A comparison of 14 soil phosphorus extraction methods applied to 50 agricultural soils from Central Europe

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ABSTRACT

Phosphorus (P) fertilization is commonly based on soil testing, for which a variety of different soil P extraction methods are in use. The aim of this study was to compare 14 soil P extraction methods in terms of their extraction yield and their relation to soil properties. Fifty contrasting agricultural topsoils were sampled from Austria and Germany. The soils were extracted with the following methods/extractants: H_2O, $CaCl_2$, LiCl, Olsen, Bray and Kurtz II (Bray II), Mehlich 3, calcium-acetate-lactate (CAL), iron oxide impregnated filter papers (Fe-oxide P_i), cation and anion exchange membranes (CAEM), acid ammonium oxalate, citrate-bicarbonate-dithionite, HCl, organic P and total P. The extracted P varied over three orders of magnitude and increased in the order H_2O < $CaCl_2$ < LiCl < Fe-oxide P_i < Olsen < CAL < CAEM < Mehlich 3 < Bray II < dithionite < organic P < HCl < oxalate < total P. This sequence is in accordance with previous studies and reflects different extraction mechanisms and P pools. The different extraction methods were generally well correlated, especially when P extraction was achieved by a similar mechanism. The soil properties most influential on P extractability were pH, carbonate content, texture as well as iron oxide content and crystallinity. Our results show that the different extraction methods extract distinct pools of soil P with strongly varying extractability, and that the extractability of a given pool may be influenced by different soil properties to different extents. If and how these relationships translate to plant P uptake requires further examination.

Keywords: solubility; plowed layer; nutrients; agriculture; plant-available; crops

Phosphorus (P) is a central element to life on Earth. Living organisms are dependent on a persistent supply of P as it is crucially involved in most major metabolic processes, e.g. in energy transfer as adenosine triphosphate (ATP). Likewise, plants rely on P to secure energy production in photosynthesis (Smil 2000, Ruttenberg 2009). Therefore, the fundamental significance of P in agriculture and food production is beyond question.

In nature, we can identify several P pools (Smil 2000, Ruttenberg 2009). The largest P reservoir in the lithosphere occurs in marine and freshwater sediments (about 800–4000 × 10⁶ Mt P). The entire phytomass (terrestrial and marine; about 570–625 Mt P)

amounts to over 90% of P stored in the totality of all living organisms. Gaseous compounds of P are not stable; therefore, only an insignificant amount (0.028 Mt P) exists in the atmosphere as dust and sea spray picked up by wind erosion. The flows of P between these reservoirs are slow (one cycle may take more than 1 million years), which is caused by the low solubility of phosphate in water and the lack of an airborne reservoir.

In soils, P derives mainly from weathering of the primary mineral apatite (Schlesinger 1997). The average total P in soils ranges from 200 mg/kg (in older/highly weathered soils) to 800 mg/kg (in younger/less developed soils) (Cross and

Schlesinger 1995); the average amount of organic P ranges between 30% and 65% of the total P (Condron and Tiessen 2005). Four major forms of soil P may be distinguished, i.e. P dissolved in soil water, P sorbed to surfaces of clay minerals or Fe and Al oxides, P in primary phosphate minerals and P in organic substances and living organisms. Accounting for these various P pools, different approaches to extract P from the soil are available and numerous soil P extraction methods have been developed. Even in routine soil testing many different extraction methods are used today. For example, the calcium-acetate-lactate (CAL) extract (Schüller 1969) is used in routine soil testing in Austria and Germany, while Mehlich 3 (Mehlich 1984) is used in the Czech Republic and in major parts of Canada and the USA. Some states in the USA use the Olsen extract (Olsen et al. 1954), while in Brazil a more recently established extraction method based on anion exchange membranes (resin P; Bissani et al. 2002) is employed.

When comparing different soil P extraction methods, many of the available studies used a limited number of soils that had been fertilized/spiked with different levels of P. By contrast, our approach was to include a wide variety of soils covering different P status and a wide range of soil properties. This shall provide a wider perspective on the comparison of different extraction methods and their dependence on soil properties. Here we compare 14 different soil P extraction methods that utilize different mechanisms of extraction in order to quantify the magnitude of P extractable by these methods and to assess the influence of soil properties on P extractability.

MATERIAL AND METHODS

Soil sampling and basic soil characterization. Soil samples were taken from the plowed layer (0–30 cm depth) of agricultural fields in Austria and Germany in March/April 2011. The sites were chosen to cover a wide spectrum of soil properties and P levels. Prior to sampling, the soils were managed according to common farm practices, and no further measures were taken (e.g. specific P fertilization) to obtain different levels of P. Most soil samples were only represented once in this study. However, approximately one third of the samples were part of different long-term

experiments; in this case, the soils were similar and amended with different levels of P over time while otherwise managed equally. All other soils were exposed to diverse tillage, fertilization and crop rotations prior to sampling for this study.

