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## Phosphatase activity, microbial phosphorus, and fine root growth in forest soils in the Sierra de Gata, western central Spain

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**Abstract** Acid phosphatase activity (APA), labile P fractions and fine root growth were studied in an oak (*Quercus pyrenaica* Willd.) forest in the Sierra de Gata, in western central Spain. Soils in the region are acid and rich in organic matter, with low levels of extractable inorganic P but with a high proportion of organic P. In such soils, the activity of phosphatase enzymes is likely to be important for the control of P mineralization and P cycling and, consequently, can affect the availability of P for plant uptake. The biomass of fine roots was about 25-fold that of leaf litter, demonstrating a high allocation of C resources to the root system in order to compensate for a low availability of soil nutrients. The study compared plots fertilized with triple superphosphate (100 kg P ha<sup>-1</sup>) to control (unfertilized) plots. Fertilizer application had no significant effect on APA and fine root density; however, there were significant differences in available and microbial P. Spatial and seasonal variations in the APA were related to plant root density and biotic demand. Seasonal differences in the APA may also be the consequence of changes in the amount of hydrolysable organic substrates at different times of the year.

**Keywords** Phosphatase · Forest soils · Phosphorus mineralization · Available phosphorus · Microbial phosphorus

### Introduction

Phosphatase enzymes are assumed to have an essential role for the cycling of P in forest ecosystems, particular-

ly where availability of P may limit plant productivity (Speir and Ross 1978). In studies of nutrient cycling in an oak (*Quercus pyrenaica* Willd) forest in western Spain, labile inorganic P (P<sub>i</sub>) in soil, and P contents of plant tissue were low (Turrión et al. 1997). Yanai (1992) found that more than 60% of the P taken up by northern hardwood forests originated from mineralization of organic P (P<sub>o</sub>), whereas only a minor part could be traced to weathering. P mineralization may be both *biological*, where mineralization of soil organic matter (SOM) by heterotrophic soil microorganisms releases P<sub>i</sub> together with other nutrients bound to organic matter in an unspecific way; and *biochemical*, where roots and microorganisms selectively release P from SOM through the production of phosphatase enzymes (Tate 1984). Phosphatases catalyse the hydrolysis of ester bonds between phosphate and C compounds in organic substrates. Increased production of phosphatase enzymes by plant roots and by microorganisms can be induced when P is limiting. Consequently, an increase in phosphatase activity may reflect a high demand for P. However, phosphatase activity in the soil may also be limited by the amount of hydrolysable substrate (i.e. the forms of P<sub>o</sub>) and by soil moisture and temperature effects.

The objective of the present study was to measure acid phosphatase activity (APA) in an organic-matter-rich forest soil and to assess the effects of additions of P fertilizer on APA.

### Materials and methods

#### Site description

The study site was at Navasfrías (NF), in the Sierra de Gata range in the western part of the Central Iberian System (40°2′N, 30°W). The autochthonous vegetation of these mountains are forests of deciduous oak (*Quercus pyrenaica* Willd.), but large tracts of degraded oak forest and abandoned agricultural fields have been replaced with plantations of *Pinus pinaster* L. The climate of the area is characterized by rainy autumns and springs, with hot, dry summers and occasional dry winters, and is classified as temperate Mediterranean. Mean annual precipitation is 1.570 l m<sup>2</sup> year<sup>-1</sup> and the mean annual temperature is 11.3°C (Turrión et al. 2000).

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**Table 1** Chemical properties of the Navasfrías soil.  $C_o$  Organic C,  $N_t$  total N,  $P_t$  total P,  $P_o$  organic P,  $P_{ret}$  phosphate retention of the soil

Depth (cm)	pH (H <sub>2</sub> O)	$C_o$ (mg g <sup>-1</sup> )	$N_t$ (mg g <sup>-1</sup> )	C/N	$P_t$ (mg kg <sup>-1</sup> ) <sup>a</sup>	$P_o$ (mg kg <sup>-1</sup> ) <sup>a</sup>	P ret. (%)
0–10	5.2	87.4	4.8	18.4	494	350	57
10–20	5.1	69.7	4.1	17.2	466	327	70
20–40	5.1	50.3	3.3	15.3	444	322	69

<sup>a</sup> Determined according to Saunders and Williams (1955)

