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Evaluation of the phosphorus status of P-deficient podzols in temperate pine stands: combining isotopic dilution and extraction methods

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Abstract Phosphorus (P) is often a limiting factor of forest growth but our knowledge of the processes governing P availability in forest soils is rather limited. In the present work, we combined a isotopic dilution method with extraction methods to evaluate the P status in *Pinus pinaster* plantation forests on highly P-deficient soils. Total, organic, and inorganic P, dissolved and diffusive P, i.e. ionic P species that can be transferred from the solid phase to the soil solution due a gradient of concentration, were determined to a soil depth of 120 cm in a gradient of 18 forest sites (seven humid sites, five mesic sites, and six dry sites). Our objective was to assess the potential contribution of organic and inorganic P to plant available P. Based on results and our original assumptions, we observed that the contribution of organic P fractions (mineralization of soil organic P) to P availability related to the contribution of inorganic P fractions (diffusive P for durations up to 1 year) was predominant in litter, less important in top soil horizons, and negligible at depths below 30 cm. This was partly due to a decreasing proportion of organic P and an increasing proportion of diffusive P with soil depth. Owing to a very low amount of diffusive P in the top soils in dry sites, the relative contribution of organic P was actually higher in these sites than in humid and mesic sites, despite a lower overall organic P fraction. The combination of extraction and isotopic dilution methods in our study shed new light on P status in this forest range. In particular, these methods enable assessment of both the size of the pools and their dynamic fractions.

Keywords Available phosphorus · P-deficiency · Phosphorus stocks · *Pinus pinaster* · Podzol

Introduction

Phosphorus (P) is an essential element for plants and is often the first or second limiting factor of plant growth in forests, grasslands, and cultivated lands (Attiwill and Adams 1993; Aerts and Chapin 2000; Comerford et al. 2002). Maintaining adequate levels can be a challenging task. For economic, environmental, and biomass production reasons, appropriate diagnostic systems are needed to assess the P supply capacity of the soil over the long term and to adapt management practices particularly fertilisation accordingly. Up to now, most diagnostic tools for the evaluation of plant available P in forest soils have been based on single extraction methods (Bonneau

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et al. 2003) or sequential chemical extraction procedures (Chen et al. 2000; Comerford et al. 2002). These methods have proved useful for routine analyses of available P. However, such extraction methods are not based on comprehensive or even basic concepts of which processes are actually involved in making soil P potentially available to plants. Such concepts have resulted in the elaboration of process-oriented modeling of changes in plant-available P due to different management practices in agricultural croplands and grasslands (Morel et al. 2004; Stroia et al. 2007b) but not in forests. The rationale behind these process-based studies is that plant roots absorb P mainly as ionic species from the soil solution, which in turn, is replenished by the soil solid phase. The release of ionic P species from soil constituents to soil solution is potentially governed by several physicochemical (dissolution–precipitation, adsorption–desorption and diffusion) and biological (mineralization-immobilization, root and rhizosphere activity) mechanisms (Frossard et al. 2000; Hinsinger 2001; Morel 2002). Among the former, the use of an isotopic dilution method has demonstrated that the diffusion of ionic P species at the solid-to-solution interface, i.e. movement of P ions between solid and liquid phases of soil over time due to a concentration gradient (Jungk and Claassen 1997) was the prevailing process governing P availability in soils under agricultural cropping (Frossard et al. 1994; Morel and Plenchette 1994; Fardeau 1996). Among biological mechanisms, mineralization of organic P plays a key role in forests and grasslands since organic P generally represents a high proportion of total P in these systems (Harrison 1983; Attiwill and Adams 1993; Ross et al. 1999; Chen et al. 2003a). Hitherto the most important process in agricultural contexts—i.e. the diffusion of phosphates at the soil-to-solution interface—has never been extensively studied in forests, and has never been adequately compared to the relative contribution of the organic fractions. Ideally, for successful evaluation, the forest soils studied should be in a context of high P-deficiency to enable expression of the full response of P biogeochemical functioning. Furthermore, the soils should have different quantities of organic P so that the relative importance of organic P can be assessed.

The forest range of the Landes of Gascogne in southwest France covers a surface of about 900,000 ha and is essentially composed of highly productive stands of maritime pine (*Pinus pinaster* Ait in Soland). Soils are P-deficient sandy podzols and P fertilization has been a common management practice since the 1960s (Trichet et al. 2000). Due to a difference in topology related to the hydraulic architecture of the surface water streams, the development of soils and their associated understorey have diverged over time. Soils range from humid moorlands, via mesic moorlands to dry moorlands, and more organic material has accumulated in the more humid sites (Augusto et al. 2006). Due to this gradient in organic matter, this forest range provided an excellent opportunity to compare the assumed relative contributions of organic and inorganic P fractions for P availability to the trees.

Our objective was to assess the potential contribution of organic and of inorganic P to plant-available P in this forest range. To this end, we used a gradient encompassing the representative site classes of this forest area and evaluated contents and stocks of total, organic and inorganic soil P, and soluble and diffusive ionic P species down to a soil depth of 120 cm. Soil organic P mineralization was also evaluated. We hypothesized that the contribution of organic P to total P stocks and/or P fluxes would be larger in humid moorland sites with more litter and deeper organo-mineral horizons than in drier sites, relative to the respective contribution of inorganic P. Furthermore, we hypothesized that the relative contribution of organic P fractions would be negligible below a depth of 30 cm due to assumed very low amounts of organic material below this depth (Augusto et al. 2006; Achat et al. 2008). To our knowledge, this is the first large-scale evaluation of P status in forests that combines the use of an isotopic dilution method to assess the diffusive P status, with an assessment of total P and organic P pools by ignition extraction procedures.

Materials and methods

Site characteristics and soil sampling

The study soils were all located in the forest range of the Landes of Gascogne in southwest France. Mean annual temperature averages 12.5°C and mean annual precipitation is about 900 mm. Topography is nearly flat with slopes generally less than 2% and elevations ranging between 10 and 105 m above sea level. Four distinct site classes can be distinguished in the region: humid moorlands (37% of the surface area), mesic moorlands (41%), dry moorlands (14%) and dunes (7%) featuring differences in soil formation and types of understorey vegetation (Trichet et al. 1999; Augusto et al. 2006). Soils have developed from sandy ($\sim$95% fine and coarse sands) aeolian parent material deposited in the Pleistocene and Holocene and are acidic and highly organic (Trichet et al. 1999; Augusto et al. 2006). They can be classified as Entic to Albic Podzols (FAO/IUSS 2006), depending on the depth of the water table, and lenses of a cemented spodic horizon can occur between 40 and 100 cm of soil depth (Trichet et al. 1999). A typical moorland soil profile has a humus layer, an organic A horizon, an eluvial E horizon and a cemented Bt horizon before reaching the sandy parent material, but the depth, composition and continuity of these horizons vary between site classes. Soil profiles are generally richer in humus and have a thicker organic layer (A horizon) in the humid moorlands than in drier sites (Trichet et al. 1999; Jolivet et al. 2003; Augusto et al. 2006). At the littoral fringe Arenosols are also frequent and Gleyic Podzols occur at some undrained sites.

Soils were sampled at 18 different sites (seven humid sites, H; five mesic sites, M; six dry sites, D). The dry sites include two coastal dunes, one inland dune site and three dry moorlands. All mesic and humid sites were moorlands. At every site, systematic sampling was conducted over an area of 280 m^2 with reasonably uniform vegetation and with no recent disturbance of the soil surface. Sampling locations were arranged on two orthogonal diagonals of 25 m. At 12 core sample points, litter (sum of L + F + H layers, H layer not always present in the studied soils) and mineral soils were sampled to a depth of 30 cm using a soil corer (internal diameter of 8 cm). At six points out of the 12, soils from 30 to 120 cm were sampled using a percussion drill (internal diameter of gouge 4 cm). Mineral soil samples were divided into 15 cm or 30-cm thick layers: 0–15, 15–30, 30–60, 60–90, and 90–120 cm. The depth of the litter and the mineral soil horizons was measured at each point. The 6 or 12 samples of a given layer in a given site were grouped and mixed to obtain a composite single sample. In total, 18 litter samples and 90 soil samples were collected for analysis. Coarse fresh plant debris was removed by sieving to 2 mm and soils were dried at 70°C before analysis. The litter samples were dried at 65°C for 48 h, weighed and ground. All results are expressed based on dry weight. Under storage conditions, the residual moisture of the soil samples was lower than 1% irrespective of soil depth. These soils samples (108 mineral soil or litter samples) constitute a first series that was used for the evaluation of most of P status variables (total, organic and inorganic P and dissolved and diffusive ionic P species) as a function of soil depth and site class.