After sampling, the soils were dried at 50°C, sieved to pass 2 mm, and stored at room temperature until further analyses. Soil pH was determined in 1 mol/L KCl at a soil-to-solution ratio of 1:5, and electrical conductivity (EC) was measured in a water saturation extract near the Atterberg 'upper plastic limit' (= liquid limit; soil-to-solution ratios: 1:0.3 to 1:1.2). Total carbon and nitrogen were quantified by dry combustion (Tabatabai and Bremner 1991), and carbonate was measured gas-volumetrically (Soil survey staff 2004). Organic carbon (C_{org}) was calculated as the difference of total and carbonate carbon. Soil particle size distribution was determined by wet-sieving and sedimentation in a sedigraph after adding H_2O_2 to remove organic matter and dispersing with sodium metaphosphate (Soil survey staff 2004). The potential cation exchange capacity (CEC_{pot}) was determined by extraction with a 0.4 mol/L LiCl solution buffered at pH 8.2 with triethanolamine and HCl (Husz 2001). To this end, 50 mL of the solution was shaken with 40 g of water-saturated soil for 2 h, centrifuged and the supernatant transferred into a flask; this step was repeated and the supernatant again added to the flask. The solution was membrane-filtered, exchangeable cations were measured with inductively coupled plasma atomic emission spectroscopy (ICP-AES), and the CEC_{pot} calculated as the sum of exchangeable cations. The base saturation was calculated in percent of CEC_{pot}. Oxidic iron was extracted with dithionite-citrate-bicarbonate (Fe_d) according to Mehra and Jackson (1960) and also with acid ammonium oxalate (Fe_o; extracting only the poorly crystalline forms) according to Schwertmann (1964). The extracted iron was measured by atomic absorption spectroscopy (AAS).

Soil P extraction. The tested soil P extraction methods are summarized in Table 1 with respect to extracting agents, soil-to-solution ratio, extraction time and method of P determination. The different methods utilize different mechanisms of P extraction as briefly outlined below.

Water extraction (H_2O): The water extraction was conducted using a saturation extract near the Atterberg 'upper plastic limit' (= liquid limit;

Table 1. Overview of the studied soil phosphorus (P) extraction methods, summarizing the composition and pH of the extracting solution, the soil-to-solution ratio, the extraction time and the method of measurement used

Method	Extracting solution	Solution pH	Soil-to-solution ratio	Extraction time	Method of measurement
H_2O	distilled H_2O	unbuffered	1:0.3–1:1.2	12 h	photometer
$CaCl_2$	0.01 mol/L $CaCl_2$	unbuffered	1:10	2 h	photometer
LiCl	0.4 mol/L LiCl	unbuffered	1:1.8–1:4.0	2 × 2 h	ICP-AES
Olsen	0.5 mol/L $NaHCO_3$	8.5	1:20	30 min	photometer
Bray II	0.03 mol/L NH_4F, 0.1 mol/L HCl	1.0	1:7	40 s	photometer
Mehlich 3	0.2 mol/L CH_3COOH, 0.25 mol/L NH_4NO_3, 0.015 mol/L NH_4F, 0.013 mol/L HNO_3, 0.001 mol/L EDTA	2.5	1:10	5 min	photometer
CAL	0.3 mol/L CH_3COOH, 0.05 mol/L $C_6H_{10}CaO_6$, 0.05 mol/L $(CH_3COO)_2Ca$	4.0	1:20	2 h	photometer
Fe-oxide P_i	(0.01 mol/L $CaCl_2$)	unbuffered	1:40	16 h	photometer
CAEM	(distilled H_2O)	unbuffered	1:40	16 h	photometer
Oxalate	0.08 mol/L $(COOH)_2$, 0.11 mol/L $(COONH_4)_2$	3.0	1:50	4 h	ICP-AES
Dithionite	0.2 mol/L $NaHCO_3$, 0.12 mol/L $Na_2S_2O_4$, 0.24 mol/L $C_6H_5Na_3O_7$	8.5	1:50	16 h	ICP-AES
HCl	0.5 mol/L HCl	< 1	1:10	2 h	ICP-AES
Organic P	(1 mol/L HCl)	< 1	1:100	16 h	photometer
Total P	1 mol/L HCl (after calcination)	< 1	1:100	16 h	photometer

ICP-AES – inductively coupled plasma atomic emission spectroscopy; CAL – calcium-acetate-lactate; Fe-oxide P_i – iron oxide impregnated filter paper; CAEM – cation and anion exchange membranes; Organic P was determined as the difference of P extracted with 1 mol/L HCl from an ignited (550°C; 1 h) and unignited sample

Zehetner and Wenzel 2000) and thus involved varying soil-to-solution ratios (Table 1) dependent on soil properties, notably texture. The water extraction determines P in the soil solution, i.e. dissolved or readily soluble forms of P.

