

Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce [*Picea abies* (L.) Karst.] trees

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Summary. Inorganic and organic phosphates (P) were measured in bulk soil, rhizosphere soil and mycorrhizal rhizoplane soil of Norway spruce. Various methods of P extraction and estimation were compared. In addition, acid phosphatase activity and mycelial hyphae length were determined. In soil solutions from various locations, about 50% (range 35%–65%) of the total P was present as organic P. Compared to the bulk soil, the concentrations of readily hydrolysable organic P were lower in the rhizosphere soil and in the rhizoplane soil; this difference was particularly marked in the humus layer. In contrast, the concentrations of inorganic P either remained unaffected or increased. A 2- to 2.5-fold increase was found in the activity of acid phosphatase in the rhizoplane soil in comparison to the bulk soil. There was a positive correlation ($r = 0.83^{***}$) between phosphatase activity and the length of mycelial hyphae. The results stress the role of organic P and of acid phosphatase in the rhizosphere in the P uptake by mycorrhizal roots of spruce trees grown on acid soils.

Key words: Organic phosphates – Rhizosphere – Mycorrhizal roots – Acid phosphatase – *Picea abies* (L.) Karst. – Norway spruce

In most agricultural soils, organic P comprises 30%–70% of the total P. In forest soils the proportion of organic P may rise to 80%–95% of the total P (Zech et al. 1987), including 7%–18% located in mycelial hyphae (Baath and Söderström 1979), mainly of the ectomycorrhizae fungi. In mycelial hyphae, P is most likely stored as polyphosphate (Lapeyrie et al.

1984). Polyphosphates are hydrolysed by roots, with rates being higher under non-sterile than under sterile conditions (Savant and Racz 1972; Dick and Tabatabai 1986). Organic-P compounds such as sugar phosphates, lecithin or phytin are hydrolysed by plant roots (Doumas et al. 1986; Tarafdar and Jungk 1987; Tarafdar and Claassen 1988). This hydrolysis is mediated by root-borne acid phosphatase (Tarafdar and Jungk 1987; Tarafdar and Claassen 1988), fungal acid phosphatase (Doumas et al. 1986; Mousain and Salsac 1986) or bacterial alkaline phosphatase (Tarafdar and Claassen 1988). The activity of acid phosphatase in *Salix rotundifolia* roots infected with ectomycorrhiza strongly depends on the fungal species (Antibus et al. 1981). Under non-sterile conditions in the rhizosphere, hydrolysis of organic P may therefore be mediated by both plant root and microbial phosphatases.

Compared to the bulk soil, the phosphatase activity in the rhizosphere soil is considerably higher, depending on plant species and fungal (vesicular-arbuscular mycorrhizae) species (Helal and Sauerbeck 1984; Dodd et al. 1987; Tarafdar and Jungk 1987). Phosphatases are adaptive enzymes, and activity increases when the plants are deficient in P (Barrett-Lennard and Greenway 1982), at the root surface of maize (Helal and Sauerbeck 1988) and of Sitka spruce under field conditions (Alexander and Hardy 1981) and in mycorrhizal fungi (Doumas et al. 1986; Moussain and Salsac 1986). Therefore, particularly in a forest soil with organic P as the dominant form of P, rhizosphere phosphatase activity may play an important role in P acquisition by trees.

In the present study, in stands of 60- to 100-year-old Norway spruce trees, soil P was extracted with different methods and phosphatase activity was measured in bulk soil, rhizosphere soil and the rhizoplane soil associated with short mycorrhizal roots, in order to provide information on the dynamics of P in the

rhizosphere and the use of organic P by mycorrhizal roots of trees.

