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Four soil phosphorus (P) tests evaluated by plant P uptake and P balancing in the Ultuna long-term field experiment

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ABSTRACT

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Soil phosphorus (P) availability was assessed with four different soil P tests on seven soils of the Ultuna long-term field experiment (Sweden). These four soil P tests were (1) P-H_2O (water extractable P); (2) P-H_2O_{C10} (water extractable P upon 10 consecutive extractions); (3) P-AL (ammonium lactate extractable P) and (4) P-C_{DGT} (P desorbable using diffusive gradients in thin films). The suitability of these soil P tests to predict P availability was assessed by correlation with plant P uptake (mean of preceding 11 years) and soil P balancing (input vs. output on plot level for a period of 54 years). The ability to predict these parameters was in the order P-H_2O_{C10} > P-C_{DGT} > P-H_2O > P-AL. Thus, methods considering the P-resupply from the soil solid phase to soil solution performed clearly better than equilibrium-based extractions. Our findings suggest that the P-AL test, commonly used for P-fertilizer recommendations in Sweden, could not predict plant P uptake and the soil P balance in a satisfying way in the analysed soils.

Keywords: soil testing; macronutrient; phosphorus desorption; nutrition; fertilization; saturation index

The sufficient provision of phosphorus (P) as an essential nutrient is required to guarantee stable crop yields in agricultural systems. Many soil P tests exist to estimate the soil P status (Yli-Halla et al. 2016, Nawara et al. 2017), in order to allow specific fertilization recommendations avoiding an over- or under-supply of easily available P in soil. Among the different soil P tests, there are differences in the mechanisms how available P is determined. Soil P tests using soil extractions target a specific fraction of P in soil, depending on the strength and mode of action of the extracting agent. By using water (Van Der Paauw 1971) or dilute salt solutions (e.g. 0.01 mol/L $CaCl_2$, Houba et al. (1998)), the immediately available soil P is determined (intensity measure). If stronger extractants are used, such as acids, bases or complexing agents (Olsen et al. 1954, Egnér et al. 1960, Mehlich 1984), more soil P is released (Jordan-Meille et al. 2012) providing a measure of the amount of P potentially available over time (quantity measure). Other soil P tests rely on a strong sink such as anion exchange resins (Amer et al. 1955) or zero sink gels in case of diffusive gradients in thin films (DGT) (Zhang et al. 1998) to quantify desorbable P from the solid phase into the soil solution. Alternatively, the soil solution can be continuously replenished by e.g. repeated extraction (Sharpley et al. 1981, Indiati and Sharpley 1996, Lair et al. 2009) or constant

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renewal of soil water (Frossard et al. 2014), thereby gently forcing new P to desorb from the soil solid phase into soil solution.

Given the multitude of existing soil P tests, different tests have become standard to evaluate the soil P status in various countries. For most of Scandinavia and several other countries, the ammonium lactate extractable P (P-AL) has become one of the most commonly used soil P tests to base P fertilizer recommendations upon (Egnér et al. 1960). There is, however, little information provided about alternatives to the P-AL test, which could potentially describe soil P availability more adequately for Scandinavian soils. The aim of this study was to test four different soil P tests for their ability to predict plant P uptake and P availability, which were determined in a P balance assessment for different fertilization treatments of a Swedish long-term field trial.

MATERIAL AND METHODS

Site description and soil sampling. The Ultuna long-term field trial located in Uppsala (59°48'37''N, 17°39'5''E), Sweden, was started in 1956 on a Eutric Cambisol (WRB) developed from granitic glacial sediments to study the effect of organic amendments and nitrogen (N) fertilizers on soil organic matter. Mean annual temperature is 5.8°C and mean annual precipitation 542 mm. The trial consists of 60 plots, 2 × 2 m each and 15 treatments replicated four times in a randomised design. Tilling depth was 20 cm throughout the experimental period and carefully controlled using the same spade depth. Bordering between plots through iron sheets, reaching 30 cm into the soil and 10 cm above the surface, was permanent. All organic manures were applied every second year in autumn after harvest. Each plot has received 20 kg P/ha as superphosphate annually. Soil properties are shown in Table 1. Top soils (0–20 cm depth) of seven different treatments (four replicate plots) were sampled in autumn 2011 after the cropping period. From each plot, five subsamples were taken after removing crop residues. Soils were dried (105°C) and passed through a 2 mm sieve prior to analysis.