Calcium chloride ($CaCl_2$): The $CaCl_2$ extraction was prepared as described in Houba et al. (2000) and is based on the principle of a (dilute) salt solution inducing some ion exchange, but nevertheless relies mostly on the dissolution of P in the extraction solution (Van Raij 1998).

Lithium chloride (LiCl): The LiCl extraction was carried out according to Husz (2001). This method is based on a pH-neutral salt solution suitable for a wide range of soil properties. Due to the characteristics of Li (small ionic radius, thick hydration shell,

weak electrostatic charge), LiCl mainly extracts easily exchangeable cations and soluble P forms.

Olsen: This extraction method was introduced by Olsen et al. (1954); our extraction was carried out according to Sims (2000c). In general, the Olsen method is preferably used for calcareous soils (> 2% $CaCO_3$), but studies have shown that this method gives reasonable results also for non-calcareous soils (Frank et al. 1998). The Olsen extract lowers the concentration of Ca^{2+} in solution as $CaCO_3$ precipitates and therefore promotes Ca phosphate dissolution; the high pH further enhances phosphate desorption from Al and Fe oxide surfaces (Sims 2000c, Schoenau and O'Halloran 2007).

Bray and Kurtz II (Bray II): This method was performed following the procedure of Bray and

Kurtz (1945). In the Bray II extract, fluoride (F^-) enhances P release from Al phosphates by decreasing Al activity in solution through the formation of Al-F complexes and simultaneously suppresses phosphate re-adsorption by soil colloids (Kuo 1996), while the dilute HCl dissolves phosphates from Ca-, Al- and Fe-bound forms (Sims 2000a).

Mehlich 3: This method is considered suitable for a wide range of soils varying in their physico-chemical properties (Frank et al. 1998, Sims 2000b). The method was introduced by Mehlich (1984); however, for the extraction of our soil samples, the protocol described by Sims (2000b) was used. As Mehlich 3 is a multi-nutrient extraction method, included P, K, Ca, Mg, Na, Cu, Zn, Mn and Fe, several extraction mechanisms are combined. An acetic acid solution is used to extract P by promoting the dissolution of Ca-phosphates; the addition of ammonium fluoride further releases P from Al phosphates through the formation of Al-F complexes in solution. The elements K, Ca, Mg and Na are extracted by ammonium nitrate and nitric acid, while Cu, Zn, Mn and Fe are dissolved and complexed by ammonium nitrate and EDTA, which functions as a chelating agent (Kuo 1996, Sims 2000b, Ziadi and Sen Tran 2007).

Calcium-acetate-lactate (CAL): This extraction method was carried out based on Schüller (1969) who introduced the method. According to Schüller (1969), the CAL method extracts only readily soluble and exchangeable phosphates as well as easily dissolved Ca phosphates from fertilizers, neglecting the P of apatitic phosphates. However, it is not well-suited for soils with a pH below 6 as in acidic soils the extracted phosphate might get re-adsorbed on Al and Fe oxides.

Iron oxide impregnated filter paper (Fe-oxide P_i): This method was performed according to the protocol by Chardon (2000). It is based on an infinite P sink approach. The Fe oxide filter papers serve as a P sink imitating the plant root. Through sorption of P from the solution to the filter paper a further desorption and re-supply from the soil is induced (Myers et al. 2005).

Cation and anion exchange membranes (CAEM): The method used in our study was performed similarly to protocols described by Nuernberg et al. (1998), Fernandes and Coutinho (1997) and Bissani et al. (2002). The cation and anion exchange membranes were obtained from Membranes International Inc. and cut to 1 × 7.5 cm strips.

The average mass of the anion strips was 0.377 g per strip with a total exchange capacity of 1.3 ± 0.1 $mmol_c$/g (for cation strips: 0.443 g with a total exchange capacity of 1.6 ± 0.1 $mmol_c$/g). Before extraction, the strips were conditioned in a 0.5 mol/L $NaHCO_3$ solution for several days. For extraction, 40 mL of distilled H_2O was added to 1 g of soil; one anion strip and one cation strip were added to this suspension and the suspension shaken end-over-end for 16 h. After rinsing with distilled H_2O, the anion strips were placed into 40 mL of 0.5 mol/L HCl and shaken for 90 min to elute the adsorbed P. Similarly to the Fe oxide impregnated filter paper, this method is based on a sink concept. The exchange membranes simulate the root system in the soil, keeping the P solution concentration (and also that of cationic nutrients) low and thus inducing re-supply from the soil solid phase (Kuo 1996, Van Raij 1998).