For the estimation of soil organic P mineralization rate, a complementary series of soils was used. In these, the mineralization fluxes in samples from top soils of dry sites and humid sites were determined during an incubation experiment. Soils were sampled with a soil corer (8 cm diameter of corer) in 11 humid sites and 10 dry sites from the top mineral soil layer to a depth of 0–15 cm after removing the litter layer. For each site, six soil samples were mixed to obtain one composite sample. In the laboratory, coarse fresh plant debris was removed by sieving at 4 mm and moist soil was stored at 4°C before incubation. Subsamples of the soil for each of the sites were dried at 70°C and sieved at 2 mm before determination of total carbon (C; NF ISO 10694; Afnor 1999), total P and organic P.

Determination of total, organic and inorganic P

Total P content was determined by ICP following calcinations at 450°C and wet-digestion by concentrated fluoric acid (HF) and perchloric acid ($HClO_4$) of litter and soil samples (NF X 31-147, Afnor 1999). This was preferred to other extractants as HF extracts considerably more P from the soil. For instance, in the 90 samples of our soils, total P-HF was 65% higher than P determined after H_2SO_4 extraction and calcination (P-HF = 1.65 P-H_2SO_4, $R^2 = 0.79$, $P < 0.0001$). Organic P content was determined in triplicate for each sample using the Saunders and Williams's (1955) procedure. One gram of mineral soil or litter was ignited at 550°C for 4 h. Both ignited and non-ignited soil samples were extracted with 50 ml of 0.2 N H_2SO_4 for 16 h. After filtration at 0.2 μm, the P concentration in solution was determined using the green malachite colorimetric method (van Veldhoven and Mannaerts 1987) with a spectrophotometer (Camspec M330) at a wavelength of 610 nm. The organic P content was calculated as the difference between the ignited and the non-ignited samples. Subsequently, inorganic P was defined as the difference between total P-HF minus organic P.

Gross amount of diffusive P and soil solution P ions concentration

The gross amount of diffusive P ions that equilibrates P ions in solution was determined as a function of time using an isotopic labeling and dilution procedure (Fardeau 1993, 1996; Frossard and Sinaj 1997). This was performed in two steps. The first step consisted of preparing a soil suspension that was left to equilibrate for 16 h to reach a steady state (constant Cp = constant P ion concentration in the solution) for the few subsequent hours during which isotopic dilution analysis was carried out. The P ions in the solution of pre-equilibrated soil suspensions were isotopically labeled by introducing a known amount of ^{32}P as phosphate ions (*R*, about 30 kBq) into the solution. This isotopic tracer is then uniformly and instantaneously dispersed. Since the amount *R* of carrier-free ^{32}P ions is negligible compared to the total amount of ionic P in solution (*R* is generally 10^5 fold smaller than Pw, the amount of unlabeled P ions initially present in the solution), the Pw value does not change. In such a steady state, the gross rate of P desorption from the soil solid phase to solution is equal to the gross rate of P sorption onto the solid phase. We further assumed that no isotopic discrimination occurs between the two P isotopes ($^{31}PO_4$ and $^{32}PO_4$) during the exchange between the solution and the soil solid phase. It is thus possible to measure the amount of unlabeled soil P newly transferred to solution, by determining the Pw value and the ratio of radioactivity to the total amount of P ions in the solution, called the isotopic composition ratio (IC). The amount (*E*) of P ions in which *R* is diluted, called the amount of isotopically exchangeable P, includes both Pw and the gross amount (Pr) of P ions transferred between solid and liquid phases of soil in suspension (Hamon et al. 2002). By definition, all fractions of *E* have the same IC, giving:

$$\mathrm{IC} = {}^{R}\!/_{E} = {}^{r}\!/_{\mathrm{Pw}} = {}^{(R-r)}\!/_{\mathrm{Pr}} \tag{1}$$

where r/Pw and $(R - r)/\mathrm{Pr}$ are the IC ratio of Pw and Pr, respectively, and *r* is the radioactivity remaining in solution at time *t*. The *E* and Pr values were calculated as follows:

$$E = \frac{\mathrm{Pw}}{{}^{r}\!/_{R}} \tag{2}$$

$$\mathrm{Pr} = \frac{\mathrm{Pw}(R-r)}{r} = \mathrm{Pw}\left(\frac{1}{r/R} - 1\right) \tag{3}$$

The total amount of inorganic P and the difference total inorganic P minus Pw are the maximum values (limit values) of *E* and Pr, respectively.

Seven soil suspensions were prepared (one replicate for each isotopic dilution period: 10, 40, 100, 400, 1,000, 4,000, and 10,000 min) for each of the mineral soil and litter samples. Fewer than seven replicates were prepared for 4 litter samples because the amount of litters collected in the field was not sufficient to produce all the suspensions. Soil samples were added to distilled water at a soil:solution ratio of 10 g:14.9 ml including 0.1 ml of a biocide (‘Micro-O-protect’ containing bromo-nitro-dioxane 2-methylisothiazolone and ethanol, Product 1585 720, Boehringer Mannheim Corporation, Indianapolis, IN, USA) to avoid microbial activity. The mass-to-solution ratio for the litter samples was 1 g:9.9 ml. The mineral soil and litter suspensions were then equilibrated for 16 h at 20 ± 2°C, gently stirred on a roller (40 cycles min^{-1}). The volume (0.1 ml) of carrier-free ^{32}P ions (*R*) solution was introduced into the soil suspension at time zero, giving soil-to-solution ratios of 10 g:15 ml and 1 g:10 ml for mineral soil and litter, respectively. Approximately 2.5 ml of soil suspension were sampled with a plastic syringe after 10, 40, 100, 400, 1,000, 4,000, and 10,000 min and immediately filtered through 0.2 μm membrane filters. The filtered solution was used to determine the $^{32}PO_4$ radioactivity (*r*) remaining in solution for a given time of isotopic dilution. A given soil suspension was sampled only one time. In parallel, the same procedure was carried out in distilled water containing the biocide only (without soil) to determine *R* at any given time. The *r* and *R* were counted together in the counter (Packard TR 1100) using a liquid scintillation cocktail and the isotopic dilution ratio (*r*/*R*) was calculated.

A theoretical equation, adapted from Fardeau (1993), was used to fit the experimental values of the isotopic dilution ratio:

$$r/R = m\left(t + m^{1/n}\right)^{-n} \text{for } r/R \geq r_\infty/R \qquad (4)$$

where r_∞/R corresponds to the maximum possible dilution of the isotope considering that all inorganic P can take part in the isotopic dilution. The value of r_∞/R is calculated as the Pw to total inorganic P ratio (Fardeau 1993). The parameter m is the coefficient that accounts for immediate physical–chemical reactions. It represents the fraction of radioactivity (r/R) remaining in solution after 1 min (Fardeau et al. 1991; Fardeau 1993). The parameter n accounts for slow physical–chemical reactions and describes the rate of disappearance of the tracer from the solution after 1 min (Frossard and Sinaj 1997). It corresponds to the coefficient, i.e. the slope, of the regression of log r/R with log t (Fardeau et al. 1991; Fardeau 1993). The theoretical n parameter ranges from 0 to 0.5. When n parameter is zero, there is no diffusion at the solid to solution interface whereas when 0.5 is reached, diffusion is theoretically the greatest. In practice, n could reach values up to 0.65. These parameters are useful since they permit an evaluation of phosphate reactivity with the solid constituents or phosphate mobility in the soil–water system (Frossard et al. 1989). Parameters m and n can also provide an estimation of the fixing capacity and the P buffering capacity of phosphate anions (Frossard et al. 1989; Morel 2002; Chen et al. 2003b). P reactivity and P fixing and P buffering capacities increase with decreasing m and/or increasing n. In addition, m and n allow an estimation of the gross amount Pr for more relevant time scales using successively Eqs. 3 and 4.

