

Desorption of Water Soluble Phosphorus from Soil – Development of a Consecutive Extraction Method

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ABSTRACT

A consecutive extraction method was developed which allows to determine the total amount of water soluble phosphorus in soil. For that, soil was shaken with deionized water, which was removed and replaced after one hour; this procedure was repeated ten times. The concentrations measured at each extraction step allow to quantify desorption of total water soluble phosphorus.

Seven soils of the Ultuna long term soil organic matter experiment, Sweden, were used to test the method. Two additional soils from a horse paddock were used for method development. Soils treated with farmyard manure and sewage sludge showed the highest release of total water soluble phosphorus, whereas calcium nitrate and ammonium sulfate showed the lowest. Fallow, green manure and peat treatments showed intermediate release. The amount of total water soluble phosphorus was controlled by pH, total P and P-AL. The increase in potentially releasable water soluble P is about 20 per cent of total P but 55 per cent of P-AL in average among all soils tested. Data were compared with an earlier phosphorus fractionation of four of the soils used showing that all resin P and part of sodium bicarbonate P was released by consecutive extraction with water. The relative decline in consecutive P release was inversely related to the P quantity/intensity ratio.

The estimation of total water soluble phosphorus obtained by the method showed that the actual availability of P in soil to plants seems not to be limited by chemical binding and release of P to the soil solution, but by the ability of plants to obtain P from the soil solution.

PREFACE

We may be able to substitute nuclear power for coal, and plastics for wood, and yeast for meat, and friendliness for isolation - but for phosphorus there is neither substitute nor replacement.

Asimov, 1974

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TERMS AND ABBREVIATIONS

P	Phosphorus.
P _{ini} -H ₂ O	Initial water soluble phosphorus concentration. Unit usually in mg P kg ⁻¹ soil. Concentration of P obtained in the first of several extraction steps. In most cases, this value is equal to P _{max} -H ₂ O.
P _{max} -H ₂ O	Maximum water soluble phosphorus concentration. Unit usually in mg P kg ⁻¹ soil. The maximum concentration of P obtained among several extraction steps. Only in rare cases where maximum concentration of P is not obtained in the first extraction step does P _{max} -H ₂ O differ from P _{ini} -H ₂ O; otherwise they are equal to each other.
P _{rel} -H ₂ O	Potential release of water soluble phosphorus. Unit usually in mg P kg ⁻¹ soil. The value is based on the extrapolation of accumulated concentrations of P which are derived from various consecutive extraction steps.
P _{min} -H ₂ O	Minimum water soluble phosphorus concentration. Unit usually in mg P kg ⁻¹ soil. The value is based on the extrapolation of the desorption curve. Extrapolation is performed until the extraction step where P _{rel} -H ₂ O is reached.
P-H ₂ O	Water soluble phosphorus. Phosphorus extractable with water.
P-AL	Ammonium lactate phosphorus. Phosphorus extractable with ammonium lactate.
Al-P	Aluminium oxide bound phosphate. Phosphate ions can be bound to aluminium oxides in soil. There, phosphate is reversibly bound by anion exchange. The more ions are exchanged, the stronger phosphate is bound to the aluminium oxide.

1. INTRODUCTION

1.1 Phosphorus in the soil – plant system

Phosphorus, P, is an essential nutrient for all living cells, required for the build-up of the genetic code and the energy transport system within a cell. After nitrogen, P is the most limiting growth factor in many terrestrial ecosystems, such as agricultural land (Smil, 2000). The native P content in soil is often rather low, compared to other macro nutrients. Total P concentrations in top soil range normally between 0.005 and 0.15 per cent (Havlin et al., 1999) mostly depending on the following factors

- parent material
- degree of soil weathering
- extent of P-loss by leaching and
- land management.

All native P in soil derives from apatite minerals. After weathering, P released from apatite can enter the biological cycle. As a rule, 500 to 800 mg P kg⁻¹ dry weight of soil are normally present in soils. Concentrations of P are highest in the soil surface due to accumulation of organic material. Intensive cultivation can deplete P contents in soil rather quickly (Stevenson and Cole, 1999).

Phosphorus can be found in both organic and inorganic forms in soil. While the former fraction is located in humus and plant residues, the latter is released by weathering processes from parent material and adsorbed on soil particles. The ratio between organic and inorganic P compounds in soil may vary strongly. Normally, the size of the fraction is assumed to be equal, but ratios ranging from 5:1 up 1:5 for organic to inorganic P were observed (Stevenson and Cole, 1999).

The phosphorus cycle in soil is complex and dependant on many factors. There are several sources and sinks of P in soil which can enrich or deplete soil solution. Mineralization is the process whereby P is released from organic P to soil solution. The reverse process is called immobilization, mainly performed by micro-organisms. The weathering of primary minerals leads to a continuous one-way flow to replenish soil solution. Secondary compounds release P via dissolution. Desorption means release of P from mineral surfaces such as clays or iron-oxides. Also reverse processes, adsorption and precipitation, can take place and deplete soil solution. When P precipitates (due to a changing pH regime mainly) secondary compounds are formed. Leaching of P is normally low but can gain significance if excessive amounts of P are applied through fertilizer or manure. Atmospheric deposition can also contribute to P input to soils, but the sources of atmospheric P deposition are still not well determined and analysed (Brady and Weil, 2008).

1.2 Phosphorus in soil solution

In soil solution, the speciation of phosphate ions is pH dependent. Plants mostly require inorganic P such as H₂PO₄⁻ and HPO₄²⁻, which occur abundantly at pH around 6 (Schachtman et al., 1998). The average concentration of P in soil solution ranges from <0.01 to 0.1 mg P L⁻¹, differing strongly among soils (Pierzynski et al., 2000). Note that the proportion of P in soil solution is most commonly less than 0.1 per cent of total P content in soil (Blume et al., 2010). Plants can only take up P from soil solution. All kinds of exchange processes from one form into another take place via the soil solution. The plant's need for P is high, compared to the available amount of P present in the liquid phase. It has been estimated that soil solution has to be replenished 10 to 20 times per day to satisfy plant's need for P. If

replenishment is not guaranteed adequately, plants suffer from P deficiency (Syers et al., 2008).