Experimental plots were established in a 60- to 80-year-old forest of *Q. pyrenaica*. Tree density at the site was 820 trees ha<sup>-1</sup>, with a mean diameter at breast high of 15.2 cm and a mean tree height of 13 m. The total aboveground biomass is 64.5 Mg ha<sup>-1</sup> and the leaf area index approximately 1.8 m<sup>2</sup> m<sup>-2</sup> (Martin et al. 1995). The understorey vegetation is dominated by bracken (*Pteridium aquilinum*), with scattered individuals of *Cytisus scoparius*, *Erica arborea* and *E. australis*, and a sparse cover of a few herbaceous species. Soils are classified as Humic Cambisols (FAO 1989) that have developed on Precambrian schists.

#### Experimental design and soil sampling

In May 1992, triple superphosphate was applied by hand at a rate of 100 kg P ha<sup>-1</sup> to four replicate blocks (50 m × 50 m). As the site had a gentle slope, fertilized plots and adjacent control (unfertilized) plots were arranged parallel to each other down the slope to avoid contamination of the control plots by superficial transport of fertilizer P. Soil samples were taken at each season during 1993 and 1994 from three depths (0–10 cm, 10–20 cm and 20–40 cm). For each replicate plot, samples were taken from several points and bulked by depth.

Annual fine root production of the oaks was estimated by the in-growth core method (Persson 1990). Soils cores were taken with an 8-cm-diameter steel corer, and roots sieved from soil. The sieved soil was then replaced in the original hole. Cores in the field were covered with a plastic mesh that impeded the growth of herbaceous vegetation, but permitted the exchange of water and gases. After 14 months (1 year, plus 2 months to allow for equilibration of the cores after disturbance), fine roots were extracted with a 3.8-cm steel corer driven into the centre of the refilled hole. Soils were sieved and the extracted fine roots were dried and weighed to give an estimate of the annual fine root production.

In addition to estimates of annual root production, the standing fine root biomass in the control and fertilized plots was sampled 4 times a year, corresponding to the hot summer, dry autumn, and wetter and cooler winter and spring seasons. Several cores were collected at four depths (0–10, 10–20, 20–30 and 30–40 cm) using an auger, and bulked for each depth by plot. Roots were separated from soil using a combination of hand-sorting and wet-sieving. Fine root length densities (FRLD) of subsamples were estimated using the line-intersection method of Tennant (1975) and a 2-cm grid. All roots were then dried and weighed.

#### Acid phosphatase activity

The APA was determined using *p*-nitrophenylphosphate (*p*-NPP) as substrate at a temperature of 30°C (Schneider et al. 2000) in a modification of the original method of Tabatabai and Bremner (1969). The spatial and seasonal variation of APA was measured and compared between control and P-fertilized plots. APA and root length densities were estimated from the same cores in order to determine if there was a correlation between the two parameters.

#### Available and microbial P in soil

Available P ( $P_{av}$ ) was extracted with iron oxide-coated paper strips (Menon et al. 1989). Microbial P ( $P_{mic}$ ) from topsoil was deter-

mined according to the fumigation-extraction method of McLaughlin et al. (1986). These measurements were made every time APA was measured.

#### General soil analyses

Standard procedures were used for soil analyses. Soil pH was measured in water at a soil:solution ratio of 1:2.5 using a glass electrode. Organic C was determined by dry combustion with a Carmograph 12 Wösthoff analyser; total N was measured by Kjeldahl digestion followed by steam distillation and final titration of ammonium. P retention capacity of soils was determined according to the method of Blakemore et al. (1981). Some physicochemical and chemical features of the soils studied are summarized in Table 1.

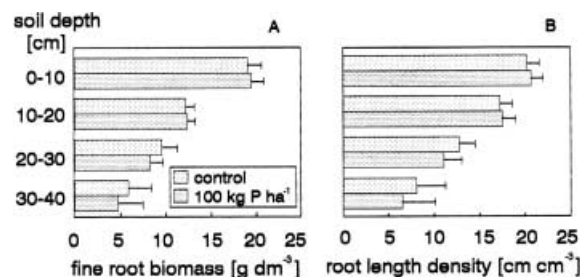
#### Statistical analysis

ANOVA was used to examine the main effects (fertilizer treatment and soil depth) on APA,  $P_{av}$ ,  $P_{mic}$ , and FRLD, and to test for differences among seasons with Student's *t*-test for pair-wise comparison of the means. Relationships between APA, P fractions and fine root growth were examined using linear regression analyses. The Statgraphics package was used for all statistical analyses. Data were log-transformed where necessary to improve the homogeneity of variances and the significance level was  $P < 0.05$  unless otherwise stated.