Oxalate: The oxalate extraction method is not a conventional P extraction method but derives from selective dissolution of soil iron forms. For this extraction, the protocol of Schwertmann (1964) was followed. With acid ammonium oxalate, poorly crystalline Fe is dissolved, which includes ferrihydrite and Fe associated with soil organic matter (Campbell and Schwertmann 1984).

Dithionite: The dithionite extraction method was introduced by Mehra and Jackson (1960) and is routinely used for the selective dissolution of soil iron forms rather than for soil P extraction. In our study, we followed the instructions of Holmgren (1967). In this extract, the Na-dithionite functions as a reduction agent and the citrate as a chelating agent. As a result, the total secondary free Fe with the exception of magnetite is solubilized (Raiswell et al. 1994).

Hydrochloric acid (HCl): Using 0.5 mol/L HCl, this method is based on the dissolution of inorganic P forms. Phosphorus originating from apatite is dissolved as well as Al- and Fe-bound P (at least partly) (Kuo 1996).

Organic P: Organic P is a calculated value. It was determined with the ignition method (Kuo 1996, with modification: 1 mol/L HCl instead of 0.5 mol/L H_2SO_4; Pardo et al. 2003) and calculated as the difference of P extracted with 1 mol/L HCl from an ignited (550°C; 1 h) and unignited sample.

Total P: Total P was extracted with 1 mol/L HCl after calcination (550°C; 1 h). Through calcination, the organic P fraction is converted to inorganic P;

thereafter, the 'total' amount of P in soil (inorganic and formerly organic) is extracted by HCl, although the extraction may not be fully quantitative.

Photometric P determination. The photometric determination of extracted P was conducted with the molybdenum blue method according to Murphy and Riley (1962). This method was further modified to allow detection of low P concentrations (detection limit of 25 µg P/L) needed for some of the samples/extracts. The following modifications were performed. The four solutions were prepared in similar concentrations as described by Murphy and Riley (1962) but their volumetric mixing ratio was changed as follows. 10 mL of 2.5 mol/L H_2SO_4 was mixed with 3 mL of 0.032 mol/L ammonium molybdate, 1 mL of 0.006 mol/L potassium antimonyltartrate and 6 mL of 0.1 mol/L ascorbic acid. 1 mL of the resulting solution was added to 5 mL of sample and measured at 710 nm after a reaction time of 15 min, as also described for the standard method by Murphy and Riley (1962).

RESULTS AND DISCUSSION

Characterization of the studied soils. The soils used for this study displayed a wide range of soil properties (Table 2). The pH (in KCl) ranged from 4.3–7.1, with base saturation spanning from 21% to 97%. The EC varied between 0.23 and 1.35 mS/cm. Two thirds of the soils were carbonate-free; the $CaCO_3$ contents of the remaining soils lay between 1% and 56%. The C_{org} contents of the soils ranged between 0.93% and 4.03%, and the C/N ratio of

the organic matter varied from 9–25. Our sample set included soils with > 50% clay and soils with > 80% sand as well as a range of soil textures in between. The CEC_{pot} of the studied soils ranged from 6.2–29.1 $cmol_c$/kg, averaging 13 $cmol_c$/kg.

The oxalate- and dithionite-extractable iron (Fe_o and Fe_d, respectively) ranged from 930–21 950 mg/kg and from 1860–42 990 mg/kg, respectively. The Fe_o/Fe_d ratio is a measure of iron oxide crystallinity (a higher ratio indicating lower crystallinity), which may be related to the degree of weathering and soil age (Lair et al. 2009). In our study, the Fe_o/Fe_d ratio ranged between 0.14 and 0.82 indicating that our samples featured soils containing mainly amorphous iron oxides (Fe_o/Fe_d > 0.5) as well as soils with primarily crystalline iron oxides (Fe_o/Fe_d < 0.5). The molar ratio P_o/Fe_o indicates the degree of P saturation of the iron oxides in the soil. In our study, this ratio ranged from very low P saturation (0.08) to very high P saturation (0.84) reflecting the diverse P status of our sample set.