Materials and methods

Site of studies. The studies were conducted in forest stands of 60- to 100-year-old Norway spruce [*Picea abies* (L.) Karst.] trees at different locations in Baden-Württemberg. The soils investigated were different Cambisols with pH (CaCl₂) of: 2.7 (Mauzenberg), 2.8 (Heidelberg unfertilized), 3.3 (Heidelberg fertilized), 3.0 (Edelmannshof), 3.3 (Lichtenstein), 3.5 (Spielberg unfertilized), and 3.9 (Spielberg fertilized). The trees were healthy or only slightly damaged (needle losses 10%–30%, except for Mauzenberg, with needle losses of 25%–60%). The Heidelberg location (Wilhelmsfeld, fertilized plot) investigated most thoroughly in this study has been previously described by Altherr and Evers (1975). In August 1986 soil cores were collected between trunks (distance 1.50 m from the trunks) with a borer (diameter 8 cm), and separated in subsamples from the different horizons: humus layer (O₁–O_h), 0–5 cm (A_{he}), 5–15 cm (A_{he}), 15–30 cm (B_{sh}). Six cores were combined into one sample, and each sample had six replicates. Sampling at the other locations was performed in the same manner.

The soil samples were sieved to 2 mm to collect the mycorrhizal fine roots (diameter < 1 mm) with adhering rhizosphere soil. Nearly 100% of fine roots from the Heidelberg location were infected with ectomycorrhiza (Dr. B. Metzler, personal communication). The different soil fractions were collected as follows. Bulk soil and rhizosphere soil were collected by shaking as described by Hendriks and Jungk (1981), and rhizoplane soil (from the surface of the mycorrhizal mantle) was collected by carefully brushing off the remaining soil from the surface of the mycorrhizal roots.

In the Heidelberg soil the proportions of total P present as organic P were 72% in the humus layer and 82% in the uppermost soil (A_{he}) layer, with organic P determined by the procedure of Saunders and Williams (1955).

Analyses. In order to obtain more detailed information on P dynamics in the rhizosphere, three different soil fractions (bulk soil, rhizosphere soil, rhizoplane soil) were studied. Water and HCl-extractable P were determined by shaking 1 ml (equivalent to 0.25 g dry weight) of humus or 1 g soil in 50 ml distilled water for 1 h (Van der Paauw 1971), followed by HCl extraction by adding 20 ml 1 M HCl to the water-extracted soil and shaking for 1 h. To characterize the binding state of P in these soil extracts and in the soil solutions (see below) the following analytical methods were compared:

Inorganic P

- (1) Murphy and Riley (1962)
- (2) Ion chromatography (Dionex ion chromatograph)
- (3) Autoanalyser (Technicon autoanalyser).

Organic P and total P

- (1) Digestion with H₂O₂ (P_{H₂O₂}); boiling with 10% H₂O₂ (v: v; 30% p.a.) according to Schlichting and Blume (1966)
- (2) Digestion with concentrated HNO₃ (P_{HNO₃}) for 6 h at 180°C under pressure
- (3) Autoanalyser (P_{AA}, Technicon method for determination of total P)
- (4) DCP measurement (Beckmann emission spectroscopy).

The concentrations of the various P fractions obtained in water extracts from different soil horizons from the location Mauzenberg are shown in Table 1. Different P concentrations were found, depending on the analytical method used (Table 1). For inorganic P, ion chromatography showed consistently lower concentrations than the other colorimetric methods (Murphy and Riley 1962; inorganic

Table 1. Concentrations of P (mg P l⁻¹) in water extracts of Mauzenberg soil samples characterized by different methods for P analysis

Soil horizon	Inorganic P ^a			Inorg. P + org. P + poly P ^b			
	P _{i chr.}	P _{i M.R.}	P _{i AA}	P _{H₂O₂}	P _{AA}	P _{HNO₃}	P _{DCP}
Humus layer	0.22	0.35	0.38	0.53	0.64	0.70	0.69
A _{ch}	0.31	0.36	0.40	0.54	0.69	0.68	0.67
A _{he}	0.18	0.20	0.21	0.36	0.41	0.43	0.42
A _{he}	0.16	0.20	0.22	0.26	0.34	0.27	0.34
B _{sh}	0.09	0.15	0.17	0.23	0.29	0.30	0.27

^a P_{i chr.}, ion chromatograph (Dionex ion chromatograph); P_{i M.R.}, analysed according to Murphy and Riley (1962); P_{i AA}, Technicon autoanalyser

^b P_{AA}, autoanalyser (Technicon method for total P); P_{DCP}, direct coupled plasma measurement (Beckmann emission spectroscopy); poly-P, polyphosphates

P by autoanalyser). Concentrations of organic P determined by H₂O₂ digestion were consistently lower than those determined by the other three methods. These differences were most probably caused by polyphosphates, which are measured with the three latter methods (P_{AA}, P_{HNO₃}, and direct coupled plasma) but not with H₂O₂ digestion. In agreement with this, the K₂S₂O₈ standard method for digestible P [Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung, Bestimmung von Phosphorverbindungen (D11), DIN 38405 (1983)], which only measures a small proportion of the polyphosphates, gave similar P concentrations (data not shown) to those of the H₂O₂ digestion method.