The phosphate concentration in clear solution (Cp, mg P l^{-1}), i.e. after filtration of soil suspension at 0.2 µm, was determined for all soil samples using the malachite green colorimetric method (van Veldhoven and Mannaerts 1987). Absorbance was measured in 1 or 10 cm quartz cells of long optical path lengths, depending on the P concentration of the samples. All concentrations were higher than the detection limit (5 µg P l^{-1} = the mean of ten blanks plus three times the standard deviation for 1 cm and 2 µg P l^{-1} for 10 cm length of optical path, respectively). Pw (in mg kg^{-1}) was then calculated using Eq. 5.

$$\mathrm{Pw} = \frac{\mathrm{Cp} \times V}{M} \qquad (5)$$

with V equal to 15 and 10 ml for mineral soil and litter, respectively, and M equal to 10 and 1 g for soil and litter, respectively.

Incubation experiment

Following Achat (2009), organic P mineralization was estimated using the C mineralization rate and the total organic P content (Eq. 6).

$$\text{Mineralized organic P} = \frac{\text{C mineralization rate} \times \text{total organic P}}{100} \qquad (6)$$

where mineralized organic P, C mineralization rate and total organic P are expressed in mg kg^{-1} $year^{-1}$, % of total C $year^{-1}$ and mg kg^{-1}, respectively.

Moist fresh soils (70% water holding capacity) equivalent to 25 g dry soil were placed in 750 ml-jars and incubated at obscurity at 28°C during 4 weeks for the determination of soil respiration. The CO_2 liberated from soil and absorbed by 10 ml of 0.5 M NaOH was measured using the back titration method (Alef 1995). Here, we used the C mineralization rate under basal (equal to constant) condition for soil respiration, i.e. after the initial microbiological disturbance resulting from soil sampling, sieving and initial sample handling. Similarly to Achat (2009) and Oehl et al. (2004), 2 or 3 weeks were necessary to reach constant soil respiration. Therefore, we used the C mineralization rate during the last week of incubation, between day 21 and day 28. Thereafter, this rate measured at 28°C and Eq. 7 describing the temperature dependence of soil respiration (Kirschbaum 1995) permitted to calculate the C mineralization rate for temperatures corresponding to field conditions (annual mean temperature in the top soils estimated at 15°C).

$$k = \exp[-3.764 + 0.204T(1 - 0.5T/36.9)] \qquad (7)$$

where k is the relative rate of C mineralization, T is the considered temperature in °C, 36.9 is the optimal temperature in °C and −3.764 and 0.204 are constants.

Data handling and statistics

To calculate diffusive P (Pr), suspensions of a given soil sample must have a constant Cp value during isotopic dilution (steady state). Since Cp values varied significantly after 1,000 min in some of the litter and topsoil samples, we did not use r/R and Cp values measured at 4,000 and 10,000 min to determine m and n parameters. The Pr values up to 10,000 min were recalculated using Eqs. 3 and 4 and the respective limit values (total inorganic P minus Pw). Values were further extrapolated to more relevant time spans (i.e. 1 year) for the discussion. In total, organic, inorganic and diffusive P, the phosphorus stocks (in kg ha^{-1}) were calculated from the P contents (in mg kg^{-1}), the thicknesses of soil layers and the bulk density. For P ions in solution, the P stock was calculated from Cp (in mg l^{-1}), the thicknesses of soil layers and the available water capacity. The available water capacity (AWC in mm) and the bulk density (BD in Mg m^{-3}) were calculated using pedo-transfer functions (Chossat 1992 for AWC; L. Augusto and DL. Achat unpublished data for BD) that were fitted on soil samples from the same region. Primary data were used to compute elaborate variables (contents in mg kg^{-1} and stocks in kg ha^{-1}, isotopic dilution kinetic parameters) that were used to perform tests and to compute means, standard errors, ranges (minimum and maximum) and coefficients of variation (CV = standard deviation × 100/mean). Since P status values between two successive soil layers were not independent in a given site, a mixed linear model with site as a random effect was performed to test the general effects of depth and site class and their interaction for each P status variable. Analysis of variance (one-way ANOVA) was performed to test the site class effect in a given layer. The Bonferroni adjustment was used to assess the differences between soil depths within or among site classes. ANOVA was performed after log- and arcsine-transformations of the amounts and the percentages, respectively. Non-linear regressions were performed to determine parameters of isotopic dilution kinetics (r/R as a function of time) and to analyze the relationships between the different variables (Cp versus n and m parameters and Pr versus Cp). Statistical analyses were carried out using SYSTAT software (SYSTAT version 10, 2000) and SAS software (SAS Institute Inc., 1999).

Results

Conditions and parameters for the isotopic dilution method

The experimental conditions relative to the use of the isotopic dilution method were the following. Concentrations of P ions in solution (Cp in mg l^{-1}) were at steady state up to 1,000 min of isotopic dilution (linear regression of Cp with time, $P > 0.05$). The data for all 18 sites and six mineral soil or litter layers show that Eq. 4 closely ($R^2 \geq 0.99$) and significantly ($P < 0.0001$) fitted the r/R values decreasing with time in the experiment. The isotopic exchangeable P (E in mg kg^{-1}) and the gross amount of diffusive P (Pr in mg kg^{-1}) increased over time at rates that drastically decreased over time (power function). Isotopic dilution parameters (m and n, Table 1) were affected by soil depth, site class and their interaction and could vary by as much as two or three orders of magnitude within a given soil layer. In general, m significantly decreased and n increased with soil depth (Table 1) but both did so more rapidly in H and M sites than in D sites. The parameter n was more variable in surface layers (litter, 0–15 and 15–30 cm) than in subsoil layers (30–120 cm). In the 60–90 and 90–120 cm layers, n was always close to 0.5. With respect to site class, the m parameter generally reached its highest values and the n parameter its lowest values in D sites (Table 1).

P ions in solution and diffusive P

The concentration of P ions in the solution (Cp) of soil suspensions was significantly affected by soil depth, site class and their interaction (Table 2). For all sites, Cp drastically decreased with soil depth from 7.6–9.9 mg l^{-1} in litter to 0.003–0.01 mg l^{-1} in 90–120 cm. In a given layer, Cp varied from one to three orders of magnitude, and site class had a significant effect on Cp (Table 2). In the 0–30 cm soil layer, Cp was significantly higher in D sites than in M sites (Table 2). There was a close and negative relationship between Cp and n ($R^2 = 0.81$, $P < 0.0001$, Fig. 1a) regardless of site class. Similarly, there were significant positive relationships (power functions, $P = 0.001$ or $P < 0.0001$) between m and Cp in each soil layer except for the litter layer. The gross amount of diffusive P (Pr) increased

Table 1 Estimates of m and n parameters that describe the isotopic dilution kinetics

Site class	m		n	
Litter				
Humid	1.01 (0.01) a	A	0.01 (0.003) a	A
Mesic	1.10 (0.06) b		0.03 (0.02) b	
Dry	1.00 (0.001) a		0.0004 (0.0004) a	
0–15 cm				
Humid	0.60 (0.15) a	B	0.22 (0.07) a	B
Mesic	0.27 (0.07) a		0.50 (0.08) b	
Dry	1.12 (0.09) b		0.05 (0.03) a	
15–30 cm				
Humid	0.42 (0.16) a	BC	0.33 (0.04) ab	B
Mesic	0.08 (0.02) a		0.39 (0.02) a	
Dry	1.29 (0.25) b		0.18 (0.05) b	
30–60 cm				
Humid	0.07 (0.02) a	BD	0.46 (0.02) a	C
Mesic	0.05 (0.01) a		0.46 (0.01) a	
Dry	1.14 (0.46) b		0.38 (0.04) a	
60–90 cm				
Humid	0.08 (0.02) a	D	0.46 (0.03) a	CD
Mesic	0.05 (0.01) a		0.52 (0.03) a	
Dry	0.52 (0.26) a		0.44 (0.01) a	
90–120 cm				
Humid	0.11 (0.03) a	CD	0.52 (0.03) a	D
Mesic	0.05 (0.01) a		0.52 (0.02) a	
Dry	0.75 (0.41) a		0.46 (0.02) a	
Depth	<0.0001		<0.0001	
Site class	0.0006		<0.0001	
Depth × site class	0.0004		<0.0001	