Leaching of P in subsurface flow is low in most cases and below eutrophication thresholds of aquifers. This is due to the relatively high immobility of phosphate ions (Pierzynski et al., 2000), which is also in contrast to other nutrients, e.g. nitrate. However, intensive application of animal manure, combined with artificial soil drainage can lead to significant P outflow and thus detrimental effects on aquatic ecosystems. The Chesapeake Bay (USA) as well as the Baltic Sea (Scandinavia) are both well studied examples on the effect of P leaching from intensively P-fertilized agricultural soils, which results in a decrease in water quality of these aquifers (Ator et al., 2001; Larsson et al., 1985).

1.3 Determination of phosphorus in soil solution

Various analytical methods have been developed to determine the amount of easily plant available P fraction, commonly called the “labile phosphorus pool”. Plant available P is considered to be loosely bound to aluminium (Al) and iron (Fe) ions or mineral surfaces (Syers et al., 2008).

1.4 Ion exchange resin

Ion exchange resins are used since the early 1960s to determine the labile P fraction in soil. In principle, the resin works as a sink absorbing ions in close vicinity. By doing so, the soil solution is depleted and more P is released, which consequentially will be absorbed again by the resin. Simply spoken, the resin mimics the process of a root, taking up P (Tan, 2005).

In comparison to water soluble P extraction, resins do not face the drawback of stagnating P release from soil into soil solution due to quick saturation. However, the capacity of resins to adsorb P is limited. To mitigate this problem, resins are renewed regularly when temporal P release is studied. Total amount of released P can be estimated by adding up the amounts of P adsorbed by each individual resin used.

1.5 Water soluble phosphorus

The determination of water soluble P is one of the most often applied techniques to estimate plant availability of P in soil. Water is used as extracting agent since it is close to conditions of soil solution. It is expected that ions soluble in water are easily available for plant uptake. In principle, soil is shaken for a specific time period and the amount of released P is determined.

Unfortunately, there is no uniform procedure for this method. Authors suggest varying soil to water ratios, ranging from 1:1.25 to 1:105 as well as different shaking times, ranging from 5 minutes to 15 hours. This creates difficulties in comparing results on water soluble P released. Fuhrman et al. (2005) made an excessive description on the variety of water soluble P determination parameters. The finding was that, the wider the soil to water ratio, the more P is released into soil solution. This is not surprising, since water is easily saturated with P and a higher amount of water consequently coincides with higher P content in the extract. With this method it remains, however, unclear how much additional P would have been releasable from soil by water, had it not been saturated. Studies, assessing the total amount of releasable P use therefore ion exchange resins or infinite sinks such as Fe oxide-impregnated paper (Frossard et al., 2000). To our knowledge there is no study assessing the total amount of P releasable by using water only.

1.6 Aim and hypotheses

Knowing the amount of total water soluble P would provide information on the potentially plant available P fraction in soil. It is common understanding that diffusion is the rate limiting step in the replenishment of soil solution with P (Blume et al., 2010). Suppose, this factor is neglected, it could be estimated how much P is potentially available for plant uptake. This data could be related to plant's need of P, being potentially an additional measure for plant available P forms in soil. Besides that, consequences for land management could be drawn in regard of improved fertilization recommendations. Finally, a tool to estimate risks of P leaching could be developed from the obtained values of total water soluble P.

That was the motivation to create a new method, aiming to describe the potential total release of water soluble P from soil into soil solution. For that, a consecutive P extraction method has been developed and applied to various soil samples of the Ultuna long-term soil organic matter experiment in Sweden. Long term experiments provide a valuable basis for investigation, since many parameters are well studied and new data can easily be related to them.

Hypotheses are

- i) Desorption of inorganic P from soil can be determined by consecutive extraction of P with water.
- ii) Desorption data can be used to determine the potential amount of P releasable by water.
- iii) Desorption dynamics can be used to gain further knowledge of inorganic P binding in soil in relation to total P (Tan, 2005), P-AL (Egnér et al., 1960) and the quantity/intensity ratio.

2. MATERIALS AND METHODS

For the present study, a number of soils from the Ultuna long-term soil organic matter experiment were chosen. In addition, two soils from horse paddocks used in other investigations at the department were included for the development and test of the desorption method, which are described in the appendix I.

2.1 Study site and soils used

Fifteen different treatments with four replicas each are maintained at the long-term experiment in Ultuna, Sweden (see Figure 1 and Table 1). Seven of those were chosen for this study.

- Bare fallow; no crops are grown on the plot, weeds are removed regularly.
- Calcium nitrate; $\text{Ca}(\text{NO}_3)_2$ fertilizer, equal to $80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.
- Ammonium sulphate; $(\text{NH}_4)_2\text{SO}_4$ fertilizer, equal to $80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.
- Green manure; pure grass without any legumes, equal to $2,000 \text{ kg C ha}^{-1} \text{ yr}^{-1}$
- Peat; commercial sphagnum peat without added fertilizer, equal to $2,000 \text{ kg C ha}^{-1} \text{ yr}^{-1}$
- Farmyard manure; solid cattle manure from various farms in the region of Uppsala, equal to $2,000 \text{ kg C ha}^{-1} \text{ yr}^{-1}$.
- Sewage sludge; from the Kungsängen sewage plant of Uppsala, equal to $2,000 \text{ kg C ha}^{-1} \text{ yr}^{-1}$. Iron(III)chloride, FeCl_3 , is used for phosphorus removal, which means that this agent is as well present in the sewage sludge.

In appendix II, further chemical properties of these treatments can be found.

Soil samples were taken in late autumn from the topsoil at 20 cm depth. Sampling was performed after all crop residues have been removed. From each individual plot five subsamples were taken with a soil corer and combined to a composite sample. In total, four replicate samples from each selected treatment were used for the experimental work.