Based on the results obtained by the various methods, the following procedure was used to characterize the binding state of P in the water and in the HCl soil extracts.

Water extract. To 1 ml humus or 1 g soil, 50 ml H₂O_{dist.} was added. The samples were then shaken for 1 h, filtered, and analysed for inorganic P (autoanalyser Technicon II) and for organic and total P by two separate methods (H₂O₂ digestion and autoanalyser). For the H₂O₂ method, the samples were digested with 10% H₂O₂ (v: v) by boiling for 30 min followed by P determination by autoanalyser. The second (autoanalyser, P_{AA}) method comprised ultraviolet digestion for 10 min and hydrolysis at 95°C for 10 min, followed by P determination (autoanalyser Technicon II).

HCl extract. To the water-extracted soil or humus, 20 ml 1 M HCl was added. The samples were then shaken for 1 h, filtered, and analysed for inorganic, organic, and total P as described for the water extract.

For simplification the P determined as P_{AA} (difference between P_{H₂O₂} and total P) is characterized in the following as organic P although it includes polyphosphates. The binding state of P in the soil solution (see below) was determined with the same procedure as in the soil extracts.

Acid phosphatase was assayed according to Tabatabai and Bremner (1969). Phosphatase activity is expressed as enzyme units (EU); one unit of acid phosphatase is the amount of enzyme that hydrolyses 1.0 μmol *p*-nitrophenyl phosphate h⁻¹ at pH 5.4 (0.1 M acetate buffer) and 35°C.

Fungal hyphae length was determined by a slight modification of the agar film method from Baath and Söderström (1979). The agar film was placed in Petri dishes, air-dried, stained with anilin blue, and measured with the intersection method (Olson 1950) under the microscope (200×).

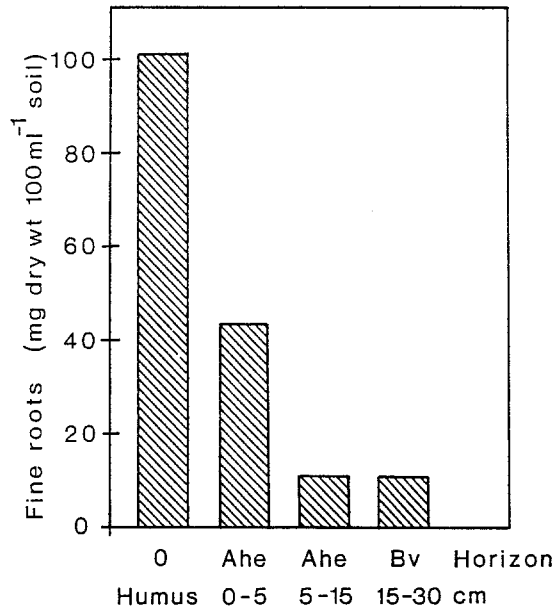


Fig. 1. Fine root density (dry weight 100 ml⁻¹ soil or humus) in different soil horizons. Location: Heidelberg, fertilized

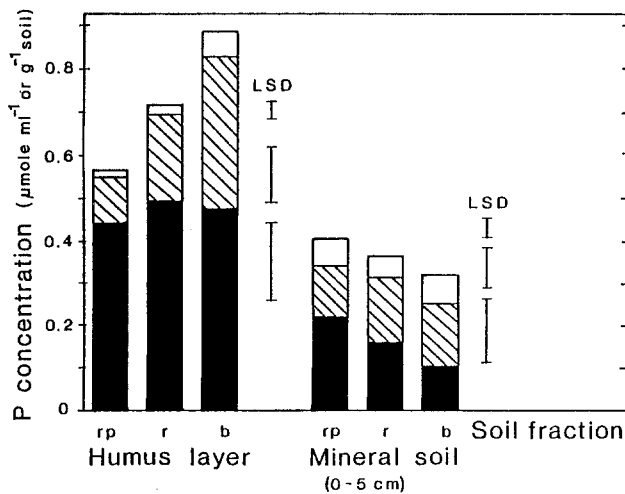


Fig. 2. Water-extractable inorganic (P_i , ■) and organic P [$P_{H_2O_2}$ (▨), autoanalyser (P_{AA} , □)] in different soil fractions (rp , rhizoplane; r , rhizosphere; b , bulk soil) in two horizons. Location: Heidelberg, fertilized

