

University of South Bohemia in České Budějovice  
Faculty of Science

**MEASUREMENT OF IN SITU PHOSPHORUS AVAILABILITY IN ACIDIFIED SOILS USING  
IRON-INFUSED RESIN**

RNDr. Thesis

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▼ **Annotation**

A hybrid anion resin was tested for *in situ* phosphorus (P) availability measurement in soils of two stands recovering from acidification and having different P-sorption characteristics. The phosphate (P-PO<sub>4</sub>) sorption capacity of the resin (before saturation) was 48 μmol g<sup>-1</sup>. Sorption and elution were tested under P-PO<sub>4</sub> concentrations common in acidic soils (0– 0.42 mmol l<sup>-1</sup>) either with or without the presence of sulfate (0.2 mmol l<sup>-1</sup>). The efficiency of P-PO<sub>4</sub> sorption was independent of the sulfate and was 100 ± 0.2% (n = 56, ± SD). The P-PO<sub>4</sub> recovery stabilized after six elution steps (each: 50 ml of 0.5 M sodium hydroxide, resin/solution 5:1). The efficiency of P-PO<sub>4</sub> recovery was 80 ± 7% and was used to evaluate field measurements. We determined the amount of P-PO<sub>4</sub> in the field using resin bags in three consecutive years. The results indicate that bioavailable P is negatively related to the soil ability to retain P.

▼ **Declaration [in czech]**

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I hereby declare that Mgr. Petr Čapek optimized the resin method, participated in the field measurements, analysed the resins in laboratory and participated in data assembly and writing of the manuscript.

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Karolina Tahovská

## Measurement of *in situ* Phosphorus Availability in Acidified Soils using Iron-Infused Resin

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### ABSTRACT

A hybrid anion resin was tested for *in situ* phosphorus (P) availability measurement in soils of two stands recovering from acidification and having different P-sorption characteristics. The phosphate (P-PO<sub>4</sub>) sorption capacity of the resin (before saturation) was 48 μmol g<sup>-1</sup>. Sorption and elution were tested under P-PO<sub>4</sub> concentrations common in acidic soils (0–0.42 mmol l<sup>-1</sup>) either with or without the presence of sulfate (0.2 mmol l<sup>-1</sup>). The efficiency of P-PO<sub>4</sub> sorption was independent of the sulfate and was 100 ± 0.2% (n = 56, ± SD). The P-PO<sub>4</sub> recovery stabilized after six elution steps (each: 50 ml of 0.5 M sodium hydroxide, resin/solution 5:1). The efficiency of P-PO<sub>4</sub> recovery was 80 ± 7% and was used to evaluate field measurements. We determined the amount of P-PO<sub>4</sub> in the field using resin bags in three consecutive years. The results indicate that bioavailable P is negatively related to the soil ability to retain P.

### ARTICLE HISTORY

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Acidification; aluminium; forest soil; ion exchange resin; iron; phosphorus availability

### Introduction

Although we have good information about the distribution of phosphorus (P) in chemically meaningful fractions in acidic soils (Kaňa et al. 2011; SanClements and Fernandez 2010; Kaňa and Kopáček 2006; Goldberg, Davis, and Hem 1996), the data about biologically available P (bioavailable P) are scarce. Moreover, the word *available* is often used with a broad meaning from P extractable by water and diluted solutions (ammonium chloride, ammonium fluoride, sodium bicarbonate) to tightly adsorbed P fractions extractable by sodium hydroxide (supposedly available). It can lead to overestimation of bioavailable P, especially when extractants are used in inappropriate soil (Sims et al. 2000; Fixen and Grove 1990).

Beyond fractionation methods, anion exchange resins and membranes may be used for estimation of available P in soils (Meason and Idol 2008; McDowell et al. 2001; Rubaek and Sibbesen 1993; Sharpley 1993; Roboredo and Coutinho 2006). Methods based on ion exchange are nondestructive and are not influenced by soil type (Qian, Schoenau, and Huang 1992; Schoenau and Huang 1991), but they can alter soil pH depending on the counterion used (Freese, Lookman, and Merckx 1995). The P-sorption capacity of resin can be reduced by competitive adsorption of other anions present in the soil solution, especially divalent sulfate, but also nitrate and chloride in acidified soils and sites exposed to marine aerosol deposition. Under such conditions the anion resin behaves as a dynamic exchanger rather than a sink (Turrion, Gallardo, and Gonzalez 1999; Cooperband and Logan 1994). All these disadvantages can be avoided by using hydrous iron oxide-impregnated resin, where the sorption of P is based on binuclear bridges with hydroxide (OH) groups (Lehmann et al. 2005). When exposed

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*in situ*, its advantage is mainly in its ability to integrate all the equilibria and processes which influence the amount of P in soil solution (e.g., P release from soil minerals and components, microbial P activity, and P demand by vegetation).

