The Quantitative Estimate of Bioavailable Inorganic Phosphorus Content in Forest Soils by the Modification of the Anion-Exchange Resin Method

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Abstract: The anion-exchange resin (AER) method for the estimation of bioavailable phosphorus was slightly optimised for soils with high contents of readily soluble organic compounds as well as for those having very different clay and sand contents. The method based on a strong base anion exchanger, the active group of trimethyl benzyl ammonium, the field moist soil, and the enclosure of resin beds in a bag was very different soils under relatively uniform outer conditions where the determination of the absorbance of (blue) phosphate-molybdate complex at 870 nm is used. The motivation of the proposed method comes from distinctive features of forest soils where marked differences are commonly encountered between clay/silt/sand contents, pH-values, and the contents of organic substances. A modestly new methodology was applied for the evaluation of seasonal phosphorus dynamics in a forest environment. The recommended rapid assay for available phosphorus was statistically compared with other procedures. Considerable seasonal effects on the bioavailable P contents were demonstrated.

Keywords*:* soil phosphorus; forest soils; available P determination

Most of the phosphorus (P) content measurements are based on quantitative determinations of the extractable agent by spectrophotometry: the use of the anion-exchange resin (AER) method is presently the most common approach for the determination of inorganic available P content (Oliveira *et al.* 2000) even though P fractionation has to be seen as an important alternative approach to a detailed characterisation of particular forms of soil P (Potter *et al.* 1991; McDowell & Sharpley 2004). The problem that justifies the introduction of a modestly new methodology comes from specific features of forest soils rich in organic matter and commonly contain a very high amount of either sand or clay particles. The use of strong extractants for the samples of forest soils that are extremely rich in humic substances leads to a great discoloration by the compounds. In addition, the traditional AER methods using exchange resin impregnated membranes (Lee & Doolittle 2002) were developed for agricultural soils.

The method optimised here was primarily introduced by Amer *et al.* (1955) who continued in the approach of Fried and Dean (1952). Later, the AER method was refined by Lathwell *et al.* (1958), Cooke and Hislop (1963), and Gunary and Sutton (1967). A fundamental advancement occurred with the routine use of polyester-netting bags (Sibbesen 1977) after the separation of the resin beads from the soil particles by using bags was primarily introduced by Vaidyanathan and Nye (1970) and Zunino *et al.* (1972, 1973). The use of resins in bicarbonate forms, in addition, was introduced by Sibbesen (1978). In mid-1970s, it was proved (Walmsley & Cornforth 1973;

Elrashidi *et al.* 1975; Sibbesen 1978) that AER phosphorus correlates highly with the uptake of phosphates by plants. Methodological development of the bioavailable phosphorus content measurements using AER in bags is useful for forest soils from the viewpoint of their distinctive features such as a greater quantity of humic substances with markedly different qualities, easily soluble organic compounds, conspicuous organic horizons, distinctive biota, frequently a higher degree of rockiness and stoniness, lower soil reaction, and more extreme mobility of elements. In addition, each of the methods for bioavailable P determinations may result in the mobilisation of not only available phosphates (Bissani *et al.* 2002) but also of part of the non-mobile phosphorus pool in the soil sample. Alternatively, there exist available methods that eliminate this disadvantage, out of which the use of isotopical solutions of ^{32}P (Olsen & Sommers 1982), water-extraction methods based on deionised water (van der Paauw 1971), and AER methods are the most relevant for the routine use. The use of isotopical solutions of P is negatively influenced by both short-term and long-term phosphate resorptions, the amount of phosphates extracted by deionised water being extremely low and the anion-exchange resin methods before usage of polyester-netting bags being time consuming. With respect to the facts and to the distinctive features of forest soils, resin-extractable phosphates were studied. The particular analytical steps were derived from the facts that the amount of anion resin-extractable phosphorus used depends upon the time required for adsorption, the concentration of the resin, and the concentration of phosphates.

The following analytical limitations of particular extractions were tested:

(i) the extremely low amount of phosphates extracted by deionised water,

(ii) the slowness of AER methods without the use of netting bags,

(iii) the long-term resorption of phosphates with the use of isotopical solutions of ^{32}P,

(iv) the treatments of fresh and air-dried samples,

(v) the different times required for adsorption,

(vi) the chemical reduction by ascorbic acid and the procedures without reduction,

(vii) the different types and changing concentrations of the resin.