Standard error of the mean is given in parentheses (number of sites was 7, 5 and 6 for humid, mesic and dry sites, respectively). General effects of depth, site class and their interaction are presented. Different small letters denote significant differences between site classes at $P < 0.05$ or lower for a given layer. Different capital letters denote significant differences between layers at $P < 0.05$ or lower

significantly with isotopic dilution time in each soil layer (Table 2). Values were 0.5–12.5 mg kg^{-1} in litter and 1.3–39.2 mg kg^{-1} at a depth of 0–15 cm after 10,000 min of isotopic dilution. In the mineral soil $\mathrm{Pr}_{10{,}000\ \mathrm{min}}$ attained values up to 47–90 and 50–96% of the limit values (total inorganic P − Pw) in H and M sites, respectively and this was significantly higher than the 6–35% in D sites (Table 2). Pr was negatively and significantly correlated to Cp (Fig. 1b) in each layer ($P \leq 0.017$) with the exception of litter ($P = 0.57$; data not shown on graph) and the 90–120 cm layer ($P = 0.91$). Isotopically exchangeable P (E, sum of Pw and Pr) amounted to 92 ± 7 mg kg^{-1} in litter and 19 ± 4 mg kg^{-1} in the 0–15 cm layer after 10,000 min and this value decreased with soil depth.

Total, organic and inorganic P contents

Total P (P-HF), organic P and inorganic P contents were all significantly affected by soil depth ($P < 0.0001$) and site class ($P \leq 0.004$). But only total P and organic P were affected by their interaction ($P \leq 0.0023$). Total and organic P were highest in the litter layer (285 ± 10 and 225 ± 8 mg kg^{-1}, respectively; $n = 18$) and decreased significantly with depth (Fig. 2; data not shown for organic P but evolution with depth similar to that of total P). In the 0–15 cm layer this was 60 ± 5 mg kg^{-1} for total P and 32 ± 3 mg kg^{-1} for organic P. At 90–120 cm soil depths, these values were 40 ± 3 and 9 ± 1 mg kg^{-1}, respectively. This decrease was significantly faster for both variables and occurred at shallower depths in D sites than in M and H sites (Fig. 2). Inorganic P contents were significantly higher in litter (56 ± 7 mg kg^{-1}) than in the 0–120 cm soil profile ($P < 0.0001$) and did not change significantly from 0–15 to 90–120 cm. Inorganic P ranged from 2 to 46 mg kg^{-1} in the entire soil profile with a mean value of 27 ± 1 mg kg^{-1} ($n = 90$). Total, organic and inorganic P contents varied considerably in any given soil horizon (CV% up to 55%) with the exception of the litter layer. For total and organic P, this variability was essentially related to different site classes with significantly lower amounts in D sites than in M and H sites (Fig. 2). The lowest values for the D sites corresponded to the two coastal dune sites. For inorganic P, the site class was not significant for any of the soil depths ($P > 0.08$).

Relative proportions of P fractions

The proportion of organic P to total P (Fig. 3) followed the same trend with depth as that of total P or organic P contents (Fig. 2) and the values were highest at the surface (79 ± 2% in litter, 55 ± 3% in 0–15 cm; $n = 18$). This proportion decreased

Table 2 P ion concentration in soil solution (Cp) and gross amount of diffusive P (Pr) as a function of time

Site class	Cp (mg l^{-1})	Pr				Limit value of Pr	
		10 min mg kg^{-1}	100 min	1,000 min	10,000 min	mg kg^{-1}	%$Pr_{10,000\ min}$
Litter							
Humid	9.12 (0.60) a	0.40 (0.22) a	1.26 (0.57) a	3.09 (1.22) a	3.09 (1.22) a	3.09 (1.22) a	100
Mesic	9.90 (1.91) a	0.79 (0.43) a	5.65 (1.46) b	12.52 (3.17) b	12.52 (3.17) b	12.52 (3.17) b	100
Dry	7.59 (0.91) a	0.15 (0.10) a	0.54 (0.19) a	0.53 (0.26) a	0.53 (0.26) a	0.53 (0.26) a	100
0–15 cm							
Humid	1.47 (0.63) ab	1.20 (0.24) a	2.82 (0.67) a	6.36 (1.82) a	13.70 (4.47) a	20.21 (4.96) a	62 (12) a
Mesic	0.15 (0.09) a	1.84 (0.38) a	6.17 (1.43) a	21.25 (7.89) b	39.20 (4.58) b	44.15 (2.88) a	88 (7) a
Dry	2.32 (0.59) b	0.17 (0.04) b	0.38 (0.08) b	0.70 (0.20) c	1.30 (0.45) c	20.72 (2.30) a	6 (1) b
15–30 cm							
Humid	0.26 (0.12) ab	1.29 (0.30) a	3.26 (0.74) a	7.89 (1.92) a	15.17 (3.03) a	20.40 (1.68) a	72 (12) a
Mesic	0.03 (0.01) a	1.55 (0.41) a	4.03 (1.17) a	10.38 (3.26) a	21.31 (5.93) a	33.78 (6.74) a	61 (15) ab
Dry	0.58 (0.22) b	0.17 (0.07) b	0.47 (0.14) b	1.03 (0.28) b	2.02 (0.62) b	19.93 (3.55) a	10 (3) b
30–60 cm							
Humid	0.02 (0.005) a	1.23 (0.20) a	3.64 (0.66) a	10.82 (2.34) a	19.28 (3.26) a	22.24 (2.96) a	90 (10) a
Mesic	0.01 (0.0004) a	1.67 (0.50) a	4.77 (1.30) a	12.92 (2.84) a	25.31 (3.53) a	29.30 (2.83) a	86 (10) a
Dry	0.06 (0.03) a	0.25 (0.10) b	0.64 (0.21) b	1.54 (0.43) b	3.66 (0.88) b	20.23 (3.47) a	21 (5) b
60–90 cm							
Humid	0.02 (0.004) a	0.95 (0.15) a	2.91 (0.57) a	9.01 (2.27) a	22.23 (4.75) a	29.61 (4.14) a	75 (10) a
Mesic	0.01 (0.0004) a	1.43 (0.36) a	4.65 (1.07) a	15.30 (3.42) a	32.37 (4.34) a	33.55 (4.16) a	96 (4) a
Dry	0.02 (0.004) a	0.34 (0.12) b	0.93 (0.29) b	2.51 (0.74) b	6.73 (1.87) b	25.13 (4.33) a	35 (12) b
90–120 cm							
Humid	0.01 (0.01) a	0.63 (0.37) a	1.96 (1.06) a	6.11 (2.98) a	13.40 (3.04) a	31.06 (4.97) a	47 (11) a
Mesic	0.003 (0.0004) a	0.44 (0.15) a	1.54 (0.59) a	5.39 (2.35) a	16.00 (6.28) a	34.85 (6.21) a	50 (16) a
Dry	0.01 (0.004) a	0.18 (0.06) a	0.51 (0.14) a	1.42 (0.33) a	3.95 (0.79) b	25.90 (4.21) a	19 (6) a
Depth	<0.0001	<0.0001	0.0005	0.002	<0.0001	<0.0001	<0.0001
Site class	0.003	<0.0001	<0.0001	<0.0001	<0.0001	0.0009	<0.0001
Depth × site class	0.03	0.02	0.0001	0.0009	<0.0001	0.06	0.03

Standard error of the mean is given in parentheses (number of sites was 7, 5 and 6 for humid, mesic and dry sites, respectively). General effects of depth, site class and their interaction are presented. Different letters denote significant differences between site classes at $P < 0.05$ or lower for a given layer. For diffusive P (Pr), there was a significant time effect for each layer ($P \leq 0.007$)

significantly with depth (26 ± 3% at 90–120 cm; $n = 18$) and the decrease with depth was steeper in the D sites. The diffusive P content expressed as a percentage of inorganic P increased along the soil profile. We used the Pr values after 10,000 min (%$Pr_{10,000\ min}$) as an example of Pr values. Results were similar for other Pr values. The proportion of inorganic P that is $Pr_{10,000\ min}$ significantly increased as an average for the 18 sites from 6% in litter to 47% in the 0–15 cm layer and 68% in the 60–90 cm layer (Fig. 4). In general, proportions of $Pr_{10,000\ min}$ in D sites were significantly lower than those in H or M sites ($P < 0.05$), while there was no difference between H and M sites (Fig. 4).