The goal of the study was to test suitability of hybrid resin [hydrous iron (Fe) oxide nanoparticles based, LayneRT, Layne, USA (formerly FerrIX A33E, Purolite, USA)] for measuring phosphate (P-PO<sub>4</sub>) availability *in situ*. First, we evaluated P-PO<sub>4</sub> sorption and elution efficiencies of the resin in laboratory. Then we used resin bags in the field to estimate *in situ* P-PO<sub>4</sub> availability in organic horizons of two acidified soils differing in their P sorption characteristics.

## Materials and methods

### Preparation of ion resin

The hybrid resin (FerrIX A33E, Purolite), which consisted of hydrous iron oxide nanoparticles impregnated into polymeric beads (diameter of 300–1200 µm) was used. Moist resin was pretreated by shaking with 0.5 M sodium hydroxide (NaOH) solution (resin to solution of 1:2 by volume) to remove impurities (P-PO<sub>4</sub> background level was less than 2 µmol l<sup>-1</sup>; data not shown). Then it was repeatedly shaken with demineralized water to remove NaOH until pH decreased to 6.

### Testing of P sorption and recovery

The P-PO<sub>4</sub> saturation capacity of the resin and number of elution steps needed for P-PO<sub>4</sub> recovery were tested with 10 g (dry weight) of the pretreated resin placed in 5-cm-long glass columns (2 cm in diameter, inbuilt sintered glass and stopcock) for both (1) high and (2) natural P-PO<sub>4</sub> concentrations: (1) Sorption capacity was determined in triplicates, with a highly concentrated monopotassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) solution in demineralized water (3.2 mmol l<sup>-1</sup>), filtered through the column at a filtration rate of 8 ml min<sup>-1</sup>. The 50-ml subsamples of filtrate were sampled, and P-PO<sub>4</sub> was determined as soluble reactive P (SRP; Murphy and Riley 1962). The resin sorption capacity was then calculated from the total amount of P retained in the column (difference between the P-flux into and from the column) until SRP in the filtrate was detected (0.003 mmol l<sup>-1</sup>) and was expressed on a dry-weight resin basis. The saturated resin was then sequentially eluted with 50 ml of 0.5 M NaOH at the same filtration rate as during the previous experiment, until the SRP concentrations in the extract stabilized at 1 mmol l<sup>-1</sup>.

(2) The P-PO<sub>4</sub> sorption ability of the resin under natural P-PO<sub>4</sub> and sulfate concentrations (common in our target soils) and the efficiency of sequential elution to extract the retained P-PO<sub>4</sub> were tested with 10 g (dry weight) of moist pretreated resin in the same columns and at the same filtration rates as described previously. Fifty ml of KH<sub>2</sub>PO<sub>4</sub> solutions in demineralized water (0–0.42 mmol l<sup>-1</sup>) with and without the addition of sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) (0.2 mmol l<sup>-1</sup>) were filtered through the column and the filtrates were determined for SRP concentrations. Then the resin was five times sequentially eluted with 50 ml of 0.5 M NaOH in each step; all eluents were combined to obtain one integrated sample, in which the recovered P-PO<sub>4</sub> was determined as SRP. Altogether, we performed 56 tests (35 and 21 for P-PO<sub>4</sub> with and without sulfate, respectively).

### Bags and their deployment

Bags for *in situ* P availability measurement (Binkley and Matson 1983) were prepared by placing 20 g (dry weight) of moist pretreated resin into open plastic rings (1 cm in height, 6 cm in diameter, area 27 cm<sup>2</sup>) covered with a polyamide net (Uhelon 160T, Silk & Progress, CZ). In the field, the bags were

placed horizontally below the litter horizon (in 1- to 5-cm depth, Ol + Of) and at 10 cm below the surface of the humus horizon; both bags were in one pit, but not below each other. There were ten pits within ~3 m in each of the two research plots. The bags were exposed for approximately 7- and 5-month periods (Szillery et al. 2006; Giblin et al. 1991), from November to May (henceforth winter) and then from June to October (henceforth summer) from 2007 to 2010. The winter exposition reflects the common persistence of snow cover in the study area. In total, we always placed 20 bags per one study site, horizon, and exposition period. For each season, absolute amounts of *in situ* available P-PO<sub>4</sub> were expressed as  $\mu\text{mol m}^{-2} \text{ day}^{-1}$ , to compare the expositions with each other. The formulation of *in situ* available P-PO<sub>4</sub> in the rest of the article means P-PO<sub>4</sub> that was available in the soil solution during particular exposition period and was adsorbed on the resin.