The modified technique starts with analysis of fresh soil samples: if the resin is free, soil samples have to be air-dried, crushed, and the sieved which leads to physical and chemical changes that affect the solubility of phosphates. Furthermore, some losses of individual resin beads during the separation can occur, resulting in underestimates of the available P content in the soil.

As a result of the literature survey, the objective came from the intention to develop a modification of the determination of plant available P in forest soils which would be accurate and simple enough to be carried out routinely. The methodological improvement of the determination of the bioavailable soil P by the AER method was tested using standard statistical criteria.

MATERIAL AND METHODS

Soils used

The study site in southern Czech Republic Ranspurk Virgin Forest (Ranspurk National Nature Reserve) was located at 48°4′ latitude, 16°6′ longitude, and 152 m above sea level. The mean annual temperature and the average annual precipitation are 9.1°C and 534 mm, respectively. The floodplain deciduous forests comprised English oak (*Quercus robur* L.), narrow-leaved ash (*Fraxinus angustifolia* Vahl.), field maple (*Acer campestre* L.), European hornbeam (*Carpinus betulus* L.), smooth-leaved elm (*Ulmus carpinifolia* Gled.), and hawthorns (*Crataegus* sp.) growing on the site where no cropping systems, no fertilising, no liming and no peat formation can be found.

Based on the current approach (Robertson *et al.* 1999; Pansu *et al.* 2001), the sampling was carried out in the frame of the design-based approach from both surface (soil layer 0–20 cm) and subsurface (soil layer 20–40 cm) horizons. The design involved collection of individual soil samples (per soil type and depth) followed by mixing them into representative samples and analysing those in replicates. Each particular soil is characterised by the data measured in four replicates of mixed samples obtained from nine individual soil samples coming from three independent sampling plots.

Two volumetric samples were taken from the soil layers of 0–20 cm and 20–40 cm from the following soil units (ISSS-ISRIC-FAO 1998): Eutric Cambisol, Haplic Fluvisol, Humic Regosol, Gleyic Regosol, Haplic Gleysol and Histic Gleysol. Eighteen experimental plots (2 × 2 m) were established for sampling the six soil units in a randomised design, each soil with three replicates. Soil samples, which are characterised by some physical and chemical characteristics (Table 1), come generally from deep, not well drained sandy-clay loams derived from mixed non-calcareous Holocene alluvial sediments of large Central European left-side tributaries of the Donau River. Even though the sampling sites were within 200 m from each other, there are six different soil units differing considerably in the percentage of sand (from 21% to 90%) and clay (from 4% to 42%), entirely in the humus content (from 0.2% to 29%) and C:N ratio (from 1.5 to 21.5), and partly in the actual soil reaction (pH/H_2O from 4.2 to 6.1) under relatively uniform outer abiotic conditions.

With the knowledge of the seasonal dynamics study, three independent sampling plots for each of the soil types were chosen four times during the year (the spring, the summer, the autumn, and the winter sampling). From each replicate plot, three individual soil samples of each of the soil layers were randomly taken and combined. The samples were placed in PE-bottles, immediately refrigerated at 4°C, and analysed within one or two weeks. All laboratory analyses were done in four replicates, pH measurements in six replicates. Particle-size classes were determined using the U.S.D.A. system (diameters of 0.002–0.05–0.1–2 mm) by pipette techniques. The content of the humic substances was determined after sulphochromic oxidation followed by spectrophotometric measurement (ISO/DIS 14235 1995). The analyses of oxidisable carbon and total nitrogen were carried out on an Autoanalyser LECO FP-2000 Makro. The soil reaction was measured in the air-dried soil-water suspension (1:5 v/v, shaken for 5 min) at room temperature (ISO/DIS 10390 1992).