The soil solutions were collected in August 1986 with suction cups (P 80 ceramic, Staatl. Porzellan Manufaktur, Berlin) installed 10 cm below soil surface. After collection of the soil solutions, the ceramic cups were removed from the soil and desorbed (0.1 M HCl) to measure adsorption of P in the cups. Only small amounts of inorganic P and no detectable amounts of $P_{H_2O_2}$ and of P_{AA} were found. Vertical bars in the figures are least significant differences (LSD) for $P < 0.05$ according to analysis of variance (SAS Institute Inc. 1982).

Results

The fine root density in different soil horizons (Heidelberg location) is shown in Fig. 1. The highest root

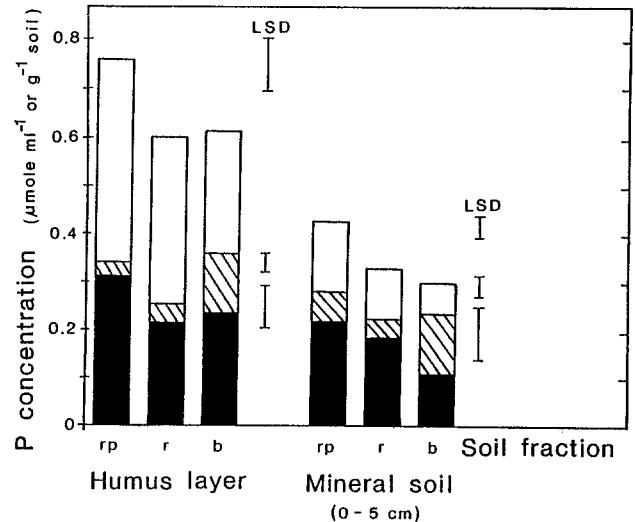


Fig. 3. HCl-extractable inorganic (P_i) and organic P [$P_{H_2O_2}$, autoanalyser (P_{AA})] in different soil fractions (rp , rhizoplane; r , rhizosphere; b , bulk soil) in two horizons. Location: Heidelberg, fertilized. Symbols as for Fig. 2

density was found in the humus layer followed by the uppermost layer of mineral soil (A_{he}). In the soil layers below 5 cm the root density dropped to a low level. Thus, at this location, the humus layer and the uppermost soil horizon (0–5 cm) are probably of most importance for mineral nutrient uptake, P in particular. Therefore, in the following, only the results from these two soil horizons are presented.

The concentrations of P in the water extract from the different soil fractions (bulk, rhizosphere, and rhizoplane soil) are shown in Fig. 2. In the humus layer the concentration of total P decreased by about 38% from the bulk soil to the rhizoplane soil. This decrease was exclusively at the expense of the organic-P fractions ($P_{H_2O_2}$; P_{AA}), whereas the inorganic-P concentrations remained unaffected. In the mineral soil layer (0–5 cm) not only the total P concentrations were lower but also the concentration gradient was reversed; it increased from the bulk towards the rhizoplane soil. This increase was confined to inorganic P.

In the HCl soil extracts (Fig. 3) the concentrations of total P were higher in the humus layer than in the mineral soil (0–5 cm). In marked contrast to the water extract (Fig. 2), in the HCl extracts a much higher proportion of the total P was found as P_{AA} , particularly in the humus layer (Fig. 3). The high P_{AA} concentrations in the HCl extracts were probably derived from polyphosphates in mycelial hyphae. Despite this, in both soil layers, higher concentrations of total P were found in the rhizoplane soil than in the bulk soil. This accumulation of P in the rhizoplane soil was due to increased inorganic P and P_{AA} , whereas $P_{H_2O_2}$ decreased.