### Study site and soil characterization

The study was conducted in an unmanaged area of Plešné (PL) and Čertovo (CT) lakes, the area's watersheds, located in the Bohemian Forest (the Hercynian crystalline mountain massive) at 49° 11' to 48° 47' N and at 13° 12' to 13° 52' E, at elevations of 1057–1334 m. Both watersheds were exposed to high sulfur (S) and nitrogen (N) deposition during the 20th century, became strongly acidified in the 1960s–1980s, and currently undergo slow chemical and biological recoveries from acidic stress (Majer et al. 2003). The watersheds are covered (>90%) with 90–160-years-old Norway spruce forest (*Picea abies*), with sparse beech (*Fagus sylvatica*), fir (*Abies alba*), and mountain ash (*Sorbus aucuparia*). The main difference between the watersheds is the bedrock, which is predominantly mica-schist (muscovite gneiss) with quartzite intrusions in the CT watershed, while the PL watershed is composed of granite. Soils are ~0.5-m-deep spodosols and spodo-dystric cambisols. The respective mean effective cation exchange capacity [ammonium chloride (NH<sub>4</sub>Cl) and potassium chloride (KCl) extractable] of the PL and CT soils (the whole profile) is 129 and 104 mmol<sub>c</sub> kg<sup>-1</sup>, their base saturation is low (15 and 9%), and contributions of exchangeable aluminium (57 and 62%) and proton concentrations (28 and 29%) are high (Kopáček et al. 2002a, 2002b). Soil pH<sub>CaCl2</sub> is lowest in the litter (O, decaying litter material) and humus (A, uppermost mineral horizon with accumulated humified organic matter) horizons (2.5–3.3) and the greatest in the mineral horizon (3.2–4.5). Bedrock of the PL watershed compared to CT bedrock is characteristic with greater P concentrations (37 vs. 13 mmol kg<sup>-1</sup>), greater release of P under acidic conditions (three times greater), and lower release of Fe (150 vs. 870 mmol kg<sup>-1</sup>). Concurrently, soils in the PL watershed have lower concentrations and pools of Fe and aluminium (Al) oxohydroxides and reduced phosphate sorption capacity (three times lower watershed weighted mean sorption capacity) compared to the CT soils (for details, see Kaňa and Kopáček 2006).

### Statistics

*In situ* data were tested for normality first (Kolmogorov-Smirnov test and histogram plots). They did not have normal distribution even after logarithmical transformation. Therefore, a Mann-Whitney test was used for evaluation. All analyses were performed using Statistica<sup>TM</sup> 9.1 for Windows (StatSoft, Inc., USA).

## Results

### P-PO<sub>4</sub> sorption and recovery

Sorption capacity of the resin was 48  $\mu\text{mol P-PO}_4$  per 1 g (dry weight) of the resin (Figure 1a), prior to the first occurrence of measurable SRP concentrations in the filtrate. The P-PO<sub>4</sub> recovery of saturated resin (Figure 1b) was 24% in the first step of elution, then declined in following steps and its cumulative value almost stabilized after the fifth step, increasing only slightly from 51 ± 4% on