Comparing the applied extraction methods. The extracted P (mean value of all 50 soils) increased in the order H_2O < $CaCl_2$ < LiCl < Fe-oxide P_i < Olsen < CAL < CAEM < Mehlich 3 < Bray II < dithionite < organic P < HCl < oxalate < total P (Figure 1). This sequence is in accordance with findings by other authors (Fernandes et al. 1999, Csathó et al. 2005, Kulhánek et al. 2009) and reflects different extraction mechanisms and P pools. The mean values of P extracted by the different methods may be grouped as follows. H_2O, $CaCl_2$ and LiCl extracted mean P amounts of below 10 mg/kg; the means for Fe-oxide P_i, Olsen, CAL

Table 2. Summary of selected physicochemical soil properties (*n* = 50)

	pH_{KCl}	EC (mS/cm)	C_{org} (%)	$CaCO_3$ (%)	C/N (molar)	Clay (%)	Sand (%)	CEC_{pot} ($cmol_c$/kg)	BS (% of CEC_{pot})	Fe_o (mg/kg)	Fe_d (mg/kg)	Fe_o/Fe_d	P_o/Fe_o (molar)
Mean	6.0	0.65	1.72	5.6	13.0	22.8	36.5	13.0	67	4040	9770	0.42	0.31
Median	6.0	0.61	1.47	0	11.7	24.4	28.1	12.4	66	3270	8370	0.42	0.25
Standard dev.	0.8	0.27	0.74	11.6	3.1	9.9	23.8	4.5	21	3320	6480	0.14	0.20
Minimum	4.3	0.23	0.93	0	9.0	4.2	1.5	6.2	21	930	1860	0.14	0.08
10% Quantile	4.9	0.37	1.02	0	10.4	12.1	13.1	7.4	40	1360	4900	0.23	0.16
90% Quantile	7.0	1.02	2.67	25.1	15.9	34.5	66.3	18.0	95	5710	14 240	0.55	0.67
Maximum	7.1	1.35	4.03	56.2	25.1	53.1	86.2	29.1	97	21 950	42 990	0.82	0.84

EC – electrical conductivity (in saturated H_2O extract); C_{org} – organic carbon; CEC_{pot} – potential cation exchange capacity; BS – base saturation; Fe_o and P_o – oxalate-extractable Fe and P; Fe_d – dithionite-extractable Fe
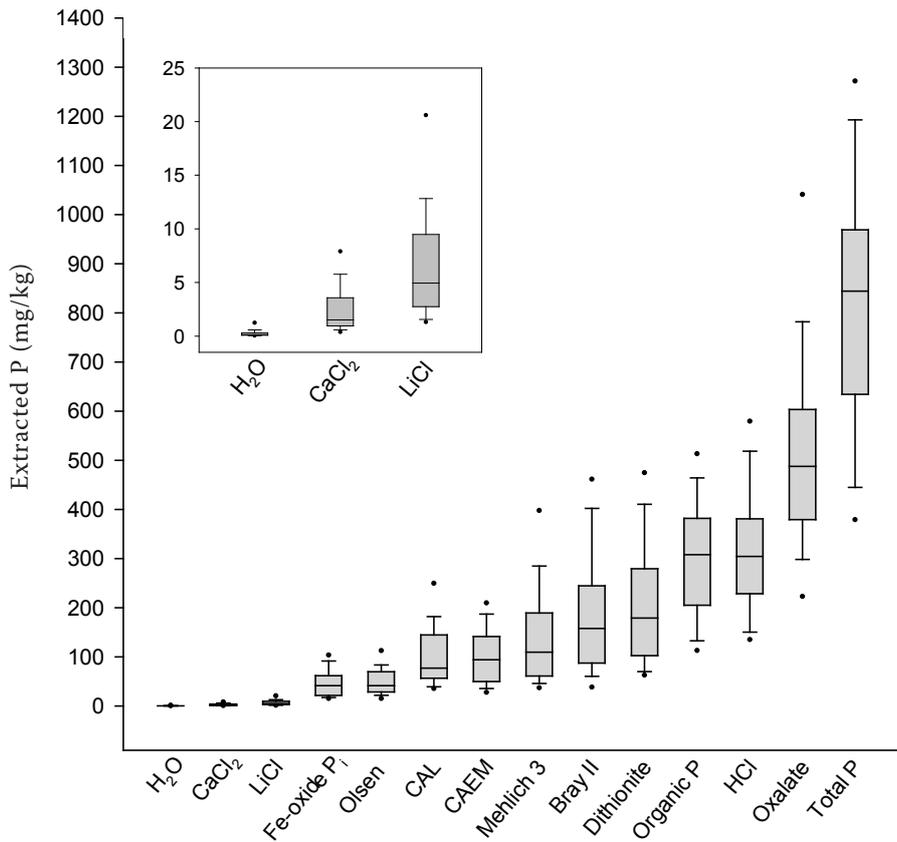
Figure 1. Phosphorus (P) extracted by the different extraction methods in the order of increasing extraction yield (*n* = 50). Fe-oxide P_i – iron oxide impregnated filter paper; CAL – calcium-acetate-lactate; CAEM – cation and anion exchange membranes

and CAEM were in the same range of around or below 100 mg/kg; Mehlich 3, Bray II and dithionite extracted mean P amounts of around or below 200 mg/kg, while organic P and HCl-extractable P were on average around 300 mg/kg; the mean values of oxalate and total P amounted to 530 and 830 mg/kg, respectively.