P stocks

For the sum of litter plus the 0–120 cm soil profile, P stocks were 817 ± 51, 321 ± 26, and 496 ± 36 kg ha^{-1} for total, organic and inorganic forms, respectively (Table 3). For the inorganic forms, 4 ± 0.2 kg ha^{-1} corresponds to P ions in solution and 271 ± 44 kg ha^{-1} to diffusive P in 10,000 min. P ions in solution represented less than 1% of total P in the soil

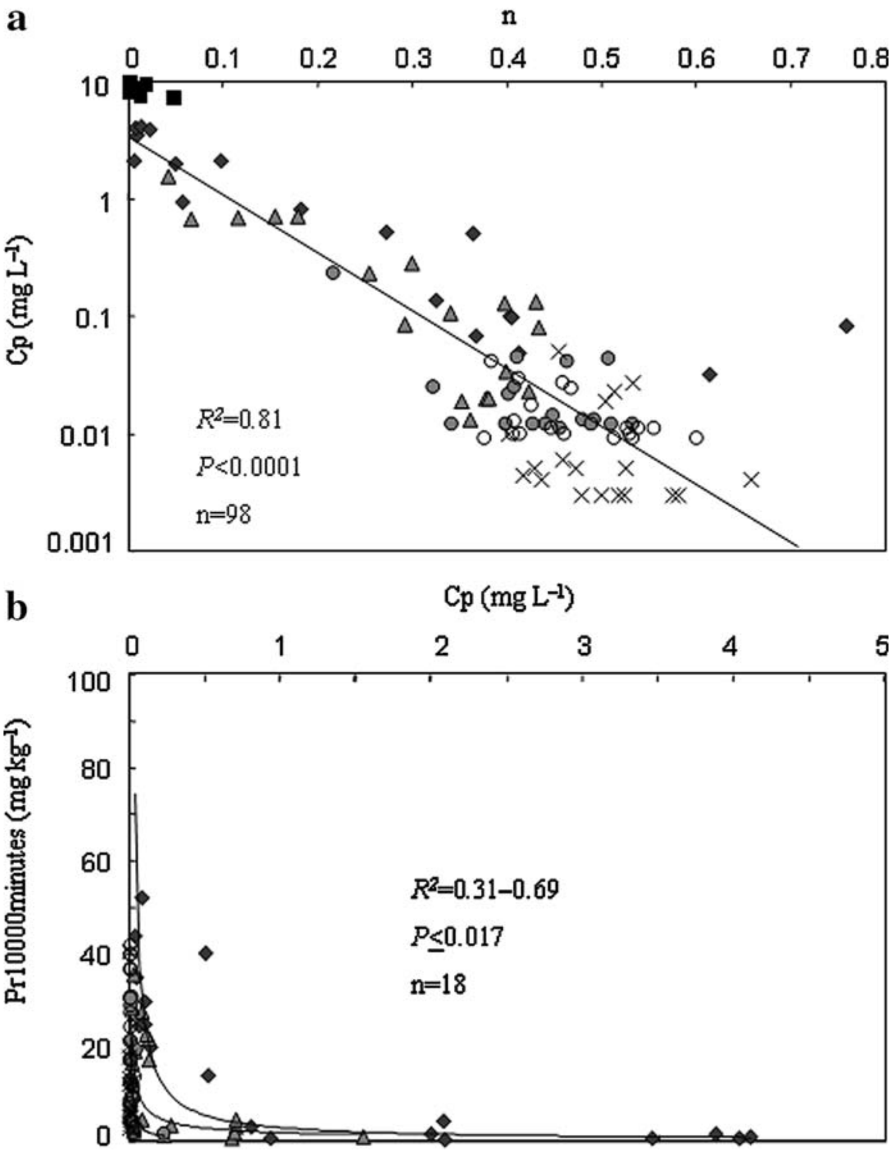

Fig. 1 Relationships between the concentration of P ions in solution (Cp) and the parameter *n* of isotopic dilution (**a**) respectively, the gross amount of diffusive P in 10,000 min ($Pr_{10,000\ min}$) and Cp (**b**). Probability and R^2 of the non-linear regressions are given. ■ litter, ◆ 0–15 cm, ▲ 15–30 cm, ● 30–60 cm, ○ 60–90 cm, × 90–120 cm

layers but about 20% in the litter layer. P stocks were generally significantly ($P \leq 0.01$) lower in D sites than in H and M sites except for total inorganic P and P ions in solution for which there was no site class effect ($P \geq 0.14$). Litter represented a small proportion of the total (litter plus 0–120 cm) stock of total, organic, inorganic and diffusive P (between 0.1 and 6%) but represented the major part of the total stock of P ions in solution (89%). In the soil, total, organic, inorganic and diffusive P stocks were distributed rather evenly between the 0–60 and 60–120 cm layers (Table 3).

Incubation experiment

P contents in the 21 top soils used in the incubation experiment (Table 4) were similar to those of the 18 other top soils (Figs. 2, 3). Total C content and daily C mineralization were in general higher in incubated soils from H sites than in incubated soils from D sites and soil respiration decreased with incubation time before reaching constant values after 14 days (Table 4). Under basal soil respiration and using values between day 21 and day 28, annual C mineralization rate was calculated to range from

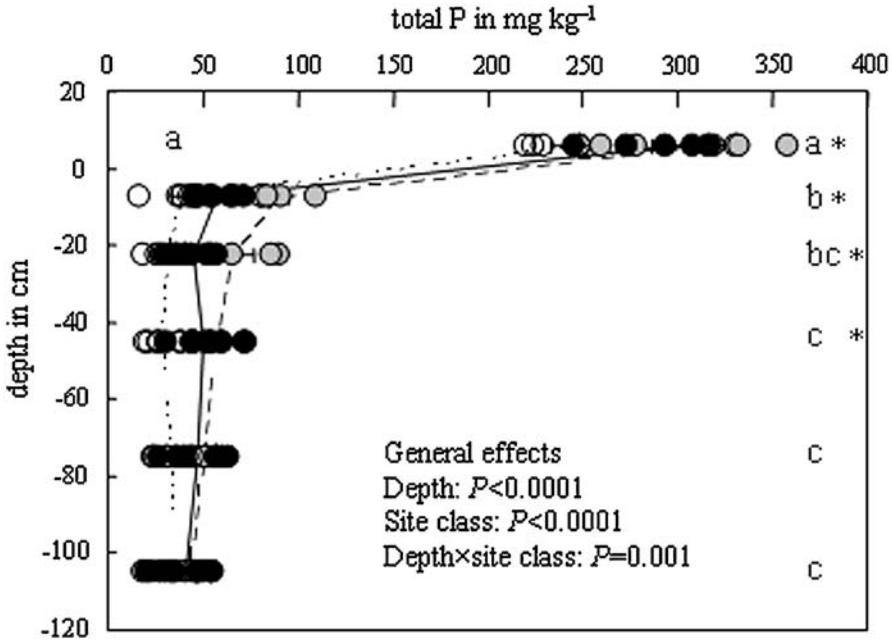

Fig. 2 Total P-HF content (fluoric acid extraction; in mg kg^{-1}) as a function of soil depth and site class. *Different letters* denote significant differences ($P < 0.05$) between soil and litter layers ($n = 18$ for each layer). The probabilities of the general site class and depth effects are given. Any significant ($P < 0.05$) site class effect for a given layer is denoted by *an asterisk*. ● Humid sites, ◍ mesic sites, ○ dry sites. *Dotted lines, dashed lines*, and *solid lines* represent means of dry, mesic and humid sites, respectively