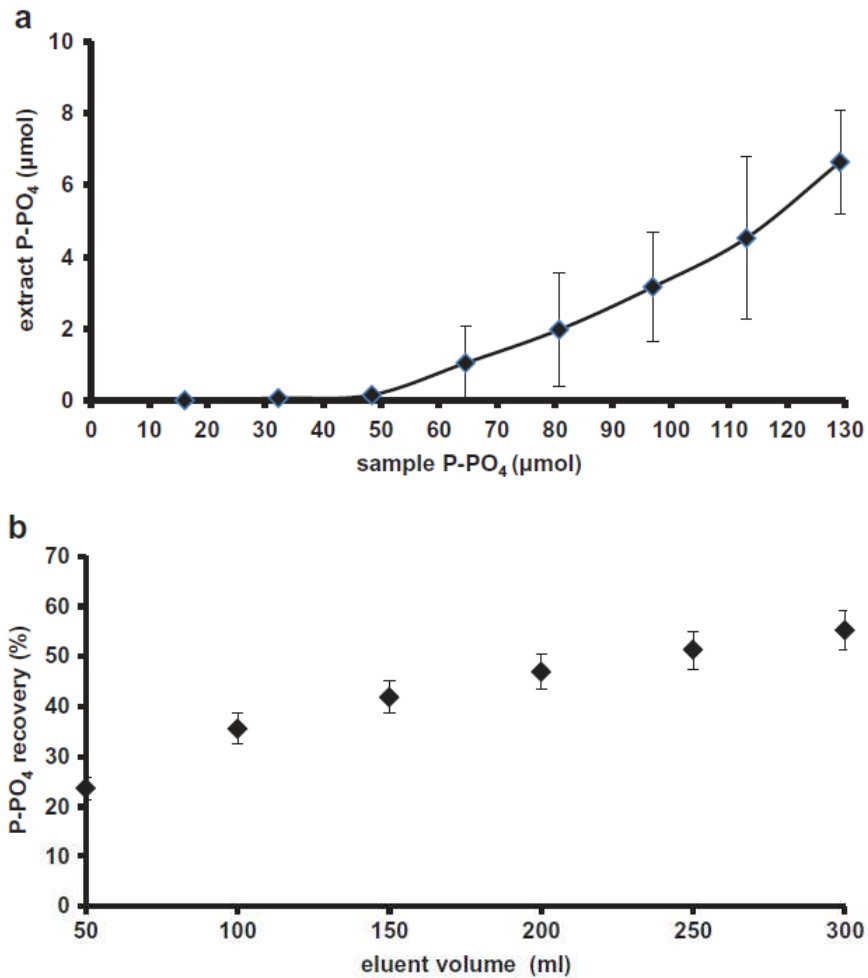


Figure 1. (a) The resin phosphate saturation curve and (b) the elution of phosphate from saturated resin.

average ( $n = 3$ ) between the fifth and sixth steps (Figure 1b). Maximal measured P-PO<sub>4</sub> recovery of saturated resin after six elution steps was 60%.

The sorption ability of the resin was 100% ( $100\% \pm 0.2$ ,  $n = 56$ ) in the natural range of soil P-PO<sub>4</sub> concentrations ( $0\text{--}0.42 \text{ mmol l}^{-1}$ ) and was independent of the presence of sulfate ions (Figure 2a). The amounts of P-PO<sub>4</sub> recovered during the five-step sequential elution were closely related to the P-PO<sub>4</sub> amounts previously adsorbed on the resin (for equation of linear regression see Figure 2b). The recovery of P-PO<sub>4</sub> ranged from 70 to 97%. Taking into account the average efficiency of recovery ( $80\% \pm 7$ ,  $n = 56$ ), the absolute amount of P-PO<sub>4</sub> ( $\mu\text{mol}$ ) accumulated in the bags during *in situ* exposition ( $A_P$ ) can be calculated from its recovered amount ( $R_P$ ) using Eq. (1):

$$AP = (R_P - 0.26)/0.80 \quad (1)$$

where coefficients 0.26 and 0.80 are intercept and slope, respectively, of linear regression between the adsorbed and recovered P-PO<sub>4</sub> in the laboratory experiments (Figure 2b).

### In situ available P

*In situ* available P-PO<sub>4</sub> was significantly greater in the PL than in the CT soils with the respective ranges of 40–100 versus 8–22  $\mu\text{mol m}^{-2} \text{ d}^{-1}$  in the litter and 46–195 versus 9–29  $\mu\text{mol m}^{-2} \text{ d}^{-1}$  in the humus horizons (Table 1). There were no overall differences between litter and humus horizons, but humus horizons were in almost all cases greater than litter horizons.

