zou X., Binkley D. and Doxtader K.G. 1992. A new method for estimating gross phosphorus mineralization and inmobilization rates in soils. Plant Soil 147: 243–250.