Soil chemical analyses. Total soil P was determined by Coupled Plasma-Atomic Emission Spectrometry (ICP-AES; Perkin-Elmer, Bodenssewerk, Germany) after digestion in 1 mol/L HNO_3 according to the Swedish Standards-028311 (SIS, 1997). Soil pH was determined using a glass electrode pH meter using a soil to water ratio of 1:5 (6 g soil in 30 mL H_2O). Acid ammonium oxalate extractable iron (Fe_{ox}), aluminium (Al_{ox}) and P (P_{ox}) were determined after the recommendation of Schwertmann (1964) using ICP-AES for quantification. The P saturation index was determined according to Van Der Zee et al. (1987) using equation (1) on a molar basis for each element:

$$\text{P saturation index} = P_{ox}/(Al_{ox} + Fe_{ox}) \qquad (1)$$

Table 1. Soil properties and phosphorus (P) characteristics of soils from the Ultuna field trial (n = 4; ± standard deviation)

Soil treatment	pH_{H_2O}	Bulk density (g/cm^3)[1]	SOC (g/kg)[1]	Total P (mg/kg)	Soil P stocks (kg/ha)[2]	Oxalate-extractable P	Oxalate-extractable Fe	Oxalate-extractable Al	P-saturation index[3]
Fallow	6.4^{e4}	1.43	10.3^{a}	1050 ± 22^{a}	3003 ± 64^{a}	964 ± 46^{a}	5043 ± 112^{ab}	1477 ± 32^{ab}	0.21 ± 0.01^{a}
Calcium nitrate	6.5^{e}	1.28	14.1^{b}	903 ± 15^{a}	2312 ± 39^{bd}	746 ± 46^{a}	5195 ± 234^{ab}	1500 ± 65^{ab}	0.16 ± 0.01^{b}
Ammonium sulphate	4.0^{a}	1.21	13.4^{b}	1045 ± 19^{a}	2529 ± 46^{cd}	1004 ± 114^{a}	6129 ± 588^{b}	2160 ± 204^{c}	0.17 ± 0.01^{bc}
Green manure	5.9^{d}	1.34	16.7^{bc}	1061 ± 16^{a}	2843 ± 44^{ac}	974 ± 50^{a}	4957 ± 463^{a}	1468 ± 103^{ab}	0.22 ± 0.01^{a}
Peat	5.4^{c}	1.12	27.9^{e}	1079 ± 36^{ab}	2417 ± 82^{bd}	935 ± 34^{a}	5117 ± 69^{ab}	1731 ± 9^{b}	0.19 ± 0.01^{ac}
Farmyard manure	6.5^{e}	1.24	20.4^{d}	1270 ± 23^{b}	3150 ± 58^{a}	1120 ± 44^{a}	4731 ± 256^{a}	1364 ± 73^{a}	0.27 ± 0.01^{d}
Sewage sludge	4.8^{b}	1.02	28.0^{e}	4899 ± 195^{c}	9994 ± 397^{d}	5618 ± 515^{b}	20 898 ± 752^{c}	3182 ± 75^{d}	0.37 ± 0.03^{e}

[1]Data from Kätterer et al. (2011); [2]Refers to 0–20 cm soil depth; [3]P saturation was calculated as the molar ratio of oxalate-extractable P to oxalate-extractable Fe + Al; [4]Within columns, mean values followed by different letters are significantly different at P < 0.05 (Tukey's *HSD* (honestly significant difference) test)

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Soil P tests. Four different soil P tests were evaluated in this experiment. Water extractable P (P-H_2O) was determined by extracting six grams of soil with 30 mL of deionized water on a horizontal shaker (160 rpm), followed by centrifugation at 2817 g for 20 min for phase separation. The supernatant was passed through a 0.45 µm membrane filter (Schleicher and Schüll GmbH, Dassel, Germany) and orthophosphate concentrations determined by the molybdenum blue method (Murphy and Riley 1962) using a UV-1201 SHIMADZU photometer (Japan).