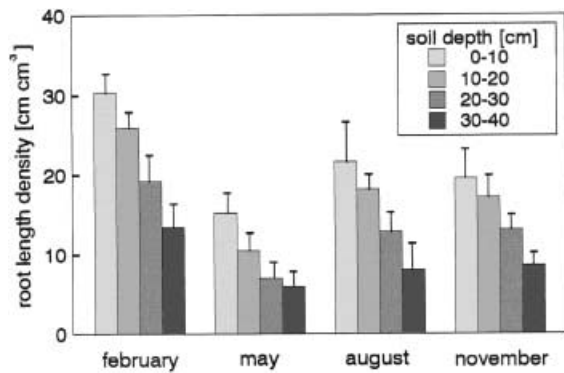
## Results

Root biomass and FRLD were very high at NF and showed the typical decrease with soil depth (Fig. 1). The FRLD exhibited a certain seasonal pattern with a maximum in winter and a minimum in spring (Fig. 2).

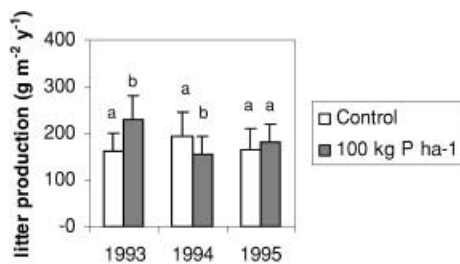
Annual leaf litter production during 3 years in control and fertilized plots can be seen in Figure 3. Significant differences in the leaf litter production between control and P-fertilized plots were observed only in the first year after fertilization (Fig. 3).



**Fig. 1** **A** Distribution of the fine root biomass (g dm<sup>-3</sup>) and **B** corresponding root length density (cm cm<sup>-3</sup>) in control and fertilized plots (100 kg P ha<sup>-1</sup>) at the Navasfrías (NF) stand. Bars indicate SEs



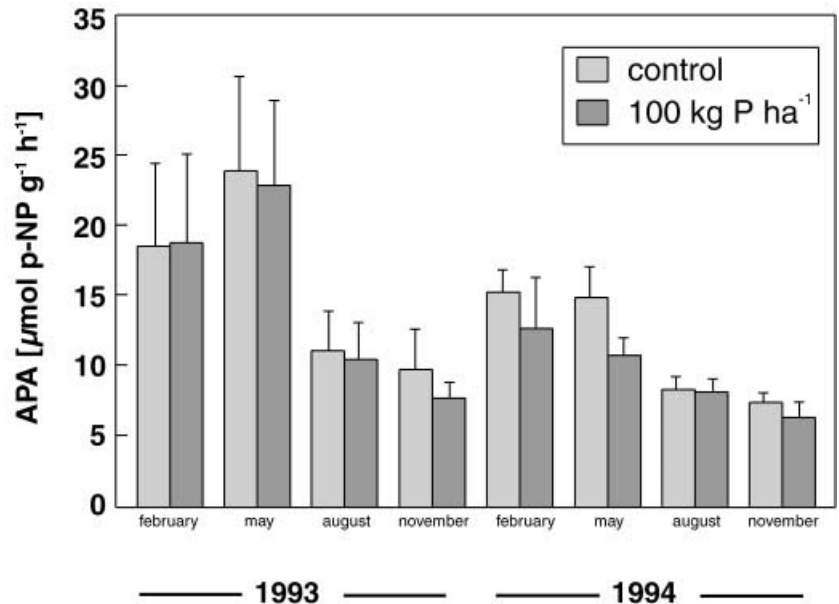
**Fig. 2** Seasonal variation of the root length density ( $\text{cm cm}^{-3}$ ) in different soil depths in unfertilized plots at the NF stand. Bars indicate SEs



**Fig. 3** Annual leaf litter production ( $\text{g m}^{-2} \text{year}^{-1}$ ) during 3 years in control and fertilized plots ( $100 \text{ kg P ha}^{-1}$ ) at the NF stand. Different letters indicate significant difference at  $P < 0.05$ , bars indicate SEs

Table 2 shows the APA,  $P_{\text{av}}$ , and  $P_{\text{mic}}$  concentrations of control and P-fertilized soils from 0–10 cm soil depth at the NF site. The APAs were very high in both the control and the fertilized plots, and no significant differences between treatment means were detected by Student's t-test.