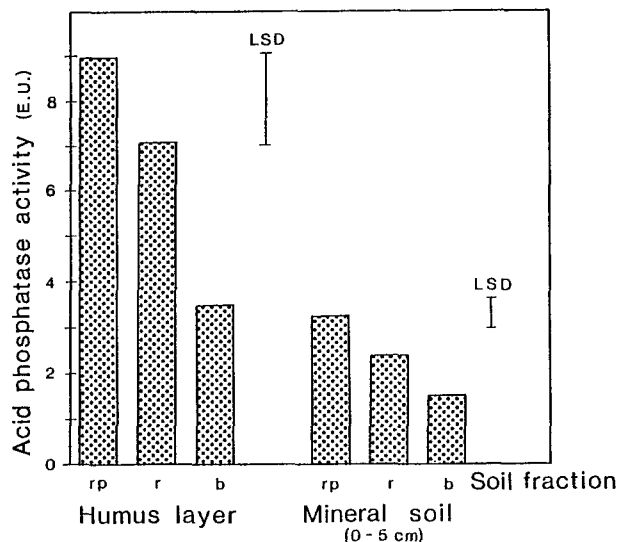


Fig. 4. Phosphatase activity in different soil fractions (*rp*, rhizoplane; *r*, rhizosphere; *b*, bulk soil) in two soil horizons. Location: Heidelberg, fertilized

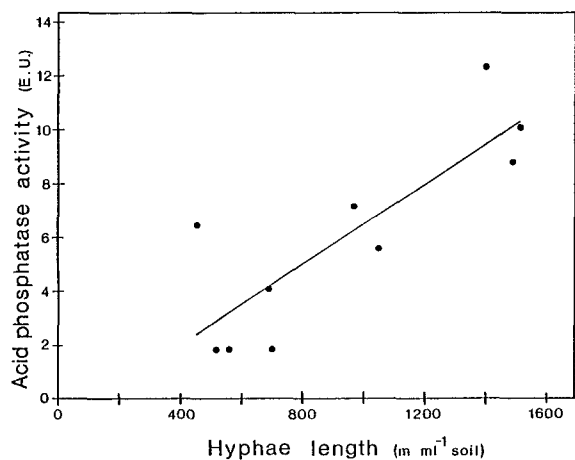


Fig. 5. Correlation between mycelial hyphae length and phosphatase activity (*E. U.*, enzyme units) in the humus layer of bulk and rhizosphere soil. Location: Heidelberg, fertilized ($r = 0.83^{***}$)

Acid phosphatase activity was higher in the humus layer than in the mineral soil, and increased in both soil horizons from the bulk soil towards the rhizosphere and rhizoplane soil by a factor of 2–2.5 (Fig. 4). This increase in phosphatase activity was, at least for the rhizosphere soil fraction, highly positively correlated with an increase in mycelial hyphae length (Fig. 5).

Against expectations, the hyphae length (Fig. 6) was lower in the rhizoplane than in the rhizosphere soil. The lower hyphae length in the rhizoplane soil could be the result of disruption of hyphae from the rhizoplane when the roots were shaken to obtain

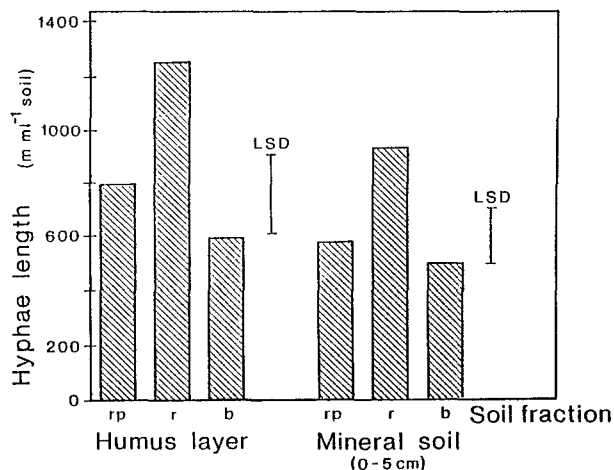


Fig. 6. Mycelial hyphae length in different soil fractions (*rp*, rhizoplane; *r*, rhizosphere; *b*, bulk soil) in two soil horizons. Location: Heidelberg, fertilized