The widest range between lowest and highest value was found for Bray II, where the highest measured value (628 mg/kg) was about 200-fold greater than the lowest value (3.13 mg/kg). The narrowest range was observed for the HCl extraction, where the maximum extracted P (622 mg/kg) was less than 5 times higher than the minimum value (129 mg/kg). Saggar et al. (1999) suggested that a wider range of extracted P was advantageous because of easier categorization in P status groups. This applies especially for routine soil testing where the results provide the basis for fertilizer recommendations.

As expected, the lowest extractable amounts of P from soil were found for the H_2O extraction method and the highest amounts were extracted by the method for total P (Figure 1). According to Kulhánek et al. (2007), $CaCl_2$ may extract lower

amounts of P than the H_2O method due to the formation of Ca-phosphate. The opposite trend in our study may result from the application of a saturation water extract and hence much lower soil-to-solution ratios compared to the $CaCl_2$ extract (Table 1). Likewise, the lower water-extractable P in our study compared to the range of water-extractable P reported by Marschner (1995; 0.8–8.0 mg/kg) may have the same reason. The total P values in our samples ranged from 298–1680 mg/kg. By comparison, Zehetner et al. (2008) reported a mean total P concentration of 713 ± 58 mg/kg for young floodplain soils in Austria; Cross and Schlesinger (1995) found total P values from 64–908 mg/kg in 88 different soils globally. Figure 1 shows an average organic P content of approximately 36% of the total P in the studied soils. This is at the lower end of the common range reported by Condron and Tiessen (2005), i.e. 30–65%.

For Mehlich 3, similar results as in our study were previously found by Zbíral and Němec (2002) who reported values from 4–643 mg/kg with an average of 107 mg/kg over a very broad range of soils. Yet, the direct comparison of our results with

other studies entail some problems; in some cases the soils were very different regarding physico-chemical parameters (e.g. tropical soils; Mamo et al. 1996, Bortolon and Gianello 2012); or the focus of other studies was on other factors e.g. the influence of various fertilizer applications on P extraction methods (e.g. Menon et al. 1989a, 1991). Menon et al. (1989b) found Fe-oxide P_i and Olsen-P to be of similar magnitude; this was confirmed by our results, as shown in Figure 1.

Plants and other organisms in soil mainly assimilate P that is dissolved in the soil solution. However, the concentration of dissolved P in the solution is generally low (< 5 μmol/L) as P compounds generally have low solubility (Condron and Tiessen 2005). Blume et al. (2010) report a common range of soil solution P concentrations of between 0.001 and 0.1 mg/L for unfertilized soils and of between 0.1 and 5 mg/L for fertilized topsoils. This is approximately the same range as extracted by the water saturation extraction in our study (0.04–3.37 mg/L). For optimum plant yields, Blume et al. (2010) deem soil solution P concentrations of between 0.3 and 0.8 mg/L as necessary.

When correlating the different extraction methods with each other, the overall picture indicates that most extraction methods were well-correlated (Table 3). Yet, organic P and total P were exceptions to this general trend; organic P only correlated with the extraction methods oxalate, dithionite and total P (positively) and with $CaCl_2$ (negatively), and total P correlated (positively) with only half of the methods (Olsen, CAL, CAEM, oxalate, dithionite, HCl and organic P). It was notable that correlation coefficients between the weaker extraction methods, i.e. H_2O, $CaCl_2$ and LiCl, on the one hand and the other extraction methods on the other hand declined with increasing extracting strength of the other methods (Table 3, Figure 1). The oxalate and dithionite extraction methods showed little to no correlation with any of the weaker extraction methods. The highest correlation coefficient was found between Bray II and Mehlich 3 (0.944), the lowest for total P and $CaCl_2$ (–0.040). The close correlation of Bray II and Mehlich 3 is likely due to the underlying similar mechanism for P extraction (phosphate release through ligand exchange with F^-). It is notable that the H_2O method best correlated with LiCl, while the $CaCl_2$ method was most closely

Table 3. Pearson correlation coefficients between the different soil P extraction methods