Experimental protocol

The soil samples were tested using the proposed modification and six other common methods (Olsen *et al.* 1954; Amer *et al.* 1955; Egnér *et al.* 1960; van der Paauw 1971; Mehlich 1984;

Table 1. Physical and chemical soil characteristics of the single study site

	Sand (%)	Fine s. (%)	Silt (%)	Clay (%)	pH/H_2O	Humus (%)	C_{OX} (%)	N_t (%)	C/N
Soil layer 0–20 cm									
Eutric Cambiso9l	79.0	8.7	8.2	4.0	5.83	28.83	16.72	0.79	21.4
Haplic Fluvisol	64.7	9.2	22.0	4.0	5.73	14.64	8.49	0.41	20.3
Humic Regosol	45.5	14.7	27.0	12.7	4.48	8.66	5.03	0.32	15.7
Gleyic Regosol	41.2	19.5	20.5	18.7	5.48	7.25	4.21	0.50	8.3
Haplic Gleysol	39.0	6.2	30.7	24.0	4.88	2.13	1.30	0.14	9.0
Histic Gleysol	33.5	16.0	23.5	28.0	4.24	3.21	1.84	0.13	13.8
H statistics	21.669	18.771	22.371	22.02	31.282	22.019	22.019	22.4	21.789
Confidence interval (*p*)	0.0006	0.0021	0.0004	0.0005	0.0000	0.0005	0.0005	0.0004	0.0006
Soil layer 20–40 cm									
Eutric Cambisol	90.0	3.0	3.2	3.7	5.26	2.44	1.41	0.13	10.6
Haplic Fluvisol	55.0	12.0	29.0	4.0	5.90	2.21	1.28	0.10	13.5
Humic Regosol	52.7	5.5	43.0	10.7	5.35	4.46	2.59	0.16	16.0
Gleyic Regosol	20.7	37.7	16.0	25.5	4.77	1.02	0.59	0.40	1.5
Haplic Gleysol	33.0	8.0	25.5	33.5	5.47	0.33	0.19	0.04	4.2
Histic Gleysol	28.5	10.0	20.0	41.5	6.13	0.20	0.12	0.03	3.6
H statistics	22.059	19.557	20.793	21.649	30.206	22.4	22.4	22.052	20.668
Confidence interval (*p*)	0.0005	0.0015	0.0009	0.0006	0.0000	0.0004	0.0004	0.0005	0.0009

i.e. Mehlich III extraction; Soon 1990) for the bioavailable P content determination. The methods were compared by means of the approach based on the relative values expressed by particular errors of the methods used – such approach was applied on the comparison of the laboratory techniques which cannot be compared by the traditional way of comparing their absolute values. The operational conditions in these two AER methods were the following: the original procedure of Amer *et al.* (1955) using 1 g of air-dried soil, 1 g of resin, 10 ml of distilled water and the leaching of phosphates with sodium chlorite, and of Soon (1990) using 2 g of air-dried soil, 2 g of resin beads, and shaking for 18 hours. The experimental protocol for the application of the modification of AER method involved three other particular analytical steps – a different ratio between soil and resin, different ways of leaching inorganic P assessed by resin, and a different shaking time. The operational conditions for the optimising of AER methods included the use of the ratio of 1 g of air-dried soil to 3 g (weight) of resin beads by Cooke and Hislop (1963), the ratio of 4 g of air-dried soil to 5 cm^3 (volume) of resin beads by Sharpley *et al.* (1989), the leaching of phosphates with sulphates and hydroxides after using AER as P-sink by Gunary and Sutton (1967), the leaching with a mixture of sodium chloride, hydrochloric acid, and distilled water by Bache and Ireland (1980), 2 hour-shaking by El-Nennah (1978), 16 hour-shaking by Zunino *et al.* (1972), and 24 hour-shaking by Sibbesen (1977). Both Cl-saturated (Amer *et al.* 1955) and HCO_3-saturated (Sibbesen 1978) resins were examined.