2.2 Chemical analyses

2.2.1 Consecutive extraction of water soluble phosphorus

The method developed aims to deplete a soil from the entire instantly available water soluble P fraction, whereby other processes than desorption such as weathering and mineralisation are excluded. For that, a soil sample is sequentially shaken with water being the extracting agent. Since water is quickly saturated with P, it is removed and replaced with new water within a short time to ensure constant removal of P. Measuring P concentrations in water released from soil allows the following:

- Estimation of the potential release of water soluble phosphorus ($\text{P}_{\text{rel-H}_2\text{O}}$) from soil: By adding up the concentrations of each individual extraction step, the total amount of water soluble phosphorus released from soil can be approximated with the aid of a mathematical function.
- Estimation of the minimum concentration of phosphorus ($\text{P}_{\text{min-H}_2\text{O}}$) in soil solution: This value represents the lowest P concentration in soil solution after consecutive removal of P released from the soil. This concentration can be calculated if the extraction steps required to reach $\text{P}_{\text{rel-H}_2\text{O}}$ are known. In principle the desorption curve derived from consecutive P extraction is extrapolated.

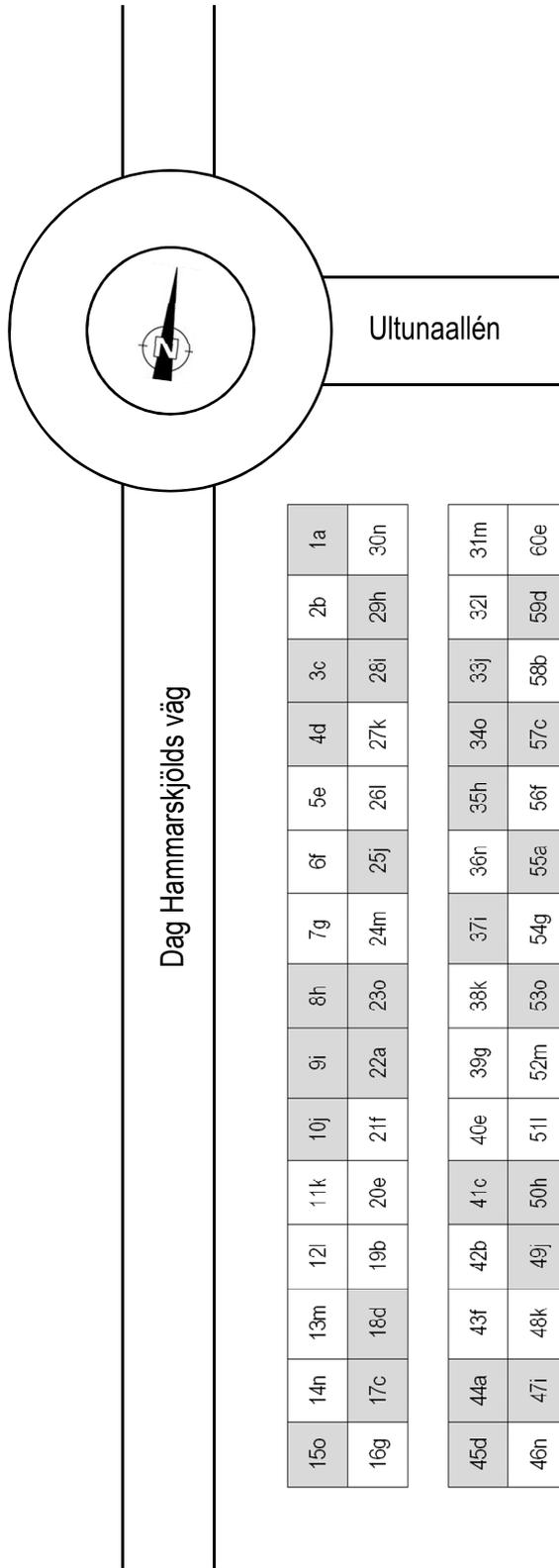


Figure 1 Design and layout of the experimental plots of the Ultuna long-term soil organic matter experiment. Plots marked in grey were analysed for this study.

Table 1 Basic data of the Ultuna long-term soil organic matter site. Source: Kätterer et al., 2011.

Location	59.82°N, 17.65°E, ca. 8 km south of Uppsala, Sweden
Soil, FAO taxonomy	Eutric Cambisol. Clay: 36.5%, Silt: 41%, Sand: 22.5%
Mean precipitation	542 mm yr ⁻¹
Mean temperature	5.8°C
Set-up	60 individual plots, 2-by-2m; 15 treatments with 4 replicas each, separated by iron plates. Organic amendments added in autumn, work performed by hand.
Fertilization at all plots	20 kg P ha ⁻¹ (superphosphate), 35-38 kg K ha ⁻¹ (KCl); applied every spring
Crops	Spring barley 17yrs, oats 1yrs, silage maize 10yrs, wheat 3yrs, others 10yrs. Crop failure in 3yrs.

A more comprehensive description of the theory behind the method is given in the appendix I. Basically, the method consists of six successive stages (see Figure 2), which were performed in a loop of extractions. Initially, air dried and 2 mm sieved soil samples were put into a 50 ml centrifugation tube. The weight of the tube, filled with soil was measured (weight 1). After that, the tube was filled with 30 g of deionised water, the exact weight of the filled tube measured again (weight 2). The tube was shaken for one hour and centrifuged for 20 minutes at 3,000 rotations per minute (2817 *g*) to separate liquid soil solution from solid soil. After centrifugation, soil accumulated in the bottom of the tube, leaving behind a clear supernatant. The liquid was filtered with filter paper to exclude floating particles and stored at 4°C for further P analysis. The weight of the emptied centrifugation tube was measured (weight 3) before it was refilled again with purified water until it reached the initial weight of filling (i.e. weight 2). The procedure was repeated ten times in total. For practical reasons it might not be possible to perform the entire consecutive extraction method in one day. However, it is assumed that storing soils after water removal in centrifuge tubes overnight at 4°C will not greatly influence P release. Microbial breakdown under this condition is not likely if the total period for analysis does not exceed 12 hours.