Table 2. Accumulation (+) or depletion (–) of P in the water extract fractions of rhizosphere soil compared to the bulk soil in Norway spruce stands at various locations in Baden-Württemberg

Location	Soil layer	P _i ^a	P _{H₂O₂} ^a	P _{AA} ^a
Heidelberg	Humus	+	–	–
unfertilized	0–5 cm	+	–	–
Heidelberg ^b	Humus	–	–	–
fertilized	0–5 cm	+	–	+
Lichtenstein	Humus	–	–	–
	0–5 cm	–	–	–
Mauzenberg	Humus	–	–	–
	0–5 cm	+	–	+
Spielberg	Humus	+	–	+
unfertilized	0–5 cm	–	+	+
Spielberg	Humus	+	±	–
fertilized	0–5 cm	+	–	+
Edelmannshof	Humus	–	–	+
	0–5 cm	+	–	–

^a P extracted by different methods: P_i, inorganic P; P_{H₂O₂}, extracted by H₂O₂ digestion; P_{AA}, autoanalyser (Technicon method for total P)

^b Willemshöhe, where the data for Figs. 1–7 were collected

rhizosphere soil. Therefore, the hyphae length in the rhizoplane soil fraction has probably been underestimated and that in the rhizosphere probably overestimated.

A comparison of the P fractions in soil water extracts from various locations under Norway spruce stands in Baden-Württemberg is shown in Table 2. Compared to the bulk soil, in the rhizosphere soil an accumulation of inorganic P was found in 8 out of 14 instances. In contrast, in the P_{H₂O₂} fraction, with only two exceptions, P depletion was found, indicating that this organic-P fraction has a particular role in the P acquisition by mycorrhizal roots. In the P_{AA} frac-

Table 3. Concentrations of inorganic P (P_i), organic P ($P_{H_2O_2}$, P_{AA}), and total P in the soil solution (suction solutions) collected at 10 cm depth from spruce stands at various locations in Baden-Württemberg. Solutions were collected in August 1986

Location	Binding state of P and concentrations (μmol)			
	P_i	$P_{H_2O_2}$	P_{AA}^a	Total P
Heidelberg unfertilized	0.7	0.4	0.3	1.4
Heidelberg fertilized	4.8	1.8	0.7	7.3
Lichtenstein	0.5	0.6	0.1	1.2
Mauzenberg ^b	14.4	5.4	0.2	20.0
Spielberg unfertilized	0.4	0.3	0.1	0.8
Spielberg fertilized	1.1	0.7	0.1	1.9
Edelmannshof	0.5	0.7	0.1	1.3

^a P determined by autoanalyser (see text)

^b Severely damaged trees

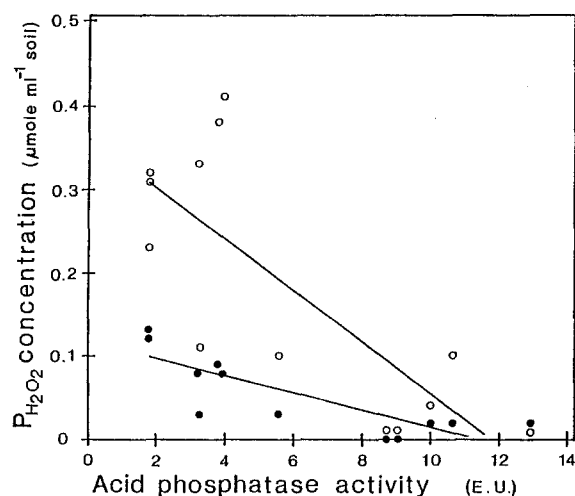


Fig. 7. Correlation between phosphatase activity (E. U., enzyme units) and $P_{H_2O_2}$ concentrations in water (O, $r = -0.78^{***}$ and HCl (●, $r = -0.33^{***}$) extracts of the humus layer. Location: Heidelberg, fertilized

tion the depletion in the rhizosphere soil was less evident.