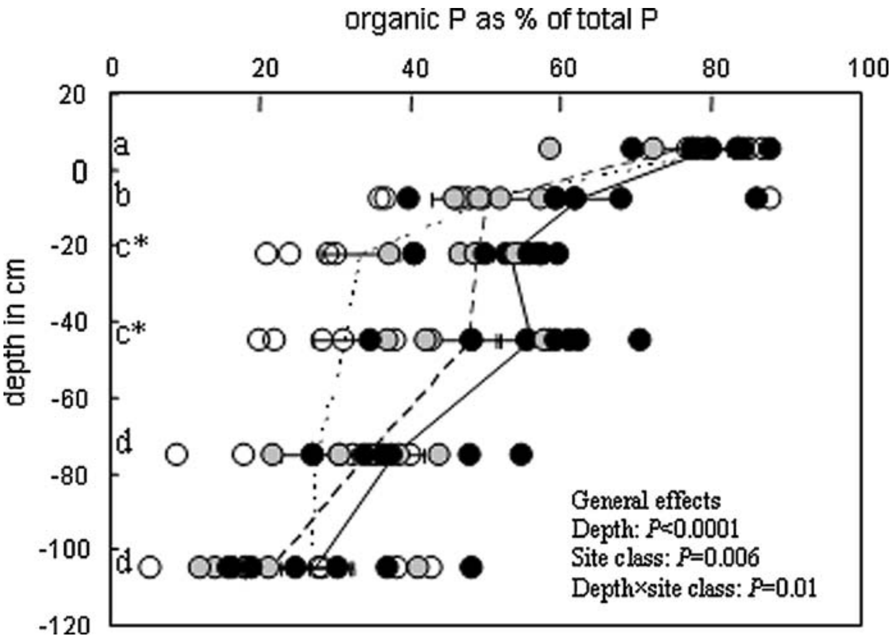

Fig. 3 Organic P as a % of total P-HF as a function of depth and site class. *Different letters* denote significant differences ($P < 0.05$) between the different soil and litter layers ($n = 18$ for each layer). The probabilities of the general site class and depth effect are shown. Any significant ($P < 0.05$) site class effect for a given layer is denoted by *an asterisk*. ● Humid sites, ◍ mesic sites, ○ dry sites. *Dotted lines, dashed lines*, and *solid lines* represent means of dry, mesic and humid sites, respectively

17–21 and 6–7% of total C year^{-1} for temperatures of 28 and 15°C, respectively. These rates were not significantly different between the two site classes. Assuming that the C and organic P have similar annual mineralization rate (in % of total organic content), organic P mineralization is estimated to be 2 mg kg^{-1} year^{-1} ($T = 15$°C; Table 4).

Discussion

General considerations in the study of P status in forests

The isotopic dilution method was successfully applied to assess the diffusive P status of our forest

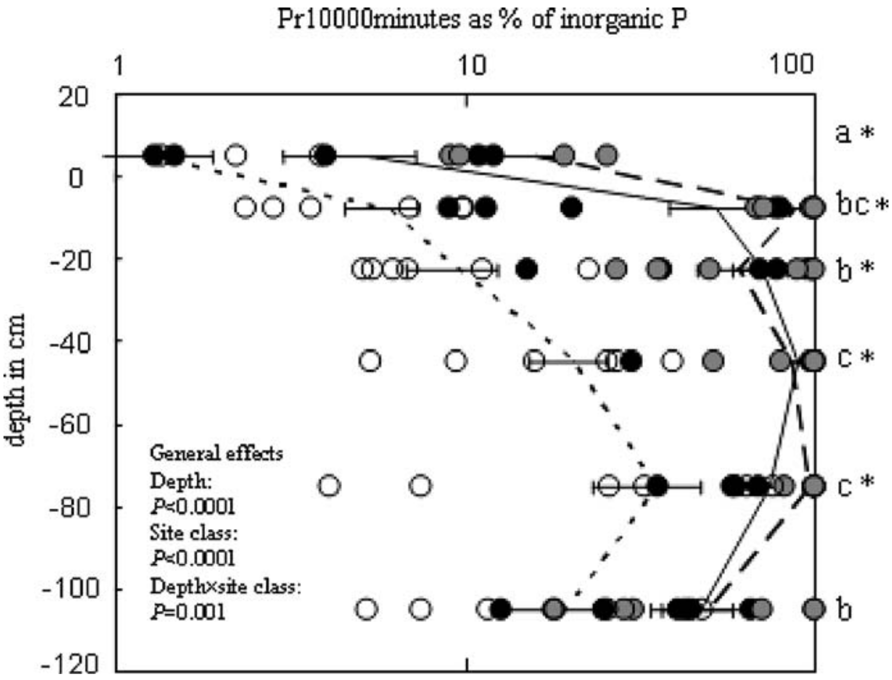

Fig. 4 $Pr_{10,000\ min}$ as a % of inorganic P as a function of soil depth and site class. *Different letters* denote significant differences ($P < 0.05$) between the different soil and litter layers ($n = 18$ for each layer). The probabilities of the general site class and depth effect are shown. Any significant ($P < 0.05$) site class effect for a given layer is denoted by *an asterisk*. ● Humid sites, ● mesic sites, ○ dry sites. *Dotted lines, dashed lines*, and *solid lines* represent means of dry, mesic and humid sites, respectively

Table 3 Phosphorus stocks in kg ha^{-1} in the entire litter plus soil profile (0–120 cm) and % in litter, in 0–60 cm and in 60–120 cm soil layers

	Total P	Organic P	Inorganic P		
			Total	Ionic P in solution	$Pr_{10,000\ min}$
Total stocks in litter plus 0–120 cm					
Range	479–1,133	136–495	206–706	2.4–5.9	32–667
Means					
All sites	817 (51)	321 (26)	496 (36)	4.0 (0.2)	271 (44)
Humid sites	858 (75) ab	383 (28) a	475 (56) a	4.4 (0.3) a	311 (50) a
Mesic sites	990 (65) a	390 (26) a	600 (55) a	4.1 (0.5) a	449 (65) a
Dry sites	626 (58) b	191 (17) b	435 (67) a	3.5 (0.3) a	76 (16) b
% of total stocks in litter, in 0–60 cm of soil or in 60–120 cm					
% in litter	3 (0.3)	6 (1)	1 (0.1)	89 (3)	0.1 (0.04)
% in 0–60 cm	48 (2)	58 (2)	42 (2)	11 (3)	43 (3)
% in 60–120 cm	49 (2)	36 (2)	57 (2)	0.4 (0.1)	57 (3)

Standard error of the mean is given in parentheses. Number of sites was 7, 5 and 6 for humid, mesic and dry sites, respectively. Different letters denote significant differences ($P < 0.05$) between site classes

podzols in a large range of experimental conditions. These encompassed a gradient of sites, both litter and mineral soils down to a soil depth of 120 cm. These soils cover the entire theoretical range of soils with respect to P reactivity (Table 1) and include both soils that were nearly not reactive (i.e. dilution parameter $n = 0$) and soils that had the highest possible reactivity (n close to 0.5; Fardeau et al. 1991). The values obtained for P ion concentration in solution in the top soil are comparable to those reported in the literature (Table 5). Conversely, values for diffusive P in our soils were in a lower range than values reported in the literature for grasslands and agricultural croplands (Table 5); no data for forest contexts were available for comparison. Values of diffusive and dissolved P ions in our

Table 4 C and P contents in the top soils (0–15 cm) and C and organic P mineralizations during the incubation experiment

	Humid sites ($n_{\mathrm{sites}} = 11$)	Dry sites ($n_{\mathrm{sites}} = 10$)
C and P contents		
Total C (g kg^{-1})	33.2 (2.3) a	21.1 (2.7) b
Total P (mg kg^{-1})	60.9 (4.8) a	41.1 (2.9) b
Organic P (mg kg^{-1})	41.7 (3.3) a	27.2 (3.1) b
Organic P (% of total P)	68.7 (2.5) a	64.8 (3.9) a
Daily soil respiration (mg C kg^{-1} day^{-1}) measured for different time intervals ($T = 28°C$)		
Day 0–day 2	31.7 (3.5) a AB	19.1 (1.8) b AB
Day 2–day 4	25.4 (2.3) a AC	25.8 (3.5) a AB
Day 4–day 7	39.3 (2.3) a B	30.2 (1.9) b A
Day 7–day 14	20.4 (1.6) a CD	15.7 (1.6) b BC
Day 14–day 21	15.0 (1.5) a D	10.2 (1.4) b C
Day 21–day 28	15.0 (1.2) a D	11.7 (1.5) a C
Annual C mineralization rate (% of total C $year^{-1}$) as a function of soil temperature[a]		
$T = 28°C$	17.1 (1.5) a	21.2 (2.3) a
$T = 15°C$	5.6 (0.5) a	7.0 (0.8) a
Estimated soil organic P mineralization (mg kg^{-1} $year^{-1}$)[b]		
$T = 15°C$	2.3 (0.3) a	1.9 (0.3) a