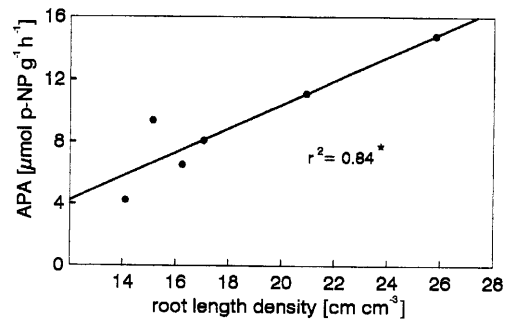
**Fig. 4** Seasonal variation of acid phosphatase activity (APA) in soil from 0–10 cm depth from control and fertilized plots ( $100 \text{ kg P ha}^{-1}$ ) at the NF stand during a 2-year period. Bars indicate SEs. *p-NP* *p*-Nitrophenol



**Table 2** Acid phosphatase activity (APA), microbial P and available P concentrations with SEs for control and P-fertilized plots ( $n=4$ )

	APA ( $\text{mg g}^{-1}$ )	Microbial P ( $\text{mg g}^{-1}$ )	Available P ( $\text{mg g}^{-1}$ )
Control	$9.7 \pm 1$	$49 \pm 12$	$8 \pm 0.4$
P fertilized	$7.7 \pm 1$	$104 \pm 14$	$16 \pm 2$
<i>P</i>	NS	*	*

\*  $P < 0.05$ , NS not significant



**Fig. 5** Relationship between the fine root length density and the APA. For abbreviations, see Fig. 4

APAs varied throughout the year and were significantly higher in late winter and spring (February and May) than in summer and autumn (Fig. 4). There was no significant difference in APA between fertilized and control plots, which may be partially attributable to the high spatial variability of APA within each treatment (Table 2, Fig. 4). Further, much of the P applied as fertilizer had been immobilized by the humic horizons and by the microbial biomass (Table 2).

When analysing the APA and the FRLD from the same soil cores, a significant relationship was found be-

tween them (Fig. 5). Therefore, the variation of the APA can be explained by differences in the spatial distribution of fine roots.

## Discussion

### Fine root growth

The biomass of fine roots (Fig. 1) was about 25-fold that of leaf litter (Fig. 3), demonstrating a high proportional allocation of C resources to the root system. A low availability of P and/or N in the soil favours the development of the root system (Ericsson and Ingestad 1988; Haynes and Gower 1995) in order to mitigate the low soil supply of these nutrients by an increase in the absorbing root surface. The high degree of ectomycorrhizal infection of the fine roots of oak, where about 60% of the fine roots in the upper soil horizon are infected (Schneider 1999), is also characteristic of forest ecosystems with low nutrient availability (particularly of N and P) and slow SOM mineralization (Read 1991).

Forest nutrient cycles are commonly shown as pools and fluxes indicating major inputs, storage pools and out pools. The high amount of biomass of fine roots obtained in the present study shows the need for accounting for the requirements for fine-root production where these forest nutrient cycles are studied.

### Acid phosphatase activity

APAs in soils at NF are higher than those reported from other acid forest soils (Harrison 1983; Trasar Cepeda and Gil Sotres 1987) and are a reflection of both the low levels of extractable  $P_i$  and relatively large amounts of labile  $P_o$  (Table 2; Speir and Ross 1978). The APA of soils generally decreases in response to fertilizer application (Haynes and Swift 1988; Clarholm 1993). However, in this study there was no significant difference in APA between control and fertilized plots (Table 2). This may be partly explained by the high P retention of the soils at NF (Turrión et al. 2000), and the resulting low impact of the fertilizer on the labile P fractions, which remained quite low (Table 2). In addition to chemical immobilization through adsorption and precipitation reactions, fertilizer P was also immobilized by the microbial biomass (Table 2). The increase in  $P_{mic}$  is a typical response after applications of P to P-deficient soils (Haynes and Swift 1988; Grierson et al. 1998), where P in the microbial biomass was several times greater than the fraction of  $P_{av}$  (Table 2). Microorganisms compete with plants for P, and the annual P demand of microorganisms can exceed that of plants (Tarafdar and Jungk 1987). In addition, the effects of fertilizer on the  $P_{mic}$  in the soil were pronounced while there was little impact on the P content of oak foliage (Schneider 1999). This suggests that microorganisms in the soil acquire P more efficiently, are more rapid in their response to an increased supply of P, or have a greater demand for P, than the oak trees.