	H_2O	$CaCl_2$	LiCl	Olsen	Bray II	Mehlich 3	CAL	Fe-oxide P_i	CAEM	Oxalate	Dithionite	HCl	Organic P
$CaCl_2$	0.795***												
LiCl	**0.851*****	0.771***											
Olsen	0.574***	0.657***	0.646***										
Bray II	0.409**	0.643***	0.649***	0.792***									
Mehlich 3	0.437**	0.701***	0.652***	0.837***	**0.944*****								
CAL	0.509***	0.556***	0.732***	0.801***	0.826***	0.761***							
Fe-oxide P_i	0.644***	**0.813*****	0.686***	0.888***	0.810***	**0.865*****	0.663***						
CAEM	0.722***	0.721***	0.752***	**0.849*****	0.658***	0.693***	0.661***	**0.868*****					
Oxalate	0.293*	0.216	0.286*	0.740***	0.519***	0.497***	0.564***	0.538***	0.551***				
Dithionite	0.194	0.193	0.173	0.664***	0.367*	0.394**	0.342*	0.528***	0.607***	0.788***			
HCl	0.327*	0.289*	0.447**	0.727***	0.505***	0.448**	0.740***	0.516***	0.622***	0.695***	0.598***		
Organic P	–0.073	–0.337*	–0.164	–0.047	–0.152	–0.210	–0.048	–0.209	–0.098	0.475***	0.378**	0.104	
Total P	0.165	–0.040	0.157	0.436**	0.212	0.139	0.400**	0.214	0.359*	**0.813*****	0.675***	0.680***	0.765***

$n = 50$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; correlation coefficients > 0.8 are highlighted. CAL – calcium-acetate-lactate; Fe-oxide P_i – iron oxide impregnated filter paper; CAEM – cation and anion exchange membranes

correlated with the Fe-oxide P_i. A tight correlation was also found between CAEM and Fe-oxide P_i, which was expected as these methods are based on the same mechanism (introducing a P sink). We repeated the correlation analyses after exclusion of carbonate-containing soils; this resulted in very similar patterns (data not shown) as for the whole dataset presented in Table 3.

Phosphorus extractability in relation to soil properties. The solubility, availability and extractability of P in the soil is influenced by the prevailing pH and $CaCO_3$ content, soil texture, mineralogical composition, total surface area, organic matter content and also by the presence of Fe and Al (hydr-)oxides (Abdu 2006). How these parameters occur concomitantly influences the solubility of phosphates and therefore the extractability by and efficiency of different extraction methods.

In our study, P extracted with $CaCl_2$, Olsen, Bray II, Mehlich 3, Fe-oxide P_i, CAEM and dithionite was negatively correlated with carbonate content and soil pH (Table 4). This trend seems reasonable because $CaCO_3$ may bind readily available P in Ca-phosphates thereby fixing P in the soil. Zbíral (2000) discussed a strong effect of the prevailing pH on acidic extraction methods, and Zorn and Krause (1999) analyzed the effect of $CaCO_3$ content in the soil on the extraction capacity of the CAL method. They suggest that higher $CaCO_3$ contents may increase the pH in the extraction solution and diminish the effectiveness of the CAL extraction. However, samples that showed reduced extraction capacity could not be identified solely based on high $CaCO_3$ contents (Zorn and Krause 1999, Zbíral and Němec 2002). As an alternative and less $CaCO_3$-dependent method, Zorn and Krause (1999) recommend the use of the Olsen extract, Zbíral and Němec (2002) propose the use of Mehlich 3. Contrary to these observations and recommendations, our results showed that CAL did not correlate significantly with $CaCO_3$ while Olsen and Mehlich 3 did (Table 4).

Soil C_{org} only correlated (positively) with P extracted by oxalate as well as organic P and total P (Table 4); reconfirming that only these extraction methods were able to extract significant amounts of P from the organic pool.

Zheng and Zhang (2012) found that soil texture and particle size distribution strongly influenced soil P fractions. The authors claimed the strong impact was due to the influence on the microbial

Table 4. Pearson correlation coefficients between soil P extraction methods and soil properties ($n = 50$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