Standard error of the mean is given in parentheses. Different small letters denote significant differences between site classes at $P < 0.05$ or lower. Different capital letters denote significant differences between time intervals at $P < 0.05$ or lower

[a] Values for $T = 15°C$ estimated using the temperature dependence function

[b] Soil organic P mineralization estimated assuming that C and organic P have comparable mineralization rate (in % of organic content $year^{-1}$)

Table 5 Comparison of available inorganic P (P ion concentration in solution Cp and diffusive P in 10,000 min $Pr_{10,000\ \mathrm{min}}$) between the top soil layer (0–15 cm) in the present study and surface layers (0–20 cm) in different contexts

	Cp		$Pr_{10,000\ \mathrm{min}}$	
	n_{sites}	mg l^{-1}	n_{sites}	mg kg^{-1}
This study	18	1.4 (0.03–4)	18	17 (0.2–52)
Forests[a]	17	0.3 (0.04–2)	–	–
Grasslands[b]	25	0.5 (0.02–3)	20	126 (19–605)
Cultivated lands[c]	31	0.3 (0.01–3)	31	75 (4–271)

Means with ranges in parentheses are presented. The number of sites is denoted by n_{sites}

[a] Harrison 1982; Andersen 1994; Parfitt et al. 1994; Grierson et al. 1998; Bünemann et al. 2007

[b] Bowman and Cole 1978; Frossard et al. 1989; Chen et al. 2003b; Bünemann et al. 2007; Stroia et al. 2007b

[c] Frossard et al. 1996; Morel 2002; Oehl et al. 2004; Rabeharisoa 2004; Bünemann et al. 2007

study varied mainly with the reactivity (inferred with *m* and *n*) of the soils (Eqs. 3, 4; Fig. 1a). In high reactive soils (high *n* values and/or low *m* values) most of the P ions were present as diffusive P bound to the soil solid phase (low P ion concentration in the soil solution). Conversely, in low reactive soils most of the P ions were present in the soil solution. Consequently, diffusive P was negatively correlated to the concentration of P ions in the soil solution. Comparable links between soil solution ionic P and the isotopic dilution parameters were found in a study comparing 15 different soils of grasslands (Chen et al. 2003b). One likely explanation for the differences in reactivity is a varying amount of iron and aluminum contents that have been reported to correlate well with parameters of isotopic dilution kinetics

(Hamon and McLaughlin 2002; Chen et al. 2003b). Variability in reactivity was limited in samples from deeper layers and these soil samples had an *n* value close to the maximum value of 0.5 (Table 1), consequently the P ion concentration in solution was always low in soils at these depths (Fig. 1).

Total P, inorganic P and organic P pools were clearly in the lower range of values reported in the literature, but proportion of total P that was organic P was comparable to those in other forest or grassland contexts (Table 6). It should be born in mind that we preferred to use HF as an extractant instead of H_2SO_4 as it extracts a larger quantity of inorganic P leading to higher total P. Consequently, methods based on H_2SO_4 result in a higher proportion of organic P in particular when soils are very reactive (i.e. strongly bound inorganic P). The use of the H_2SO_4 to compute an organic P fraction would have yielded values of 83% on average for the organic P fraction in the 0–15 cm layer in our study instead of 56%. This would place our values in the upper range of values reported in the literature. On the other hand, variations in the reported P range in Table 5 may be related to different extraction methods used.

Also, direct quantification of organic P mineralization is extremely difficult since most of mineralized P does not accumulate in solution but is sorbed onto the solid constituents (Frossard et al. 1996). However, Achat (2009) found, during a long term (515 days) incubation experiment with soils from the same region, that C and organic P mineralization rates (in % of total organic content) were comparable under basal conditions for soil respiration. Estimation of organic P mineralization via C mineralization could thus be an interesting indirect approach.

Relative importance of organic and inorganic P to plant P availability

Organic P represented a high proportion of total P and this proportion decreased with soil depth, although a relative accumulation occurred in the B horizon in humid sites. Such a distribution with soil depth has also been observed in other soils including forest podzols (Anderson 1980; David et al. 1995). David et al. (1995) reported higher pools of organic P in the forest floor and relative accumulation of organic P in spodic horizons, thought to result from the movement of dissolved organic compounds in podzols, i.e. leaching of dissolved organic carbon and therefore organic P from the forest floor and deposition in mineral horizons by complexation with Fe and Al. In the humid sites in our study area, the humus and organic top soil (A) layers are thicker than

Table 6 Comparison of total and organic P contents and of proportion of organic P between the surface soil layers in the present study and those in different contexts

	Total P		Organic P		
	n_{sites}	mg kg^{-1}	n_{sites}	mg kg^{-1}	% of total P
This study					
Litter	18	285 (220–359)	18	225 (175–292)	79 (59–88)
0–15 cm	18	60 (16–109)	18	32 (14–57)	56 (36–88)
15–30 cm	18	46 (18–91)	18	22 (7–47)	45 (21–60)
Forests[a]					
Litter	14	764 (313–1,520)	3	622 (504–741)	86 (82–92)
0–15 cm	30	557 (37–1,773)	15	527 (221–666)	67 (50–77)
10–20 cm	17	568 (75–797)	13	466 (169–573)	69 (67–76)
Grasslands[b]					
0–20 cm	71	919 (132–1,765)	31	527 (103–872)	70 (53–78)

Mean with ranges in parentheses are presented. Number of sites is denoted by n_{sites}

[a] Harrison 1982; Clarholm 1993; van Wesemael 1993; Parfitt et al. 1994; Sparling et al. 1994; David et al. 1995; Joergensen et al. 1995; Grierson et al. 1998; Ross et al. 1999; Chen et al. 2000, 2003a; Kavvadias et al. 2001; Comerford et al. 2002

[b] Bowman and Cole 1978; Sharpley 1985; Friesen and Blair 1988; Parfitt et al. 1994; Sparling et al. 1994; He et al. 1997; Ross et al. 1999; Chen et al. 2000, 2003a, b; Hayes et al. 2000; Turner et al. 2001; Stroia et al. 2007a

those in dry sites. The lower accumulation of organic matter in these dry sites (including dunes) is thought to be due to the younger geological age of the soils in these sites together with less dynamic soil formation (Legigan 1979; Righi and Wilbert 1984) and less productive vegetation (Jolivet et al. 2007). Conversely, in the humid sites, organic material accumulates more at the surface due to the presence of a water table near the soil surface in winter limiting the vertical migration of organic compounds and the formation of an eluvial horizon (Trichet et al. 1999).

The combination of extraction and isotopic dilution methods together with a few basic assumptions enabled us to establish a general concept for the relative contribution of organic and inorganic P in supplying P to the trees based on processes (Fig. 5). Relative contributions in mesic sites are comparable to those in humid sites (data not shown). To evaluate the relative contribution of the different processes, we used extrapolations of diffusive P to different time spans and we estimated the proportion of organic P that can be mineralized over a period of 1 year based on C or P mineralization rates obtained in incubation. Here, C or P mineralization was assessed only in the top mineral soil and was estimated to 6–7% year^{-1}. No data were available for the other soil layers and for the different site classes. However, for the litter layer, a P mineralization rate of 10% year^{-1} was used based on litter from the same region (Achat 2009). This rate was comparable to that obtained in other Pine forests using litter bag methods based on mass loss of litter (10–20 year^{-1}; van Wesemael 1993; Moro and Domingo 2000). In deep soil layers (60–120 cm; Fig. 5) we assumed that mineralization is negligible or even extremely low since residence time of C and stability of organic compounds have been reported to increase with soil depth in forests and grasslands (Rumpel et al. 2002; Schöning and Kögel-Knabner 2006). In accordance with this assumption, the C mineralization rate in the 60–80 cm soil layer of grassland was only 1% year^{-1} (Fontaine et al. 2007). This increased stability with soil depth is thought to result from the lack of fresh carbon supporting microbial activity (Fontaine et al. 2007), organic-mineral complexes that are less accessible to decomposers (Baldock and Skjemstad 2000; Wattel-Koekkoek et al. 2003) and generally less favorable conditions for microbes such as lack of oxygen. Organic P in forest soils and more particularly the dominant fraction of inositol phosphates, which cannot be readily hydrolyzed (McGill and Cole 1981; David et al. 1995), forms stable complexes with Fe and Al (Anderson 1980; David et al. 1995). Such oxides occur in larger amounts in deeper soil layers (B horizon).