To estimate the amount of P desorbable from soils by water in the longer term, a simple consecutive soil extraction was applied. Water extractable P upon ten consecutive extractions (P-H_2O_{c10}) was determined similarly to P-H_2O, where after centrifugation the supernatant was replaced by new extraction solution (deionized water) to start a new extraction cycle. Ten extraction cycles were applied in total. The quantity of desorbed P extracted at each single extraction step was then accumulated to obtain P-H_2O_{c10}.

Ammonium acetate lactate extractable P (P-AL) was determined after the recommendations of Egnér et al. (1960). In detail, soils were extracted in a solution of 0.1 mol/L NH_4-lactate, 0.4 mol/L CH_3COOH for 30 min at a soil:extractant ratio of 1:20. Extracted P concentration was quantified by ICP-AES.

The diffusive gradients in thin films approach (P-C_{DGT}) was used to estimate desorptive P release from soil (Zhang and Davison 1995, Menzies et al. 2005). DGT uses ferrihydrite-impregnated hydrogels for binding P and inducing P desorption from a soil paste. Soil and ferrihydrite are separated by a hydrogel and a filter membrane only exposing the membrane to the soil housed in a plastic moulding. During exposure, the concentration gradient between the soil and the ferrihydrite sink causes a steady flux of P into the sampler. The sampled mass of P can be converted to the time-averaged P concentration, C_{DGT}, in solution at the surface of the DGT device calculated as:

$$C_{DGT} = M\Delta g/DAt \quad (2)$$

Where: M – mass of P sampled by the ferrihydrite gel (µg); Δg – thickness of the diffusion layer (0.094 cm); D – diffusion coefficient of P in the hydrogel at the exposure temperature (5.27×10^{-6} cm^2/s at 20°C); A – exposed sampler area (3.14 cm^2); *t* – deployment time (86 400 s [i.e. 24 h]).

Hydrogel preparation and assembly of DGT samplers followed previously described methods (Zhang and Davison 1995, Santner et al. 2010). Saturated soil pastes were made according to Rhoades (1996) and were incubated at 20°C for 24 h prior to sampling with DGT. For DGT deployment, about 5 g of soil was exposed to one DGT sampler for 24 h. After this period, the soil was washed off the DGT samplers with deionised water. The samplers were opened, the ferrihydrite gel retrieved and eluted in 10 mL 0.25 mol/L H_2SO_4 overnight. The inorganic P concentration in the eluates was measured using molybdate blue (Zhang et al. 1998). The P mass reflects diffusion of dissolved P from the soil solution and P resupplied from the solid phase.

Soil P balancing. A topsoil P budget (Oenema et al. 2003) was calculated for each treatment of the Ultuna field trial based on mean annual inputs and outputs from the period 1956 to 2011. Inputs consisted of inorganic P fertilization (20 kg P/ha/year in form of superphosphate) plus additional P through organic amendments. Output (i.e. plant P uptake) of P was based on the aboveground plant biomass production and P concentrations in grain and straw, respectively. From the start of the field trial, several crops were grown each year on the field trial (including turnips, wheat, barley, oat and rapeseed and mustard). Since 2000, corn (*Zea mays* L.) has been the only crop grown. Plant harvest and nutrient data were only available until 2007. To cover the period 2008 to 2011, mean annual corn yields of the period 2000 to 2007 were calculated. These mean annual corn yields were used for the period 2008 to 2011.

Statistical analysis and P test evaluation. For all statistical analyses r (R Development Core Team 2010) was used. After the analysis of variances, Tukey's *HSD* (honestly significant difference) test (α = 0.05) was used to identify significant differences between treatments. Pearson's correlation coefficient was determined to identify significant correlations between soil P tests and mean plant P uptake by corn (period 2000 to 2011) as well as the P balance. The adequacy of the four different soil P tests was assessed by correlating the results obtained for each soil P test with those of the nutrient balance as well as the mean P uptake by corn.