In the soil solutions (suction solution) from the various locations the total P concentrations differed by the factor of more than 20 (Table 3). Irrespective of the large differences in total P, about 50% of total P (range from 35% to 65%) was found as organic P. High proportions of inorganic P in the soil solutions were only found where P fertilizers had been applied (Heidelberg) or where the pH of the soil was very low (Mauzenberg).

The high proportion of organic P in the soil solution stresses the potential importance of acid phosphatase in the rhizosphere for P acquisition. This importance is indicated by the correlation between the depletion of $P_{H_2O_2}$ in both the H_2O and HCl extracts and the corresponding activities of acid phosphatase (Fig. 7).

Discussion

Studies on P dynamics in the rhizosphere of field-grown plants in general, and of trees in particular, are limited by several major factors: (1) Lack of an analytical method for precise separation of the various forms of P (especially organic P); (2) incomplete mechanical separation of the different soil fractions (bulk, rhizosphere, rhizoplane); and (3) the unknown contribution of P in mycorrhizal hyphae to $P_{H_2O_2}$ and P_{AA} . Despite these limitations, some general conclusions can be drawn from this study on the P dynamics in the rhizosphere of mycorrhizal roots of spruce trees.

The activity of the acid phosphatase is higher in the rhizosphere than in the bulk soil (Fig. 4). This increase in activity can be attributed to both plant roots and fungi (Doumas et al. 1986; Tarafdar and Jungk 1987; Tarafdar and Claassen 1988). In non-mycorrhizal roots of *Pinus halepensis* the phosphatase activity was about 60% of that in mycorrhizal roots (Doumas et al. 1986).

The phosphatase activity increases with P deficiency in both plant roots (Silberbush et al. 1981; Doumas et al. 1986; Helal and Sauerbeck 1988) and mycorrhizae (Doumas et al. 1986; Mousain and Salsac 1986). The relatively small increase in phosphatase activity in the rhizosphere of spruce trees compared to the bulk soil (Fig. 3) may indicate that the P supply to the roots was sufficient at this location. This assumption is supported by the relatively high P concentration in the soil solution (Table 3, Heidelberg, fertilized) and P concentrations in the needles of 1.63 mg g^{-1} dry weight (data not shown), which are in the sufficient range (Knabe 1984).

The utilization of organic P by the mycorrhizal roots is reflected in the decrease in the $P_{H_2O_2}$ fraction, not only in the soils from Heidelberg (Figs. 2 and 3) but also in most of the other soils (Table 2). Thus, the changes in this organic-P fraction in both the water and the HCl soil extracts seem to comprise a suitable parameter for studies on the dynamics of organic P in the rhizosphere, at least of mycorrhizal tree roots. The simultaneous increase of organic P in the P_{AA} fraction in the HCl extracts of rhizosphere soil (Fig. 3) may be attributed to polyphosphates in the mycorrhizal hyphae (Lapeyrie et al. 1984).

The increase of inorganic P in the rhizosphere soil of spruce tree roots (Figs. 2 and 3, Table 2) indicates that the rates of hydrolysis of organic P ($P_{H_2O_2}$ fraction) exceeded the P-uptake rate by mycorrhizae and plant roots. In annual species the rate of hydrolysis by the root-borne phosphatase can be several times higher than the P-uptake rates by the plants (Tarafdar and Claassen 1988). High phosphatase activities in the

rhizosphere may therefore lead to an accumulation of inorganic P in the rhizosphere, particularly in acid mineral soils (Figs. 2 and 3) with highly reactive sesquioxide surfaces.

A depletion of organic P but not inorganic P in the rhizosphere has also been convincingly demonstrated by Tarafdar and Jungk (1987) in various annual species. For the P nutrition of plants, organic P may become particularly important in unfertilized soils (Sharpley 1985). The results of the present study confirm, under field conditions, the role of organic P, and correspondingly of the phosphatase activity in the rhizosphere, for the P uptake by mycorrhizal roots of spruce trees grown on acid soils.