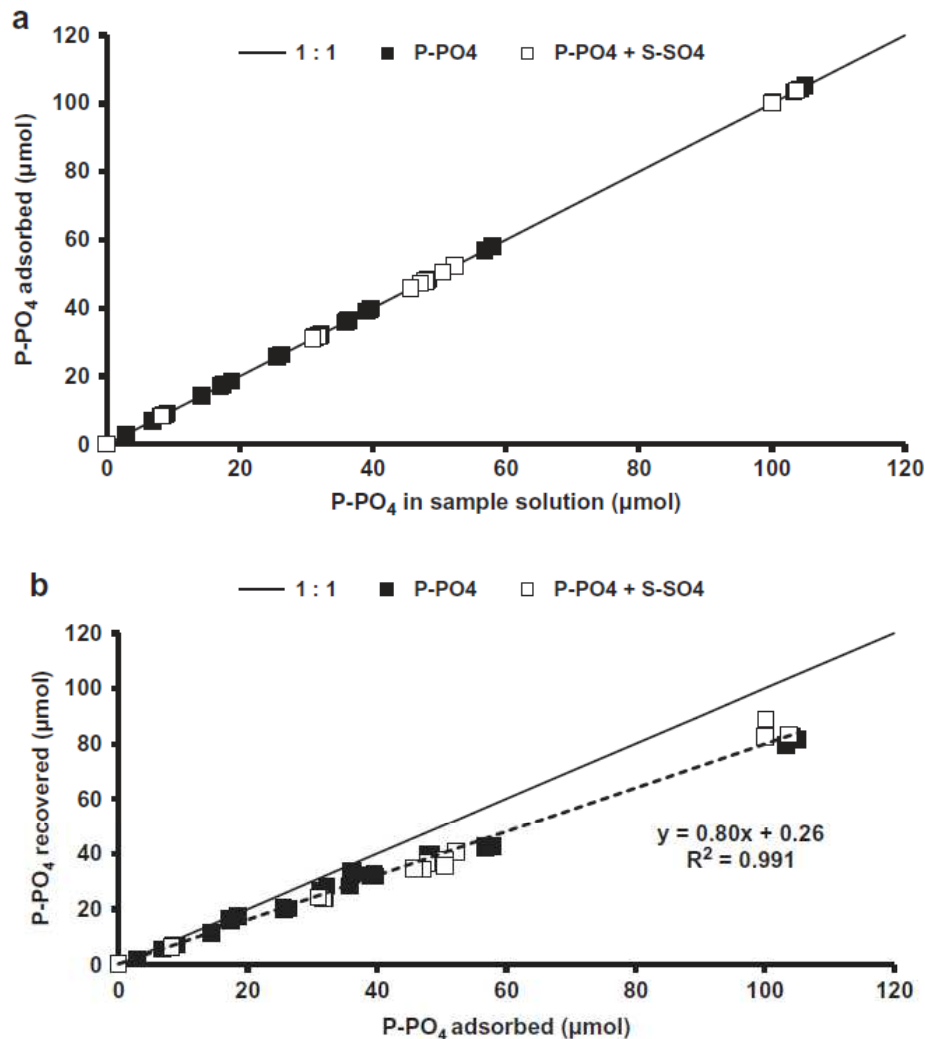


Figure 2. (a) Sorption of phosphate on the resin in the natural range of concentrations and with or without the presence of sulfate and (b) the elution of phosphate from the resin after five elution steps in the natural range of concentrations and with or without the presence of sulphate.

Table 1. *In situ*-available P-PO<sub>4</sub> (μmol m<sup>-2</sup>d<sup>-1</sup>) in litter and humus horizons of Plešné (PL) and Čertovo (CT) soils measured during summer and winter expositions from 2007 to 2010. Medians (n = 20) and quartiles (25%, 75%) are given. Asterisks mark significant differences between PL and CT soils. Mann Whitney test,  $P < 0.01$ .

		Winter 2007/2008	Summer 2008	Winter 2008/2009	Summer 2009	Winter 2009/2010	Summer 2010
Litter	PL	45 (38; 69)*	40 (26; 124)*	51 (28; 122)*	65 (55; 91)*	100 (73; 139)*	44 (34; 53)*
	CT	11 (10; 14)	8 (8; 8)	17 (13; 19)	19 (16; 20)	18 (13; 28)	22 (21; 26)
Humus	PL	67 (43; 88)*	60 (29; 137)*	57 (25; 159)*	46 (16; 68)*	195 (145; 270)*	47 (41; 91)*
	CT	12 (10; 18)	9 (8; 9)	18 (16; 32)	18 (17; 21)	29 (17; 41)	25 (22; 30)

## Discussion

### Methodological considerations

Based on high efficiency of P-PO<sub>4</sub> sorption and recovery we can confirm the suitability of tested resin for measurement of available P-PO<sub>4</sub> in acidified soils. Sorption efficiency of the resin was 100%, independent of the P-PO<sub>4</sub> concentrations. In real soil solution, the sorption of P-PO<sub>4</sub> can be



decreased by other ions, especially sulfate in acidic soils. Our testing showed that the presence of sulfate ions in sample solution did not decrease the sorption of P-PO<sub>4</sub>, which is in agreement with the proposition of Sylvester et al. (2007). The competition with sulfate and other anions (nitrate) is lower due to their low affinity to this type of resin (Sylvester et al. 2007). This supports the suitability of this resin for use in acidified areas.