APA was highly variable between seasons (Fig. 4). Fine root distribution and activity may partially explain both the spatial and temporal variability observed in this study. The excretion of phosphatases from roots or microorganisms can result in a localized concentration of phosphatase enzymes in the rhizosphere (Tarafdar and Jungk 1987; Grierson and Adams 2001). In this study, there was a strong relationship ( $r^2=0.84$ ,  $P<0.05$ ) between fine root length density and APA (Fig. 4).

Soil water availability has been assumed to be the main factor regulating the seasonal variation of APA in similar forest soils of northern Spain (Trasar Cepeda and Gil Sotres 1987). However, APA in this study remained low in November (Fig. 3), even though the soil water content had increased considerably with respect to the summer (Turrión et al. 1997). Differences in seasonal APA can be partially attributed to differences in the availability of substrate and P demand throughout the year. Litterfall occurs mainly from the end of October until February (winter) with a peak at the end of November (Martín et al. 1995). Decomposition rates of litter are approximately  $0.33 \text{ year}^{-1}$  at this site, therefore about 24 months are necessary for the degradation and the subsequent incorporation of organic matter into the upper mineral horizon by leaching and the action of soil fauna (Gallardo et al. 1998). Consequently peak amounts of hydrolysable substrate would coincide with periods of peak APA (in the late winter and spring) and are likely to increase rates of P mineralization and  $P_i$  available for uptake. APA depends on a sufficient energy supply and is limited by the availability of labile organic matter (Spiers and McGill 1979). As root growth and nutrient uptake generally precede the emergence of the leaves, usually taking place in May at NF, a high demand for P would be expected before the initiation of new growth aboveground. FRLD peaked in the late winter, illustrating a divergence between below and aboveground growth (Fig. 2). Other Mediterranean-type ecosystems also exhibit this contrasting phenology of growth, particularly where nutrients may be limiting (Spetch and Moll 1983). The high activity of soil phosphatases in the winter may also be due to the leaching of enzymes from decomposing litter by rainfall (Harrison and Pearce 1979).

Given the high quantity of P present in organic forms (Table 1), the high capacity of the soil to fix  $P_i$  (Table 1), and the limited biological mineralization of SOM as a result of the formation of complexes of  $P_o$  with active Al and Fe (Turrión et al. 2000), phosphatases play a crucial role in the P acquisition of plants and microorganisms, and thus in the cycling of P within the forest ecosystem.

In conclusion, APA plays an essential role in P cycling at the NF site, where  $P_o$  is the dominant P fraction and the amount of  $P_{av}$  is low. The transformation of organic to inorganic  $P_{av}$  mediated by acid phosphatases in the soil root intersphere is a crucial process for plants' P nutrition, and also for the cycling of P in the overall ecosystem. A very high fine root production in the studied soil (contrasting with a low aboveground production of the oaks) demonstrates a high allocation of carbohy-

drates to the development of the root system in order to compensate for a low availability of soil nutrients. An important proportion of P added by fertilization was immobilized as microbial biomass.