Second, the experimental protocol for the application of the modification of AER method came from the testing of particular anion exchange resin type. The type of resin having its functional groups basic in nature was chosen. Amino groups ($-NH_2$) are the most common functional groups in the strong base anion exchangers (Kenkel 2002) producing ionogenic groups and releasing hydroxylic ions, such as $-NH_2 + H_2O \leftrightarrow -NH_3^+ + OH^-$. Strongly basic active groups include $-N(CH_3)_3^+OH^-$, $-N(CH_3)_3^+Cl^-$ and $-N(CH_3)_3^+HCO_3^-$, weakly basic active groups include $-NH_3^+OH^-$, $-NH_3^+Cl^-$ and $-NH_3^+HCO_3^-$ (Newman & Hobot 2001). For the modified technique, two types of resin are recommended: strong base anion exchangers where amino groups are incorporated as trimethyl benzyl ammonium (Type I), or strong base anion exchangers where amino groups are incorporated as dimethyl ethanol benzyl ammonium (Type II) instead of styrene exchangers of either a microporous or a macroporous gel matrix.

The description of the methodology

Polyester-netting bags containing 2 g of resin in the form of spherical beads were prepared from anion exchange resin, Dowex 1x8 50, strong base anion exchanger with the active group of trimethyl benzyl ammonium, mesh 30–50, and polyester-netting bags, mesh < 30. The bags were placed into a 500 ml-polyester flask filled with sodium bicarbonate solution, 0.5 mol/l $NaHCO_3$, and shaken intensively for 30 minutes. The bags were taken out, put into the same flask filled with a new solution of sodium bicarbonate, and were intensively shaken for further 30 minutes. For the purpose of final spectrophotometric determination, ammonium vanadate, 0.002 mol/l NH_4VO_3, was mixed with ammonium molybdate, 0.03 mol/l $(NH_4)_2MoO_4$, in the ratio 1:1.

10 g of fresh soil was used to calibrate the PE-flasks, and 15 ml of deionised water was added. The gravimetric moisture content of these mostly organomineral samples were concurrently determined by drying the soil samples at 65°C for 24 h (Kutilek & Nielsen 1994). The soil suspensions were shaken for 30 minutes and filled up with deionised water to 20 ml. The bags with resin in the bicarbonate form were placed into the soil suspensions and shaken intensively for five minutes. After standing for 30 min, the resin bags were taken out, washed quickly in deionised water, and put into new PE-flasks. 15 ml of hydrochloric acid, 0.5 mol/l HCl, was added to the resin bags followed by 30 min of vigorous shaking. The newly prepared suspensions were filtered into beakers. 1 ml of each of these filtrates were placed in test tubes to which 1 ml of a mixture of ammonium vanadate and ammonium molybdate were added. The absorbances of the solutions were measured against the blank at 400 nm. To make the calibration curve by the preparation of standard solutions, potassium dihydrogenphosphate, initial dilution – 0.005 mol/l KH_2PO_4, hydrochloric acid, and the ammonium vanadate and ammonium molybdate mixture were used. To obtain the blank, 20 ml of distilled water was mixed with 15 ml of hydrochloric acid and the mixture was filtered. 1 ml of the filtrate was added to 1 ml of the ammonium

vanadate and ammonium molybdate mixture and 2 ml of this solution was used as the blank. The bioavailable phosphorus content was expressed as mg of P obtained from 1 kg of fresh soil/recalculated into dry matter.

The statistical methods

The correlation analysis for the comparison of different analytical methods was performed: the recommended rapid assay for available phosphorus was statistically compared with the other procedures. The results were analysed statistically (Webster 2001) on the laboratory repetitions basis by the analysis of variance. Because these sets of data did not show the normal distribution, the differences between the soil layers of the six soil units were analysed by the non-parametric Kruskal-Wallis ANOVA test where the least significant differences were calculated by multiplying the standard error of differences between the means by the t-value. H parameter (Table 1) comes from Kruskal-Wallis statistics (Kruskal & Wallis 1952) where the analysis was based on variance performed on ranks, and B parameter (Table 2) from Kendall τ statistics (Kendall 1948). The descriptive statistics and the ANOVA method for the detailed evaluation of variance was used to show the interactions between the laboratory procedures tested and the samples of particular layers. Statistical suitability of the measured data according to normality was tested by Kolmogorov-Smirnov-one-sample test. Because of the aim being the comparison of analytical methods, the seasonal dynamics in AER phosphorus content in six forest soils was not tested from the viewpoint of the differences between the seasons.

RESULTS AND DISCUSSION

A modestly modified AER method by Amer *et al.* (1955), improved by the usage of polyester-netting bags of resin (Sibbesen 1977), was compared with the original methods and, afterwards, the modified method was applied on the description of the seasonal variation of the available P content on a single site showing very different soil types.