The observed 80% P-PO<sub>4</sub> recovery was slightly less than 87% achieved by Sylvester et al. (2007), using warm elution solution. The amount of P-PO<sub>4</sub> recovered during the elution increased linearly with its amount adsorbed on the resin in the whole range of tested P-PO<sub>4</sub> (up to 420 μmol l<sup>-1</sup>). The approximate concentrations of P-PO<sub>4</sub> main flow through the ion exchange bags exposed *in situ* (μmol l<sup>-1</sup>) can be counted as the ratio of adsorbed amount of P-PO<sub>4</sub> (μmol m<sup>-2</sup>d<sup>-1</sup>) and the amount of precipitation per exposition (l m<sup>-2</sup>d<sup>-1</sup>, Kopáček et al. 2011). The concentrations of P-PO<sub>4</sub> varied from 1.7 μmol l<sup>-1</sup> (CT litter, winter 2007/08) to 35 μmol l<sup>-1</sup> (PL humus, winter 2009/2010), with the averages of 15 ± 7 μmol l<sup>-1</sup> and 4 ± 2 μmol l<sup>-1</sup> for PL and CT, respectively, which was fairly low compared to tested concentration (3.2 mmol l<sup>-1</sup>). Testing range of P-PO<sub>4</sub> concentrations and the capacity of the resin bags were highly sufficient. Because the pH<sub>H2O</sub> in the studied soils was ~4 (Kopáček et al. 2002a, 2002b) the adsorption of phosphate on the resin would not be very reversible, because of the greatest adsorption capacity of Al and Fe hydroxides and low efficiency of the organic anions on the solubility of Fe and Al hydroxides under such pH values (Oburger, Jones, and Wenzel 2011; SanClements and Fernandez 2010; Harvey and Rhue 2008; Persson, Nilsson, and Sjöberg 1996).

### **In situ available P**

Our results showed consistent differences in *in situ* available P-PO<sub>4</sub> between PL and CT soils, which was in agreement with our expectations. We found greater *in situ* P availability in the PL soils as compared to CT soils, which is most probably primarily related to the differences in bedrock composition. Granite in the PL contains three times more P than mica schist of the CT (Kopáček et al. 2002a, b; Veselý 1994) and releases P more easily under acidic soil conditions (Kopáček, Veselý, and Hejzlar 1998). Soil ability to retain P is defined by its sorption capacity, which is attributed mainly to P sorption on various Al and Fe oxohydroxides in acidic soils. These compounds are not water soluble and P is not, therefore, directly available to plants and microorganisms. It was shown that oxalate-extractable Al and Fe, usually used as a measure of Al and Fe oxohydroxides content in soils, correlate closely with oxalate-extractable P (e.g., Kaňa et al. 2011; Yuan and Lavkulich 1994). The bedrock of CT was found to liberate more Al and Fe than the granite in PL under low pH, which can affect the sorption ability of soils. According to Kaňa and Kopáček (2006) P-PO<sub>4</sub> sorption capacity of the CT soils is 3.5 times greater than that of the PL soils due to greater concentrations and pools of Al and Fe oxohydroxides. Consequently, also the concentrations of oxalate-extractable P are greater in soils of CT than of PL (Kopáček et al. 2002a, 2002b). If the soil sorption capacity is high, P-PO<sub>4</sub> entering the soil from atmospheric deposition, litter decomposition, and weathering is more effectively retained by solid phase and only a small P-PO<sub>4</sub> fraction remains in equilibrium in the soil solution (McDowell et al. 2001). In contrast, soils with a low P-sorption capacity can exhibit high P in soil solution, which is directly available to plants and microorganisms (bioavailable). This is in agreement with Giesler et al. (2004), who found that P immobilization and the related growth of microorganisms can be limited in boreal soils with a high content of Al and Fe. Because the actual soil P availability is affected by many of the abovementioned factors, the soil extraction methods provide only an estimation rather than an exact determination of *in situ* soil P bioavailability. In contrast, the iron-infused resin bag method that integrates fluctuations of P concentration in soil solution provides more straightforward answers to questions about P bioavailability than measurements of P fractions in the solid phase. We recommend this method also in cases when site-specific bedrock and soil composition could limit use of chemical extractions and misrepresent the information about bioavailable P.

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