2.2.2 Extract treatment and phosphorus analysis

The extract gained after each extraction step was filtered with 0.45 µm membrane filters (Schleicher and Schüll GmbH, Dassel, Germany) and analysed for P-H₂O colorimetrically (Murphy and Riley, 1962). In principle, acidic conditions are formed with sulphuric acid. Orthophosphate reacts with added ammonium molybdate and potassium antimonyl tartrate to phosphomolybdic acid. This is reduced to molybdenum blue, which is intensely coloured, by adding ascorbic acid. The intensity of blue colour corresponds to P content in the sample and is measured by a spectrophotometer. In this study, the UV-1201V SHIMADZU apparatus was used. For all extracts of the treatments fallow and sewage sludge, total P was measured by digesting samples with 7 M HNO₃ and determination with ICP-AES according to Swedish Standards-028311 (SIS, 1997), performed by a commercial soil laboratory.

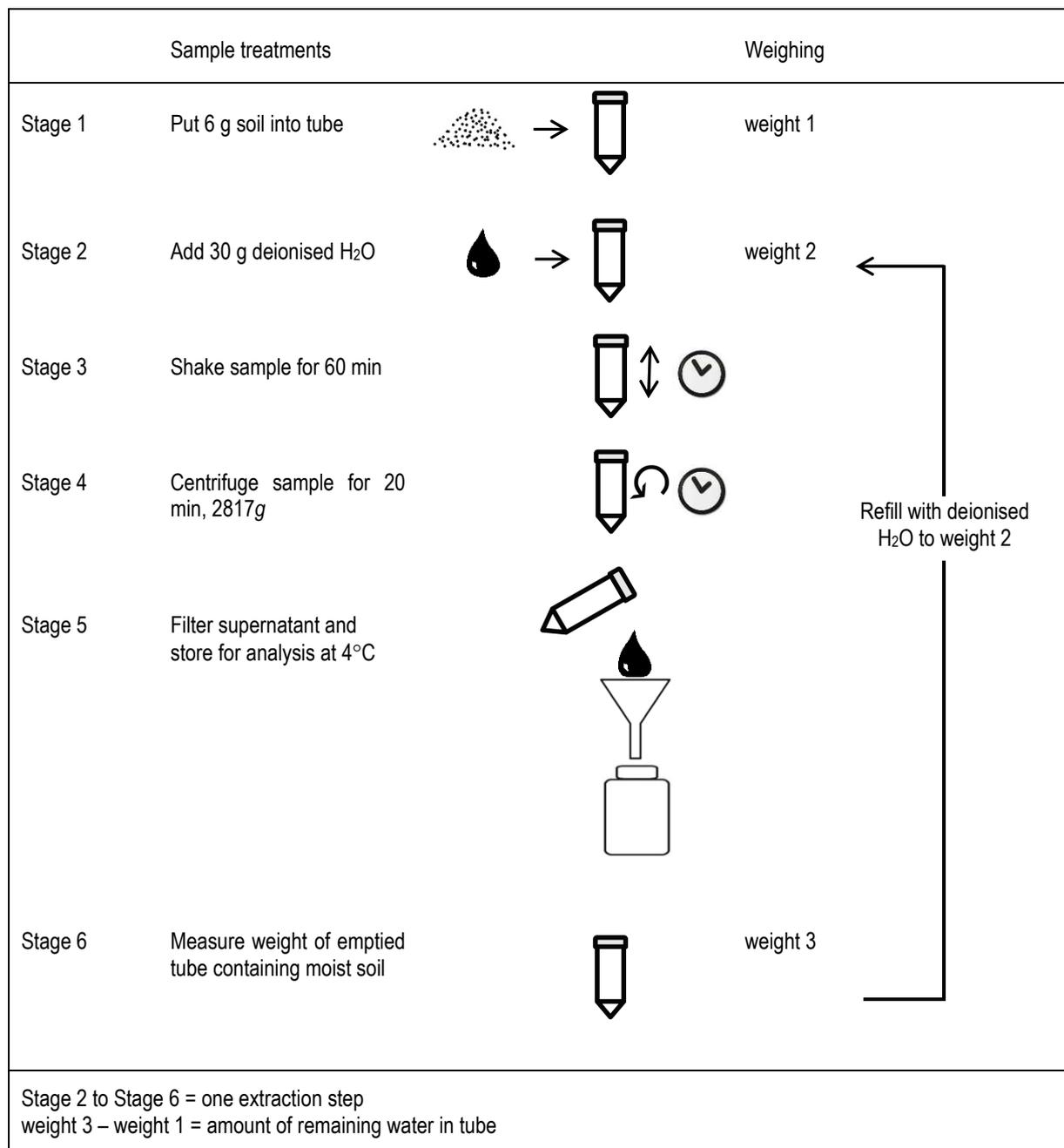


Figure 2 A schematic diagram of the different stages involved in the consecutive extraction of water soluble phosphorus from soil.

2.2.3 Correction of measured P values

The ratio of soil to purified water in the centrifugation tube is 1:5 (6 g soil and 30 g purified water). However, after extraction and centrifuging a certain amount of water remains in the tube after emptying the supernatant into storage bottles, which is about 10 per cent. When new deionised water is added for the next extraction step, remaining water in the soil matrix needs to be considered, requiring measurement of the weights (see figure 2). By subtracting the weight of the emptied tube (weight 3) from the initial weight of the tube filled solely with soil (weight 1) remaining water in soil matrix can be determined. Knowing the P concentration in the extract, the quantity of P in the remaining water can be calculated by a mass balance. The amount of P in the remaining water has to be subtracted from the P content gained in the following extraction step. Thereby, relatively accurate values of P concentration without disturbance from former extraction can be obtained. However, in practice is the difference in concentrations of corrected vs. non-corrected values hardly higher than 2 per cent.

Box 1 Example for the correction of measured P values by the use of a mass balance.