RESULTS AND DISCUSSION

Soil characteristics. The long-term application of mineral and organic fertilizers significantly

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altered several soil properties (Table 1). Soil pH decreased from 6.5 in 1956 to values between 4.0 and 5.9 in the treatments with ammonium sulphate, sewage sludge, peat and green manure. In the ammonium-N fertilized and green-manured soil, nitrification was probably the main reason for the acidification, while in the sewage sludge-treated soil, nitrification, oxidation of organically bound sulphur and high leaching losses of sulphate were likely the governing processes (Kirchmann et al. 1996). In the peat-treated soil, the acidic organic material likely decreased the pH values. In the treatments with calcium nitrate, farmyard manure and fallow, no pH changes were found. Soil organic carbon (SOC) concentrations increased (15 g C/kg soil in 1956) in treatments with organic amendments (green manure, peat, farmyard manure and sewage sludge), but declined in the fallow, calcium nitrate and ammonium sulphat treated soil (Kirchmann et al. 1994, Kätterer et al. 2011, Menichetti et al. 2015).

While total soil P concentrations were significantly increased only in treatments with farmyard manure and sewage sludge, soil P stocks showed more variability between treatments, mostly due to the differences in soil bulk densities (Kätterer et al. 2011). Soil P stocks in 0 cm to 20 cm depth ranged from 2312 (calcium nitrate) to 9994 (sewage sludge) kg P/ha (Table 1). Oxalate-extractable P (P_{ox}) concentrations were only slightly lower than the total P concentrations. This is in agreement with (Wuenscher et al. 2015) and suggests that acid ammonium oxalate is not specific to oxide-bound P but may also extract part of the primary mineral (apatite) P. No significant differences in oxalate extractable P were found between treatments except for sewage sludge being at least 5 times higher (5618 mg P/kg soil) than all other treatments. Concentrations of extractable Fe_{ox} and Al_{ox}, a measure of potential binding sites for P, varied slightly between all non-sewage sludge treatments. The lowest Fe_{ox} concentrations were determined in the farmyard manure treated soil. The high Fe_{ox} and Al_{ox} concentrations in the sewage sludge treated soil (20 898 mg Fe_{ox} and 3182 mg Al_{ox}/kg soil) were likely caused by the addition of Fe and Al compounds during wastewater treatment with the aim to precipitate P. In treatment with ammonium sulphate, a significantly higher Al_{ox} concentration was determined, probably a result of higher Al solubility due to soil acidification (pH = 4.0). The P saturation index was highest for soils treated with farmyard manure (0.26) and sewage sludge (0.36) and lowest for treatment with calcium nitrate (0.16).

P balancing and P tests. The mean annual P input ranged from 20 to 368 kg P/ha/year among treatments while P uptake by crops ranged from 6 to 13 kg P/ha/year, on average, over the period 1956 to 2011 (Table 2). All treatments received more P than was removed by crops, resulting in a positive P balance between 11 (calcium nitrate) and 355 (sewage sludge) kg P/ha/year. Mean P uptake by corn (period 2000–2011) was at times higher than average P uptake over the entire period of the trial. Indeed, plant P uptake increased strongly, after corn was introduced as crop in the field trial, explaining the higher P uptake rates since the year 2000.

The four soil P tests suggest that all treatments were well supplied with P, as suggested already in a previous study (Otabbong et al. 1997), yet differences between treatments and tests were identified (Table 2). Extracted soil P was highest in the farmyard manure treatment determined by P-H_2O, P-H_2O_{c10} and P-C_{DGT}, but not by P-AL, which was highest in the sewage sludge treatment (547 mg P/kg). The lowest extracted soil P was found in the ammonium sulphate treatment by all four soil P tests. There was a strong linear correlation between the P test results of P-C_{DGT} and P-H_2O as well as P-C_{DGT} and P-H_2O_{c10} ($P < 0.05$). However, there was no correlation between P-AL and the other soil P tests.