Table 2. The summary qualitative evaluation of methods tested in forest soil

	Method	Mean[a]	Std. error	Std. deviation[b]	K-S Z	P-level (KS)[c]	B
Bioavailable P content soil layer 0–20 cm	modification presented	0.78	≈ 0	0.16	1.5	0.0	0.58
	Van der Paauw (1971)	0.39	≈ 0	0.12	0.6	0.9	0.24
	Olsen *et al.* (1954)	1.51	≈ 0	0.32	1.1	0.2	1.12
	Egnér *et al.* (1960)	1.39	≈ 0	0.28	1.1	0.2	1.34
	Mehlich (1984)	1.39	≈ 0	0.26	1.2	0.1	1.10
	Amer *et al.* (1955)	0.62	≈ 0	0.13	0.9	0.4	0.43
	Soon (1990)	0.67	≈ 0	0.15	1.0	0.3	0.47
Bioavailable P content soil layer 20–40 cm	modification presented	0.69	≈ 0	0.15	1.3	0.1	0.52
	Van der Paauw (1971)	0.30	≈ 0	0.08	0.7	0.7	0.23
	Olsen *et al.* (1954)	1.32	≈ 0	0.27	1.1	0.2	0.98
	Egnér *et al.* (1960)	1.03	≈ 0	0.18	1.0	0.3	0.89
	Mehlich (1984)	1.35	≈ 0	0.27	1.4	0.0	1.03
	Amer *et al.* (1955)	0.52	≈ 0	0.12	1.5	0.0	0.40
	Soon (1990)	0.60	≈ 0	0.16	1.0	0.3	0.43

[a]bioavailable P content in mg P/kg; [b]descriptive statistics; [c] K-S test (test of normality); K-S Z and P-level are Kolmogorov-Smirnov statistics

Comparing the methods

Based on particular analytical data, the significance of the comparisons between the basic methods for the available P estimation, is described in Table 2 displaying the estimation of the data dispersion (standard error, standard deviation, *CV* and variance) based on the descriptive statistics for both surface and subsurface horizons.

Exploratory analyses (marginal mean estimation, ANOVA parameters estimation) for the surface organo-mineral horizons, 0–20 cm, and the subsurface mineral horizons, 20–40 cm, were performed. The data are expressed as the means of the six different forest soil units. Two factors (METHOD, SOIL) were considered for the study of the effects on the methods tested: all methods were classified as non-comparable. The error estimations, the estimation of dispersion, ANOVA results, and descriptive statistics showed a distinguished variance of the modification presented being in the centre of the variances and the standard error of the mean being one of the smallest in the modification presented. The whole dataset could be considered as being normally distributed for the surface horizons with the modification presented, and for the subsurface horizons with the methods of Mehlich (1984) and Amer *et al.* (1955).

Dealing with the actual biogeochemical cycling of P, bioavailable phosphates create a labile P-pool composed of exchangeable phosphate ions on soil colloids and in soil solutions. This pool is replenished by the dissolution of mineral phosphates and mineralisation of organic phosphates, and so P cycling is strongly influenced by (i) the mobilisation of phosphates from the products of weathering; (ii) by the rates of biological immobilisation; (iii) by the decomposition of organic matter by soil microorganisms followed by either fixation, occlusion, adsorption, and re-coagulation onto/into the soil components; and, (iv) by the uptake by plants. The depletion of phosphates begins with the transfer of non-labile and labile P from the solid soil phase into soil solutions and onto colloid surfaces. In the same way, phosphates can be depleted from the soil solution through the uptake by plants and soil biota, and by anion adsorption on the positively charged surfaces of the soil colloids. With respect to plants, the presence of the easily soluble form of labile P is a crucial factor determining P availability (cf. Sibbesen 1983). Because only low concentrations of bioavailable phosphates in soil exist, the transport of phosphates to the plant roots plays an essential role. In free soil, diffusion and mass flow are of fundamental importance (Steward & McKerchner 1982). The findings for anion-resin-extractable P, as the most plant-available P fraction, endorse the idea of the important advantages of the AER methods in comparison with the extraction methods: the resin in bicarbonate forms efficiently adsorbs phosphates without physical and chemical changes in the soil samples or the changes in their pH level.