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## References

- Blakemore LC, Searle PL, Daly BK (1981) Soil Bureau laboratory methods: methods for chemical analysis of soils. New Zealand Soil Bureau scientific report 10A. New Zealand Soil Bureau
- Clarholm M (1993) Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers. *Biol Fertil Soils* 16:287–292
- Ericsson T, Ingestad T (1988) Nutrient and growth of birch seedlings at varied relative phosphorus addition rates. *Physiol Plant* 72:227–235
- FAO (1989) Mapa mundial de suelos: leyenda revisada. FAO, Roma, 142 pp
- Gallardo JF, Martín A, Moreno G, Santa Regina I (1998) Nutrient cycling in deciduous forest ecosystems of the Sierra de Gata Mountains: aboveground litter production and potential nutrient return. *Ann Sci For* 55:749–769
- Grierson PF, Adams MA (2001) Acid phosphatase, ergosterol and microbial P relationships in a jarrah forest in south-western Australia. *Soil Biol Biochem* (in press)
- Grierson PF, Comerford NB, Kokela EJ (1998) Phosphorus mineralization kinetics and response of microbial phosphorus to drying and rewetting in a Florida Spodosol. *Soil Biol Biochem* 32:1323–1331
- Harrison AF (1983) Relationship between intensity of phosphatase activity and physicochemical properties in woodland soils. *Soil Biol Biochem* 15:93–99
- Harrison AF, Pearce T (1979) Seasonal variation of phosphatase activity in woodland soils. *Soil Biol Biochem* 11:405–410
- Haynes BE, Gower ST (1995) Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiol* 15:317–325
- Haynes RJ, Swift RS (1988) Effects of lime and phosphate additions on changes in enzyme activities, microbial biomass and levels of extractable nitrogen, sulphur, and phosphorus in an acid soil. *Biol Fertil Soils* 6:153–158
- Martín A, Gallardo JF, Santa Regina I (1995) Interaction between litter and soil epipedons in forest ecosystems of the Sierra de Gata Mountains, province of Salamanca, Spain. *Arid Soil Res Rehabil* 9:299–305
- McLaughlin MJ, Alston AM, Martin JK (1986) Measurement of P in the soil microbial biomass. a modified procedure for field soils. *Soil Biol Biochem* 18:437–443
- Menon RG, Chien SH, Hamond LL (1989) Modified techniques for preparing paper strips for the new Pi soil test for phosphorus. *Fertil Res* 19:85–91
- Persson H (1990) Methods of studying root dynamics in relation to nutrient cycling. In: Harrison AF, Ineson P, Heal OW (eds) *Nutrient cycling in terrestrial ecosystems*. Elsevier, London
- Read DJ (1991) Mycorrhizas in ecosystems. *Experientia* 47:376–391
- Saunders WMH, Williams EG (1955) Observations on the determination of total organic phosphorus in soil. *J Soil Sci* 6:254–267
- Schneider K (1999) Verfügbarkeit von Phosphor in Waldböden und bedeutung für die Ernährung von *Quercus pyrenaica* Willd. in der Sierra de Gata, W-Spanien. Dissertation. Hohenheimer Bodenkundliche Hefte, Universität Hohenheim
- Schneider K, Turrión MB, Gallardo JF (2000) A modified method to measure acid phosphatase activities in forest soils with high organic matter content. *Commun Soil Sci Plant Anal* 31:1–17
- Specht RL, Moll EJ (1983) Mediterranean-type heathlands and sclerophyllous shrublands of the world – an overview. In: Kruger FJ, Mitchell DT, Jarvis JUM (eds) *Mediterranean-type ecosystems. The role of nutrients*. Springer, Berlin Heidelberg New York
- Speir TW, Ross DJ (1978) Soil phosphatases and sulphatases. In: Burns RG (ed) *Soil enzymes*. Academic Press, London, pp 197–250
- Spiers GA, McGill WB (1979) Effects of phosphorus addition and energy supply on acid phosphatase production and activity in soils. *Soil Biol Biochem* 11:3–8
- Tabatabai MA, Bremner JM (1969) Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol Biochem* 1:301–307
- Tarafdar JC, Jungk A (1987) Phosphatase activity in the rhizosphere and its relation to depletion of soil organic phosphorus. *Biol Fertil Soils* 3:199–204
- Tate KR (1984) The biological transformation of phosphorus in soil. *Plant Soil* 76:245–256
- Tennant D (1975) A test of a modified line intersect method of estimating root length. *J Ecol* 63:995–1001
- Trasar Cepeda MC, Gil Sotres F (1987) Phosphatase activity in acid high organic matter soils in Galicia (NW Spain). *Soil Biol Biochem* 19:281–287
- Turrión MB, Gallardo JF, González MI (1997) Nutrient availability in forest soils as measured with anion exchange membranes. *Geomicrobiol J* 14:51–64
- Turrión MB, Gallardo JF, González MI (2000) Distribution of P forms in natural and fertilized forest soils of central western Spain: plant response to superphosphate fertilization. *Arid Soil Res Rehabil* 14:159–173
- Yanai RD (1992) Phosphorus budget of a 70-year-old northern hardwood forest. *Biogeochemistry* 17:1–22