P saturation index and soil P tests. The soil P saturation index (Van Der Zee et al. 1987) is a method for evaluating soils for their capacity to retain phosphorus assuming that iron and aluminium oxides are the main adsorption sites for orthophosphate. Interestingly, despite the different extraction yields of the four soil P tests, a significant correlation ($P < 0.05$) was observed between the P saturation index and each of the four soil P tests if the treatment sewage sludge showing comparatively high iron oxide concentrations (Table 1) was excluded. This suggests that irrespective of the soil P test used, desorption of P from iron and aluminium oxides likely occurs, but to different extents.

Ability of soil P tests to predict plant uptake and P balance. The aim of soil P tests is to provide the basis for informed soil P fertilization. According

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Table 2. Phosphorus (P) balance and tests for plant available P

Soil treatment	P balancing			Mean P uptake by corn[2]	Soil P test			
	P input[1]	P uptake	P balance		$P\text{-}C_{DGT}$ (µg P/L)	P-AL	$P\text{-}H_2O$	$P\text{-}H_2O_{C10}$[5]
	(kg/ha/year)					(mg/kg)		
Fallow	20	–	20 ± 0	–	728 ± 45[a]	213 ± 12[a]	20.1 ± 1.4[a]	101 ± 5
Calcium nitrate	20	9 ± 5	11 ± 5	16 ± 4	391 ± 58[b]	164 ± 16[b]	7.7 ± 0.9[b]	54 ± 5
Ammonium sulphate	20	6 ± 4	14 ± 4	6 ± 2	167 ± 17[c]	164 ± 10[b]	2.8 ± 0.6[c]	44 ± 3
Green manure	20 + 27	10 ± 6	37 ± 6	17 ± 5	682 ± 58[a]	206 ± 12[a]	19.1 ± 1.5[ad]	96 ± 7
Peat	20 + 4	7 ± 5	17 ± 5	14 ± 2	594 ± 136[a]	181 ± 14[ab]	15.5 ± 2.0[d]	93 ± 9
Farmyard manure	20 + 73	11 ± 8	82 ± 8	24 ± 6	1266 ± 46[d]	347 ± 18[c]	29.0 ± 2.2[e]	151 ± 6
Sewage sludge	20 + 348	13 ± 7	355 ± 7	21 ± 4	391 ± 21[b]	547 ± 26[d]	7.8 ± 0.7[b]	113 ± 1
Statistical analysis[3]								
Soil P test vs P balance[4]					**	*	**	**
Soil P test vs mean P uptake by corn					*	ns	ns	**

$P\text{-}C_{DGT}$ – concentration upon constant desorption by diffusive gradients in thin films; P-AL – P extractable with 0.5 mol/L ammonium acetate-lactate; $P\text{-}H_2O$ – P extracted in water after 1 h of extraction; $P\text{-}H_2O_{c10}$ – P extracted upon ten consecutive extractions with water; (n = 4; ± standard deviation). [1]The Ultuna field trial received 20 kg P/ha/year in form of superphosphate plus additional P through organic amendments; [2]Average of years 2000 to 2011. Crop failure in the year 2003 was not considered. [3]Levels of significance: $P > 0.1$ = ns; $^*P < 0.1$; $^{**}P < 0.05$; [4]Excluding sewage sludge due to a large positive P balance; [5]Concentrations for each extraction step are available in Figure 1

to our results, the P-AL test poorly described P uptake by corn (period 2000 to 2011), and inadequately explained the positive P balances in the different studied treatments (Table 2). One reason of this might be that also non-plant available forms (e.g. organically bound P) of P are detected in P-AL extracts when ICP-AES is used. The extraction of some organically bound P is likely, especially in treatments with particularly high organic matter concentrations (treatments with sewage sludge, peat and farmyard manure). The other examined soil P tests were more suitable to evaluate the P status in the studied agricultural soils. However, while differences in $P\text{-}H_2O$ were able to explain soil P balances, $P\text{-}H_2O$ could not predict corn P uptake in a satisfying way. In contrast to that, soil P tests that assessed the ability of soils to supply P to soil solution over a longer period of time