	pH (KCl)	EC	C_{org}	$CaCO_3$	C/N (molar)	Clay	Silt	Sand	CEC_{pot}	BS	Fe_o	Fe_d	Fe_o/Fe_d	P_o/Fe_o (molar)
H_2O	-0.111	0.154	-0.089	-0.234	0.062	-0.011	-0.161	0.119	-0.020	-0.129	0.051	0.010	0.199	-0.005
$CaCl_2$	-0.348*	0.387**	-0.223	-0.341*	0.452***	-0.422**	-0.462***	0.500***	-0.312*	-0.379**	-0.074	-0.106	0.232	0.122
LiCl	0.024	0.407**	-0.067	-0.238	0.184	-0.237	-0.291*	0.301*	-0.205	-0.040	-0.044	-0.091	0.196	0.174
Olsen	-0.319*	0.219	0.076	-0.363**	0.268	-0.242	-0.463***	0.427***	0.070	-0.402**	0.307*	0.146	0.627***	0.091
Bray II	-0.200	0.322*	-0.103	-0.380**	0.455***	-0.512***	-0.524***	0.579***	-0.274	-0.324*	-0.044	-0.147	0.405**	0.294*
Mehlich 3	-0.308*	0.391**	-0.081	-0.338*	0.555***	-0.549***	-0.574***	0.630***	-0.282*	-0.429**	-0.054	-0.155	0.427**	0.329*
CAL	0.110	0.267	0.142	-0.143	0.129	-0.216	-0.387**	0.360*	-0.064	0.031	0.016	-0.096	0.351*	0.316*
Fe-oxide P_i	-0.423**	0.272	-0.162	-0.478***	0.414**	-0.394**	-0.500***	0.515***	-0.136	-0.519***	0.186	0.076	0.522***	0.075
CAEM	-0.286*	0.218	-0.104	-0.500***	0.127	-0.195	-0.273	0.274	0.004	-0.382**	0.327*	0.173	0.561***	-0.100
Oxalate	-0.144	-0.117	0.291*	-0.179	-0.059	-0.010	-0.222	0.164	0.353*	-0.243	0.597***	0.455***	0.486***	0.091
Dithionite	-0.448**	-0.188	0.043	-0.466***	-0.085	-0.172	-0.246	0.251	0.243	-0.556***	0.757***	0.597***	0.573***	-0.362**
HCl	0.068	0.006	0.213	-0.163	-0.134	-0.029	-0.118	0.097	0.176	0.021	0.473***	0.296*	0.526***	-0.016
Organic P	0.193	-0.355*	0.376**	0.201	-0.440**	0.427**	0.268	-0.364**	0.529***	0.135	0.370**	0.558***	-0.285*	0.069
Total P	0.164	-0.267	0.392**	0.070	-0.413**	0.293*	0.142	-0.218	0.515***	0.075	0.592***	0.583***	0.173	0.005

EC – electrical conductivity (in saturated H_2O extract); C_{org} – organic carbon; CEC_{pot} – potential cation exchange capacity; BS – base saturation; Fe_o and P_o – oxalate-extractable Fe and P; Fe_d – dithionite-extractable Fe; CAL – calcium-acetate-lactate; Fe-oxide P_i – iron oxide impregnated filter paper; CAEM – cation and anion exchange membranes

biomass and activity in the soils. All fractions of P that were affected by microbial activity were equally affected by texture. Also, Menon et al. (1989b) found a positive correlation of P extracted with HCl and sand content. Conversely, Fernandes et al. (1999) showed that P extracted by Olsen, Fe-oxide P_i and CAEM was less dependent on soil texture. In our study, soil texture seemed to exert a strong influence on P extractability. Phosphorus extracted with $CaCl_2$, LiCl, Olsen, Bray II, Mehlich 3, CAL and Fe-oxide P_i was negatively correlated with clay and/or silt content and positively with sand content. The reverse trend was observed for organic P. Finer soil textures may contribute to stronger P binding thus reducing its extractability by most of the weaker extraction methods. On the other hand, finer textures also correspond with higher C_{org} contents ($r = 0.391$ between the fraction < 6.3 µm and C_{org}, $P < 0.01$; data not shown) and thus also with higher organic P. Our results are in contrast with those of Stewart et al. (1987) who found that a low sand content correlated with higher amounts of extractable P by methods extracting the labile forms of P.

Oxalate- and dithionite-extractable iron contents correlated (positively) mainly with P extracted by stronger extraction methods, i.e. oxalate, dithionite, HCl, organic P and total P. The Fe_o/Fe_d ratio (a higher ratio indicating lower iron oxide crystallinity) correlated positively with P extracted by Olsen, Bray II, Mehlich 3, CAL, Fe-oxide P_i, CAEM, oxalate, dithionite and HCl, but negatively with organic P. This indicates generally higher P extractability from soils with dominantly amorphous iron oxides.

Kumar et al. (1994) argue that not only soil properties but also the composition of fertilizers influences P extractability by different extraction methods. In our study, different fertilizer residues and their variable solubility may have (partly) masked soil property effects, thus reducing the significance of correlations.

In conclusion, the magnitude of extractable P by the various extraction methods increased in the order H_2O < $CaCl_2$ < LiCl < Fe-oxide P_i < Olsen < CAL < CAEM < Mehlich 3 < Bray II < dithionite < organic P < HCl < oxalate < total P. Generally, the different extraction methods were well correlated, especially when P extraction was achieved by a similar mechanism. The soil properties most influential on P extractability were pH, carbonate content, texture as well as iron oxide content and crystallinity. Owing to the different extraction mechanisms utilized, the tested extraction methods extracted different pools of soil P with strongly varying extractability and varying dependence on soil properties. Further research is needed to examine the relation of P extractability by these methods to plant P uptake in contrasting soil types.

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