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References

- Alexander IJ, Hardy K (1981) Surface phosphatase activity of Sitka spruce mycorrhizas from a serpentine site. *Soil Biol Biochem* 13:301–305
- Altherr E, Evers FH (1975) Magnesium-Düngungseffekt in einem Fichtenbestand des Buntsandstein-Odenwaldes. *Allg Forst Jagdztg* 146:217–225
- Antibus RK, Croxdale JG, Miller OK, Linkins AE (1981) Ectomycorrhizal fungi of *Salix rotundifolia*: III. Resynthesized mycorrhizal complexes and their surface phosphatase activities. *Can J Bot* 59:2458–2465
- Baath E, Söderström B (1979) Fungal biomass and fungal immobilization of plant nutrients in Swedish coniferous forest soils. *Rev Ecol Biol Sol* 16:477–489
- Barrett-Lennard EG, Greenway H (1982) Partial separation and characterization of soluble phosphatase from leaves of wheat grown under phosphorus deficiency and water deficit. *J Exp Bot* 33:694–704
- Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung DIN 38405, 3. Aufl. (1983). Verlag Chemie, Weinheim
- Dick RT, Tabatabai MA (1986) Hydrolysis of polyphosphates by corn roots. *Plant and Soil* 94:247–256
- Dodd JC, Burton RG, Jeffries P (1987) Phosphatase activity associated with the roots and rhizosphere of plants infected with vesicular-arbuscular mycorrhizal fungi. *New Phytol* 107:163–172
- Doumas P, Berjaud C, Calléja M, Coupé M, Espiau C, d'Ausac J (1986) Phosphatases extracellulaires et nutrition phosphatée chez les champignons ectomycorhiziens et les plantes hôtes. *Physiol Veg* 24:173–184
- Helal HM, Sauerbeck DR (1984) Influence of plant roots on C and P metabolism in soil. *Plant and Soil* 76:175–182
- Helal HM, Sauerbeck DR (1988) Relationship between P supply and phosphatase activity in roots of soil-grown maize (*Zea mays* L.). *VDLUFA-Schriftenreihe* 23:195–202
- Hendriks L, Jungk A (1981) Erfassung der Mineralstoffverteilung in Wurzelnahe durch getrennte Analyse von Rhizo- und Restboden. *Z Pflanzenernähr Bodenkd* 144:276–282
- Knabe W (1984) Merkblatt zur Entnahme von Blatt- und Nadelproben für chemische Analysen. *Allg Forst Z* 39:847–848
- Lapeyrie FF, Chilvers GA, Douglass PA (1984) Formation of metachromatic granules following phosphate uptake by mycelial hyphae of an ectomycorrhizal fungus. *New Phytol* 98:345–360
- Mousain D, Salsac L (1986) Utilisation du phytate et activités phosphatases acides chez *Pisolithus tinctorius*, basidiomycète mycorrhizien. *Physiol Veg* 24:193–200
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36
- Olson FCW (1950) Quantitative estimates of filamentous algae. *Trans Am Microsc Soc* 69:272–279
- SAS Institute Inc. (1982) SAS user's guide: Basics. SAS Inst Inc, Cary, NC
- Savant NK, Racz GJ (1972) Hydrolysis of sodium pyrophosphate and tripolyphosphate by plant roots. *Soil Sci* 113:18–22
- Saunders WMH, Williams EG (1955) Observations on the determination of total organic phosphorus in soils. *J Soil Sci* 6:254–264
- Schlichting E, Blume HP (1966) *Bodenkundliches Praktikum*. Parey, Hamburg Berlin
- Sharpley AN (1985) Phosphorus cycling in unfertilized and fertilized agricultural soils. *Soil Sci Soc Am J* 49:905–911
- Silverbush M, Shomer-Ilan A, Waisel Y (1981) Root surface phosphatase activity in ecotypes of *Aegilops peregrina*. *Physiol Plant* 53:501–504
- Tabatabai MA, Bremner JM (1969) Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol Biochem* 1:301–305
- Tarafdar JC, Claassen N (1988) Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatase produced by plant roots and microorganisms. *Biol Fertil Soils* 5:308–312
- Tarafdar JC, Jungk A (1987) Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biol Fertil Soils* 3:199–204
- Van der Paauw F (1971) An effective water extraction method for the determination of plant-available soil phosphorus. *Plant and Soil* 34:467–481
- Zech W, Alt HG, Haumaier L, Blasek R (1987) Characterization of phosphorus fractions in mountain soils of the Bavarian alps by ³¹P NMR spectroscopy. *Z Pflanzenernähr Bodenkd* 150:119–123

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