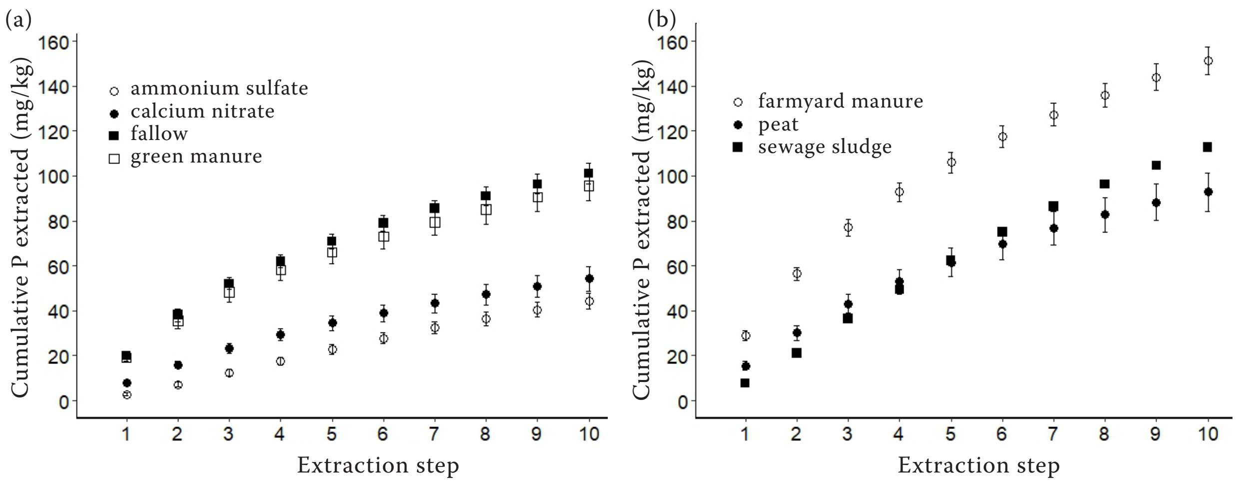

Figure 1. Data on (a) single ($P\text{-}H_2O$) and (b) consecutive ($P\text{-}H_2O_{c10}$) phosphorus (P) extraction by water (4 replicates per data point; error bars – standard deviation)

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($P\text{-}C_{DGT}$ and $P\text{-}H_2O_{c10}$) performed significantly better in predicting both the overall soil P balance and corn P uptake. Recent results show that no soil P extraction method applied to yield data from long-term field experiments in Europe was clearly superior to others (Nawara et al. 2017). Correlating several soil P tests to plant P uptake over one growing season, Zehetner et al. (2018) found that water and dilute salt solutions (both being P intensity measures) performed best, and that among several P quantity measures, only those that use a sink approach performed reasonably well. Similarly, Kulhánek et al. (2007) showed that P in soil water extracts predicted plant P uptake better than other extractants (Mehlich 3, Olsen, $CaCl_2$) on six long-term experiment soils.

In conclusion, our main finding is that measuring desorption of soil P over time ($P\text{-}C_{DGT}$ and $P\text{-}H_2O_{c10}$) was superior over a one-time extraction using water or acid (i.e. $P\text{-}H_2O$ and P-AL) in relation to long-term plant P uptake. Both evaluated quantity measures ($P\text{-}C_{DGT}$ and $P\text{-}H_2O_{c10}$) performed similarly well to predict P uptake by plants. It seems that single-step P batch extractions methods are not able to adequately account for longer-term P release regardless of the use of weak or strong extracting agents. However, methods to determine the quantity factor are laborious and time-consuming, and further development is needed to make them being an alternative. The method of consecutive soil extraction might serve as a cheap and easily applicable method to determine the soil P status in cases where costlier $P\text{-}C_{DGT}$ devices are not available. To confirm these findings, investigations with a broader set of soils with differing soil P status and soil properties should be performed.

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