The use of resin for assessing available inorganic P in soil is based mostly on chloride- or bicarbonate-saturated resin. The modestly new methodology is based on a strong base anion exchanger with the active group of trimethyl benzyl ammonium. In terms of forest soils generally characterised by low pH levels, the essential advantage of bicarbonate resin must be highlighted: unlike Cl-resin, the HCO_3-resin chosen simulates the effect of the plant roots under acid conditions more accurately (Soon 1990; Potter *et al.* 1991). Because of the important roles of the pH level of the solutions measured and the contents of arsenates, silicates, and germanium compounds, the absorbances of phosphate-molybdate complexes were recommended not to be measured after reduction with ascorbic acid.

Table 2 showed that the variance for available P in subsurface horizons was very low and the variance for available phosphorus in surface horizons was between the maximal and minimal values for the other methods tested. If all the methods tested were comprised, the marginal mean estimations for both horizons in the modified procedure would be one of the lowest. ANOVA parameter estimations fluctuated around the middle of the values obtained from the other methods. The proposed modification appeared as the lowest values of the error influence, i.e., it provided the most stable measurements in terms of the error influence. Comparing these results with the data from other comparative papers, the above-mentioned criteria supported the value of anion exchange resin methods. AER efficiently adsorbs phosphates without physical and chemical changes of the soil samples or changes of their pH level: Cajuste *et al.* (1994) and Schmidt *et al.* (1997) tested a great number of bioavailable P methods and recommended AER methods, Elrashidi and Larsen (1978) stated that phosphate depletion by either plants or AER did not change the phosphate potential of the soil. The findings of the study presented endorse the idea of the important advantages of the resin methods in comparison with other methods of the bioavailable P content determinations. Bache and Rogers (1970) and Walmsley and Cornforth (1973) tested a great number of the bioavailable P estimation methods and recommended the anion-exchange resin methods, which was confirmed by a number of other findings (El-Nennah 1978; Bache & Ireland 1980; Soon 1990).

The experiments described were performed on those types of forest habitats which show a great amount of dead organic matter overlaying mineral soil. The production of organic acids in the course of the decomposition of the plant litter forms outstandingly strong extractive environs in forest soils. The methods for the bioavailable P determinations in forest soils are still a challenge: relating the results presented, in other works, AER depletes phosphates from soil without physical-