Sample of extraction step n
vol.1 = remaining volume of H ₂ O in centrifugation tube (weight 3 – weight 1) = 3ml
conc.1 = 7 mg P L ⁻¹
Sample of extraction step n+1
vol.2 = volume of H ₂ O in refilled centrifugation tube = 30 ml
conc.2 = 5 mg P L ⁻¹
<i>What would be concentration of P in “pure” 27ml H₂O of extraction step n+1, without disturbance of extraction step n?</i>
Mass balance:
30ml * 5mg L ⁻¹ = 0.15 mg P in 30ml sample of step n+1
3ml * 7mg L ⁻¹ = 0.021 mg P in 3 ml sample of step n
0.15 – 0.021 = 0.129 mg P in 27ml sample of step n+1 without impurities of previous extraction step
0.129 / 27 * 1000 = 4.7 mg P L ⁻¹ is concentration of solution in step n+1 without interference from extraction step _n .

2.3 pH measurement

The pH of the extracts was determined using a glass electrode pH meter (PHM93 reference pH meter, Radiometer Copenhagen). The ratio of soil to water was 1:5.

2.4 Statistical evaluation and curve fitting

For the development of the method, ANOVA was used to find out up to which extraction times significant differences in P concentrations were found in the extracts (figure 10 in appendix I). Regression analyses were used to determine correlations of different P analysis of the soils with total P, P-AL and pH (data from annual soil analysis, see table 7 in appendix II). For these statistics Minitab 16 was used. A significance level of 0.05 was chosen.

In addition, curves were fitted to analytical data using the program Sigma-Plot 12. Curve fitting is a tool for finding the mathematical function that gives the best fit to measured data points. With the aid of the function, a curve can be added to a data set. The gained R²adjusted value (R²_{adj}) provides information about the accuracy of the fitted curve. The value ranges from zero to one, where higher values represent a better fit of the curve to the measured data than lower values.

The potentially releasable water soluble phosphorus (P_{rel-H₂O}) was estimated by applying an “exponential rise to maximum” function ($f=a*(1-e^{(-b*x)})$) on the accumulated concentrations of P from each consecutive extraction step. It was defined that P_{rel-H₂O} was equal to 99 per cent of the gained “a” value which represents total P release after infinite extraction steps.

Besides that, also the minimum water soluble P concentration ($P_{\min\text{-H}_2\text{O}}$) in soil solution was estimated with this method. For that, an exponential decay function ($f=d*e^{(-b*x)}$) was fitted to the concentrations of each individual consecutive extraction step. The minimum water soluble P concentration was defined by the amount of extraction steps required to reach $P_{\text{rel-H}_2\text{O}}$.

The theory behind the developed method as well as detailed description on the calculation of $P_{\text{rel-H}_2\text{O}}$ and $P_{\min\text{-H}_2\text{O}}$ can be found in the appendix I.

2.5 Quantity/Intensity estimation

The estimation of the buffer-capacity in soil is a useful concept to estimate the ability of soils to replenish soil solution with nutrients. The potential buffer capacity, PBC, is defined by the change of quantity in relation to changes in intensity.

$$\text{PBC} = \Delta Q / \Delta I$$

Quantity is a measure of the nutrient reserves in soil, whereas intensity refers to the amount of the nutrient dissolved in soil solution. Since soil solution is replenished by P from the soil reserves, decreases in total P occur when P is removed from soil solution (Brady and Weil, 2008). To give an example, a low buffer capacity of a soil is present, when a certain amount of P removed in soil solution would lead to a relative strong decrease in P content in soil. In comparison, if the same amount of P was removed in a highly buffered soil, hardly any change in the total P content would occur due to soil solution replenishment.

Following the approach of van Rotterdam-Los (2010), a quantity/intensity ratio was related to the relative decrease in water soluble P release. In two approaches, quantity was once approximated by total P and once by P-AL, whereas intensity was approximated by the highest P release ($P_{\max\text{-H}_2\text{O}}$). The gained ratio is an approximation of the buffer capacity of the individual treatments.

Relative decrease in water soluble P was approximated by the desorption coefficient, b, gained from the curve fitting process. This value describes the steepness of the P release curve slope, providing an indication on how quickly soil is depleted in P during continuous extraction.

3. RESULTS

3.1 Consecutive phosphorus extraction

Concentrations of water soluble P after each consecutive extraction step are given in table 2, showing that initial values vary from 6.3 to 29.0 mg P kg⁻¹ soil and decrease with each additional extraction step with the exception of calcium nitrate, ammonium sulphate and sewage sludge. Data from three treatments are plotted in figure 3.

3.1.1 Treatment of gained data

In figure 4 the total releasable water soluble phosphorus as well as their corresponding minimum concentration in soil are shown for three selected treatments. Also the process of calculating $P_{\text{rel-H}_2\text{O}}$ and $P_{\text{min-H}_2\text{O}}$ can be demonstrated in three steps:

- i) Extraction: The initial values of each extraction step are depicted in the lower part of the graph for three treatments. All of them have the shape of a declining curve.
- ii) Accumulation: The individual concentrations of each extraction step are accumulated, as depicted in the upper graph. Each consecutive extraction step represents the sum of all foregoing extracted P concentrations.
- iii) Extrapolation: On the created data points of accumulated P concentrations (i.e. amount of released P) curve fitting was applied using an exponential rise to maximum function. Thereby, $P_{\text{rel-H}_2\text{O}}$ can be estimated, which is reached at different extraction steps, depending on the treatment. By knowing the amount of extraction steps required to reach $P_{\text{rel-H}_2\text{O}}$, also $P_{\text{min-H}_2\text{O}}$ can be estimated. For that, curve fitting with an exponential decay function is applied on the original data of consecutive P extraction (lower part of the graph). The values after the break represent these $P_{\text{min-H}_2\text{O}}$ values, reached at the corresponding extraction step for $P_{\text{rel-H}_2\text{O}}$. Please consult the appendix I where the process of calculation $P_{\text{rel-H}_2\text{O}}$ and $P_{\text{min-H}_2\text{O}}$ is described in detail.