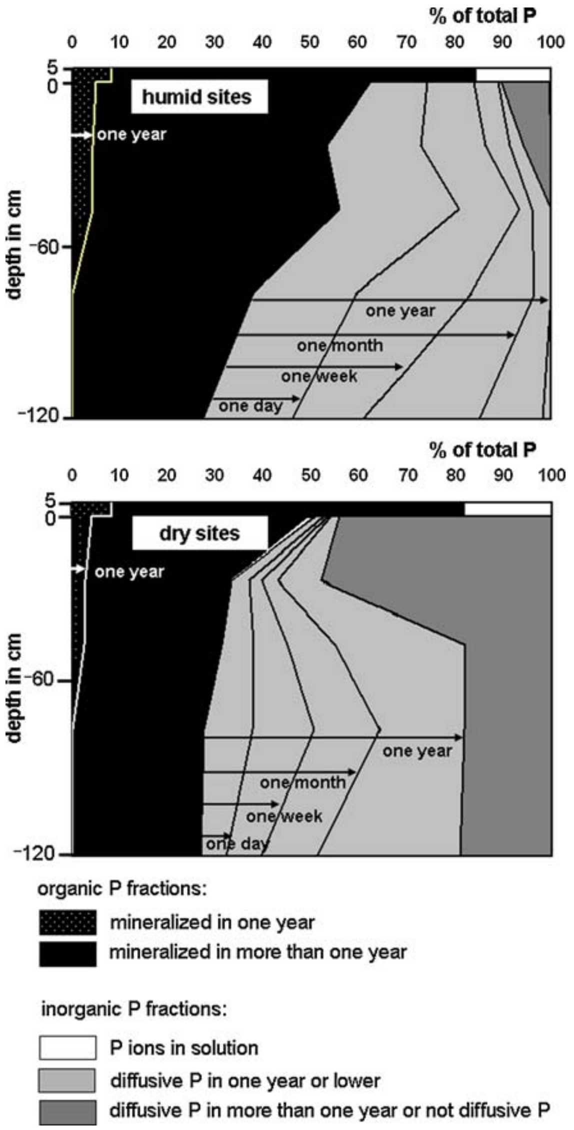

Fig. 5 Conceptual diagram of the relative contributions of organic and inorganic P fractions to total P stocks for humid sites (**a**) and dry sites (**b**). Mean values are presented for each site class

The high proportion of organic P (80–85% in the litter layer and 50–55% in 0–15 cm soil layer) suggests that mineralization plays a potentially important role in P availability. However, the decrease in the proportion of organic P and, conversely, the increase in the proportion of inorganic P that is diffusive P suggest an increase in the relative importance of diffusion with depth compared to mineralization in the P-supplying soil solution (Fig. 5). The different P pools are often measured using simple extraction methods, yet these methods may not be sufficient to assess the potential importance of the different processes (diffusion and mineralization) as the size of the pool may not be closely related to the actual fluxes from each pool. Indeed the assumed proportions of mineralized organic P in 1 year are extremely low compared to the proportions of diffusive P during the same period in all soil layers in humid sites and in deep soil layers in dry sites (Fig. 5). Mineralization and diffusion appear to be of comparable importance only in the surface layers in dry sites, as the inorganic fraction is slowly available (Table 2). In contrast to mineral soil layers, mineralization in the litter layer is assumed to be the most important process since all inorganic P is in solution (Fig. 5; Table 2). Probably P ions in solution come directly from the organic matter after mineralization in the litter layer.

The transfer of our results on the relative importance of inorganic and organic fractions in P availability to field conditions can be subject of debate. First, the shaking of the soil suspensions with a higher water/soil ratio than under field conditions, could lead to overestimates of the diffusion rates relative to undisturbed soils. However, the studied soils are essentially sandy (70–90% coarse sand) and unstructured, thus reducing the diffusion limitation in field conditions. In addition, we used a low water/soil ratio comparing to the general procedure (15 ml/10 g in this study; 10 ml/1 g in Fardeau et al. 1991; Frossard and Sinaj 1997; Morel 2002), allowing our results to be more transferable to field conditions. Secondly, organic P mineralization rates as obtained in incubation experiments were corrected for temperature to estimating whole-year values of mineralization, but soil humidity could not be considered. Presumably, due to strong variations in soil moisture in the forest between site classes and between seasons, conditions for mineralization may be less favourable there. And finally, we evaluated diffusion and mineralization processes in simplified systems, i.e. in soil–solution systems without roots. Thus root-induced pH change, release of acids-anions and phosphatases from root and ectomycorrhizal fungi and their effects on inorganic and organic P availability in the rhizosphere (Attiwill and Adams 1993; Hinsinger 2001; Neumann and Römheld 2002) were not considered. Notwithstanding these points of debate, we feel confident that our study has pointed out the most relevant stocks and processes determining P status in our context. Further research is warranted to link actual plant uptake (by roots or mycorrhizae) and the presented P status.

Implications of the study for local forest management

The evaluation of the different P stocks for the soils of the present study showed that they were extremely P-deficient compared to other contexts (Table 6). Based on forest management scenarios of different intensity, P exportation during a complete stand rotation (40 years) was estimated at 7.8–59.2 kg P ha^{-1} depending on stand management and harvest intensity (L. Augusto, unpublished data). This exportation represents between one and 9% of total P stock and between one and 14% of inorganic P stock. Moreover, probably not all of the P stock in a 0–120 cm soil profile is available to the trees considering distribution of *Pinus* roots with depth in humid and dry moorland sites (Achat et al. 2008). Fertilization may thus be necessary to maintain long-term soil fertility and productivity.

The use of the isotopic dilution method to evaluate the reactivity of the soils and to assess the dynamic inorganic fractions of the different sites classes and soil depths improved our understanding of local tree growth and forest practices. Indeed, tree growth has been reported to be higher in humid sites (Chaperon and Crémière 1994). These are the sites with the highest stocks of diffusive P (Fig. 5). Perhaps tree growth is lower in dry sites because the inorganic P is less plant available there (Fig. 5). Furthermore, P fertilization has been shown to have a positive effect on tree growth but only in humid to mesic sites (Bonneau 1995). One likely explanation is that inorganic P from fertilizers is not retained by the low P fixing capacity in dry sites (low *n* values and/or high *m* values; Table 1) but is leached from surface layers to deep layers where root exploitation is low. The capacity of the soil to adsorb phosphates should thus be taken into consideration when fertilization is envisaged.

Conclusion

The combination of extraction and isotopic dilution methods enabled us to successfully determine P contents (total, organic and inorganic) and available inorganic P (dissolved and diffusive P ions species) for a range of site classes in the forest of the Landes of Gascogne. All values were extremely low compared to those reported in the literature and estimated forest P exportations by harvests. However, P status could vary considerably in a given soil layer, mainly due to differences in site classes. Overall, the contribution of inorganic P fractions (diffusive P for durations of up to 1 year) in supplying P to the trees appeared more important than the contribution of organic P. Contrary to our first hypothesis, the relative contribution of organic P did not increase with increasing amounts of total stocks of organic P. Owing to a very low amount of diffusive P in the top soil layers of the dry sites, the relative contribution of organic P was actually higher in these sites, despite a lower overall organic P fraction. In line with our second hypothesis, the relative contribution of the organic fractions was preponderant in litter, less important in top soil horizons and negligible at depths below 30 cm. This was not only due to a decreasing proportion of total organic fractions with soil depth along with higher stability of the organic compounds, but also related to an increase in reactivity of the soil solid phase with soil depth. Thus, the combination of the two approaches yielded valuable complementary information for our forest soils not only concerning the amount of the different organic and inorganic P stocks but also their dynamic short-time fractions. In this respect, diffusive inorganic P appeared to be much more important than organic P as a dynamic plant available fraction.

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