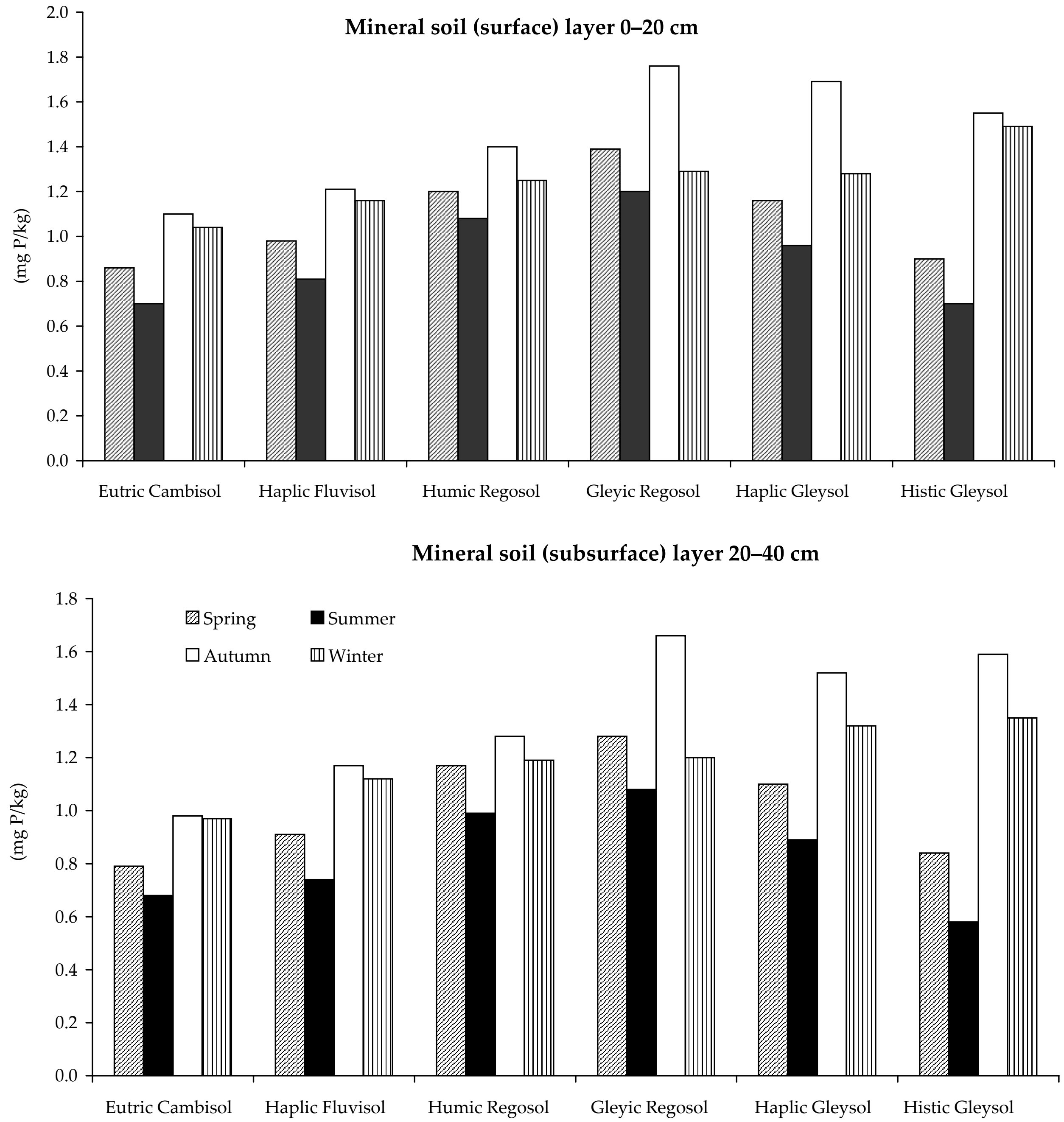

Figure 1. Seasonal dynamics in AER phosphorus content in six forest soils

chemical changes and without pH level changes. The modified technique starts with analysing fresh soil samples: if the resin is free, the soil samples have to be air-dried, crushed, and sieved which leads to physical and chemical changes that affect the solubility of phosphates. Furthermore, some losses of individual resin beads during the separations can occur, resulting in underestimates of the bioavailable P content in the soil.

An application on seasonal dynamics

In the statistical calculation of the results presented in Table 1, two soil layers were compared. The statistical significance of the differences between the soil units used was very high with all the soils used. The calculated H statistics corresponds to a certain confidence interval when p-values are much lower than 0.01 in any case. That means that the differences between groups are of a high statistical importance (the less the p-value, the higher the difference).

The data presented in Figure 1 manifest the use of the modified technique for the characterisation of the seasonal dynamics of the bioavailable P content on samples from the forest soils taken at the plots and showing very different contents of sand/silt/clay, pH values, the contents of humic substances, and oxydisable carbon:total nitrogen ratios.

The bioavailable P contents of the very different forest soils from a single site are shown in Figure 1 in which a considerable seasonal effect is demonstrated. The highest contents of HPO_4^{2-} and $H_2PO_4^-$ anions in very different soils under relatively uniform outer conditions were found in autumn when the decomposition rate of dead organic matter is most intensive. Similarly, the lowest P content was found in summer when the dead-organic-matter-pool is most depleted and the moisture content of soil cannot allow a higher activity of living soil coenoses. The P content in winter was generally higher than in spring except for Gleyic Regosol (in both surface and subsurface mineral horizons). Alloush and Clark (2001) and Feng *et al.* (2003) described significant linkages of the phosphorus uptake to the soil biological acitivity. The application of the method modified on the seasonal P dynamics showed similarly that the uptake of phosphorus at the time of the accelerated ecophysiological processes in plants is so rapid that the measurable content of the element is lower than its amount at the time of low temperatures when the biological activity of the soil considerably diminishes.

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