Fitting the data from the extraction to nonlinear graphs, data of releasable water soluble P ($P_{\text{rel-H}_2\text{O}}$) and minimal P concentration ($P_{\text{min-H}_2\text{O}}$) can be gained displayed in table 3. The starting point for calculating $P_{\text{rel-H}_2\text{O}}$ was the maximum concentration of water soluble P released ($P_{\text{max-H}_2\text{O}}$). Only for ammonium sulphate and sewage sludge, $P_{\text{max-H}_2\text{O}}$ was not reached at the first but at the third extraction. For calcium nitrate the value of the first extraction was used, since no significant difference in concentration ($p=0.05$) was found between $P_{\text{max-H}_2\text{O}}$ and the value of first extraction.

For the Ultuna soils, $P_{\text{max-H}_2\text{O}}$ and $P_{\text{rel-H}_2\text{O}}$ values were multiplied by bulk density (provided in appendix II) and thus available in mg P L⁻¹ soil unit. Assuming a ploughing depth of 20 cm (i.e. depth of sampling) $P_{\text{max-H}_2\text{O}}$ and $P_{\text{rel-H}_2\text{O}}$ were calculated on a 'per hectare' basis.

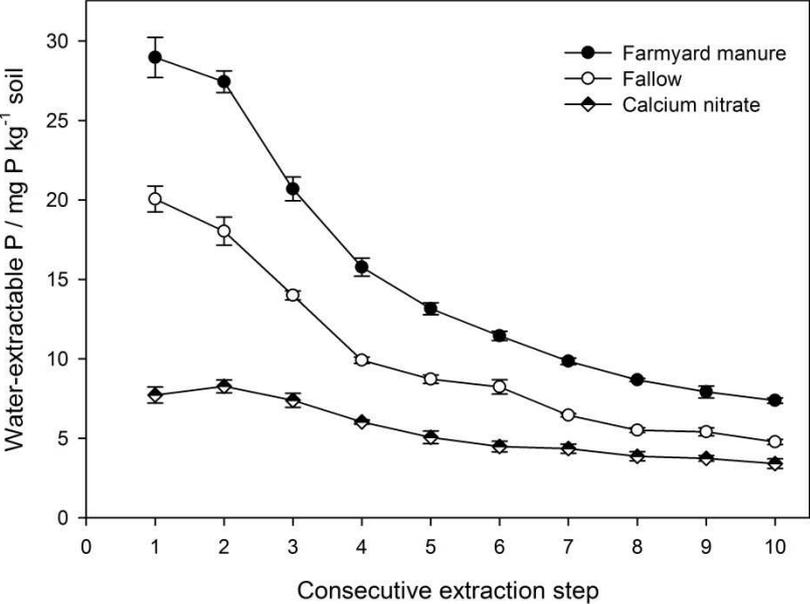


Figure 3 Water-extractable phosphorus of three soil treatments in ten consecutive extractions, with standard error, n=4.

Table 2 Data of water-extractable phosphorus after each consecutive extraction step \pm standard error, n=4. Maximum values for each soil treatment marked in grey.

Soil	Water-extractable phosphorus at extraction step \pm standard error / mg kg ⁻¹											
	1	2	3	4	5	6	7	8	9	10	11	12
Horse paddock												
Clay soil	8.55 \pm 0.17	7.89 \pm 0.08	7.20 \pm 0.19	5.87 \pm 0.04	5.76 \pm 0.07	5.75 \pm 0.19	4.85 \pm 0.12	4.63 \pm 0.11	3.90 \pm 0.05	3.58 \pm 0.03	3.48 \pm 0.04	3.19 \pm 0.08
Sandy soil	6.32 \pm 0.05	6.13 \pm 0.09	4.95 \pm 0.03	3.91 \pm 0.07	3.50 \pm 0.07	3.45 \pm 0.11	2.75 \pm 0.05	2.54 \pm 0.03	2.06 \pm 0.03	2.17 \pm 0.02	2.02 \pm 0.04	1.80 \pm 0.01
Ultuna experiment												
Fallow	20.06 \pm 0.82	18.03 \pm 0.88	13.99 \pm 0.29	9.91 \pm 0.20	8.72 \pm 0.26	8.23 \pm 0.45	6.45 \pm 0.10	5.51 \pm 0.15	5.40 \pm 0.25	4.47 \pm 0.16		
Calcium nitrate	7.72 \pm 0.51	8.27 \pm 0.40	7.39 \pm 0.44	6.03 \pm 0.13	5.06 \pm 0.40	4.48 \pm 0.34	4.35 \pm 0.29	3.87 \pm 0.29	3.74 \pm 0.18	3.41 \pm 0.30		
Ammonium sulfate	2.84 \pm 0.35	4.34 \pm 0.28	5.31 \pm 0.29	5.09 \pm 0.28	5.28 \pm 0.15	4.93 \pm 0.23	4.69 \pm 0.19	3.99 \pm 0.18	3.94 \pm 0.16	3.96 \pm 0.16		
Green manure	19.14 \pm 0.87	16.48 \pm 1.16	12.63 \pm 0.46	9.86 \pm 0.49	7.93 \pm 0.24	7.03 \pm 0.26	6.34 \pm 0.25	5.57 \pm 0.22	5.53 \pm 0.20	5.05 \pm 0.05		
Peat	15.46 \pm 1.14	14.78 \pm 0.79	12.93 \pm 0.52	9.71 \pm 0.68	8.66 \pm 0.53	8.07 \pm 0.45	7.21 \pm 0.34	5.89 \pm 0.33	5.58 \pm 0.25	4.55 \pm 0.38		
Farmyard manure	28.96 \pm 1.27	27.43 \pm 0.68	20.69 \pm 0.74	15.77 \pm 0.57	13.15 \pm 0.37	11.45 \pm 0.30	9.85 \pm 0.21	8.67 \pm 0.09	7.92 \pm 0.38	7.38 \pm 0.17		
Sewage sludge	7.78 \pm 0.41	13.34 \pm 0.41	15.25 \pm 0.31	13.02 \pm 0.22	12.79 \pm 0.34	12.87 \pm 0.36	11.26 \pm 0.21	9.93 \pm 0.31	8.34 \pm 0.28	8.08 \pm 0.33		

Table 3 Amount of potentially releasable water soluble phosphorus ($P_{\text{rel-H}_2\text{O}}$) and respective R^2_{adj} derived by using a function of exponential rise to maximum ($f = a*(1-e^{-b*x})$) where x is the number of extraction steps, b is the desorption coefficient, and a the total amount of water extractable P at an infinite number of consecutive extractions (where $P_{\text{rel-H}_2\text{O}}$ is equal to 99 per cent of parameter a); and maximal P released within ten extraction steps ($P_{\text{max-H}_2\text{O}}$) with minimum water soluble phosphorus concentration ($P_{\text{min-H}_2\text{O}}$) and respective R^2_{adj} derived by using an exponential decay function ($f = d*e^{-b*x}$) where x is the number of extraction steps, b is the desorption coefficient, and d is the hypothetical starting point or concentration of P that is potentially releasable before the first extraction step, with standard error, $n=4$.

Soil	P desorption				P accumulated release				Extraction step	
	$P_{\text{max-H}_2\text{O}}$		$P_{\text{min-H}_2\text{O}}$		$P_{\text{rel-H}_2\text{O}}$		R^2_{adj}			
	(mg kg ⁻¹)	(kg ha ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(kg ha ⁻¹)	b value	R^2_{adj}		
Horse paddock										
Clay soil	8.55 ± 0.17		0.08 ± 0.00	0.094	0.97	96.55 ± 1.43	0.093	1.00	51 ± 0.2	
Sandy soil	6.32 ± 0.05		0.06 ± 0.00	0.137	0.97	51.73 ± 0.15	0.140	1.00	35 ± 0.4	
Ultuna experiment										
Fallow	20.06 ± 0.82	57.37 ± 2.03	0.24 ± 0.01	0.186	0.97	117.85 ± 2.21	331.84 ± 5.80	0.188	0.98	25 ± 0.5
Calcium nitrate	8.27 ± 0.40	21.18 ± 0.89	0.11 ± 0.00	0.107	0.93	80.48 ± 5.08	150.05 ± 7.62	0.108	0.99	42 ± 1.8
Ammonium sulfate	5.31 ± 0.29	13.07 ± 0.52	1.40 ± 0.15	0.049	0.85	57.50 ± 1.96	141.40 ± 2.25	0.175	0.92	30 ± 1.8
Green manure	19.14 ± 0.87	51.29 ± 2.01	0.22 ± 0.01	0.181	0.96	111.95 ± 3.45	293.37 ± 8.14	0.183	0.95	26 ± 0.7
Peat	15.46 ± 1.14	34.64 ± 2.21	0.23 ± 0.03	0.139	0.98	123.04 ± 5.22	265.17 ± 7.82	0.141	0.94	33 ± 1.1
Farmyard manure	28.96 ± 1.27	71.83 ± 2.72	0.36 ± 0.01	0.181	0.97	177.61 ± 3.98	442.81 ± 6.67	0.184	0.98	26 ± 0.8
Sewage sludge	15.25 ± 0.31	31.11 ± 0.55	2.81 ± 0.11	0.086	0.92	130.40 ± 0.81	264.60 ± 1.30	0.229	0.97	21 ± 0.5

3.1.2 Potentially releasable P and minimum concentration of water soluble phosphorus

For the two horse paddock soils, almost twice as much $P_{\text{rel-H}_2\text{O}}$ was released from the clay soil than from the sandy soil. For the soils from the Ultuna experiment, four clusters of similar $P_{\text{rel-H}_2\text{O}}$ release can be identified:

- i) Farmyard manure released by far the highest amount of $P_{\text{rel-H}_2\text{O}}$, 178 mg kg^{-1} soil (440 kg ha^{-1}).
- ii) In the sewage sludge treatment, 130 mg kg^{-1} (260 kg ha^{-1}) were released but a higher $P_{\text{rel-H}_2\text{O}}$ was expected since the total P content in the sewage-sludge treated soil was almost five times higher than that of other treatments.
- iii) Fallow, green manure and peat released about the same amount of $P_{\text{rel-H}_2\text{O}}$ ranging from 112 to 123 mg kg^{-1} soil (330 , 290 and 265 kg ha^{-1} for fallow and green manure and peat, respectively).
- iv) The plots where mineral fertilizers were applied showed the lowest values ranging from 58 to 84 mg kg^{-1} soil (140 to 150 kg ha^{-1}).

The number of consecutive extraction steps required to reach $P_{\text{rel-H}_2\text{O}}$ and $P_{\text{min-H}_2\text{O}}$ differed from 21 to 51 for the different treatments. The estimated $P_{\text{min-H}_2\text{O}}$ varied, ranging from 0.06 to $2.81 \text{ mg P kg}^{-1}$ soil, where the ammonium sulfate and the sewage sludge treatments were the only treatments yielding values above 1 mg kg^{-1} . Note that for both of these treatments only seven but not ten data points were used for curve fitting, as discussed above.

The yielded b values represent the slope of the desorption curve. High values represent a steep slope of the curve (i.e. large difference between first and last extraction step) whereas lower values represent a more even run of the curve. Note that for all treatment except for ammonium sulfate and sewage sludge, the b values are very similar to the desorption and accumulation curve. The difference occurring at the two treatments is explained by the different starting points where modelling began. As afore mentioned, modelling the desorption always started at maximal P release ($P_{\text{max-H}_2\text{O}}$), whereas accumulation of values started at the first extraction step ($P_{\text{ini-H}_2\text{O}}$).

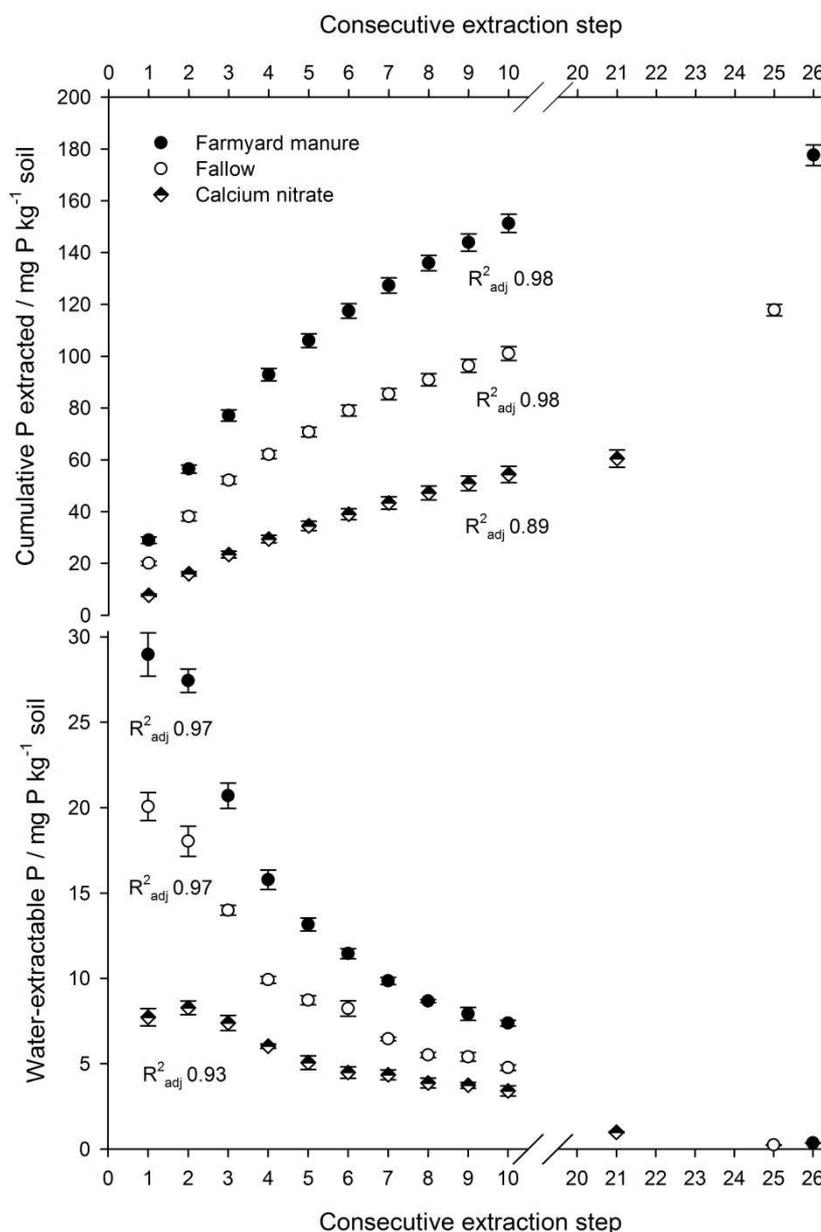


Figure 4 Curve fitting applied to data from three selected soil treatments upon consecutive extraction steps with one hour length. The upper part shows the cumulative P extracted using the following equation ($f = a*(1-e^{(-b*x)})$) where a is the maximum accumulated P, b is the desorption coefficient, and x is the number of extractions. The value after the break represents P_{rel-H_2O} . The lower part shows the corresponding water-extractable P for the treatments, values after the break represent the P_{min-H_2O} at the corresponding extraction step where P_{rel-H_2O} is reached ($f = d*e^{(-b*x)}$). All data are shown with standard error, $n=4$.

3.2 Depletion of total P and P-AL by consecutive water extraction

Consecutive water extraction yields usually high concentrations of P in the first extraction steps. In the following steps, less P is found in the extract. In the present study, three treatments deviated – maximum value were first measured after three extractions. However, differences between initial and maximum data were only significant for sewage sludge and ammonium sulphate. Despite the two deviating results, only the initial extraction concentration was used comparing treatments. The P_{ini-H_2O} value is thus a measure for P that

is immediately soluble into soil solution, i.e. at least within one hour. The $P_{\text{rel-H}_2\text{O}}$, on the other hand, is a measure of P available from the soil when the soil solution is replaced with P-free water several times (table 4).

The $P_{\text{ini-H}_2\text{O}}$ and the $P_{\text{rel-H}_2\text{O}}$ values were related to total P and P-AL contents of the soils, see table 4. The P-AL is a measure on the status of plant available P in soil.

In the fallow treatment, already in the first extraction step, 9.4 per cent of the P-AL fraction was released, but 55.4 per cent were potentially releasable with consecutive water extractions. Within one day (25 extraction steps, one hour each) one could release 11 per cent of the total P present in soil. This shows that a considerable fraction of P can be desorbed from soils without weathering processes.

Sewage sludge showed the lowest relative water solubility of P as compared to the other treatments. Related to the relatively high amounts of $P_{\text{rel-H}_2\text{O}}$ (130 mg kg⁻¹ soil) this is surprising. However, the total amount of P in the sewage sludge treated soil was almost five times higher than those of the other soils analysed, due to large additions of P through sewage sludge that was precipitated during waste water treatment.

The strength of water as a depletion agent is also evident from figure 5 providing values for three treatments showing the relative depletion of the P-AL fraction through water extraction.

Table 4 Proportion of the of P-AL and P-total released as water soluble P. $P_{\text{ini-H}_2\text{O}}$ represents initial P released by one extraction, whereas $P_{\text{rel-H}_2\text{O}}$ represents total P releasable through consecutive water extractions.

Soil	$P_{\text{ini-H}_2\text{O}}$		$P_{\text{rel-H}_2\text{O}}$	
	(% of P total)	(% of P-AL)	(% of P total)	(% of P-AL)
Horse paddock				
Clay soil	1.0	3.5	11.2	39.6
Sandy soil	0.8	4.1	6.5	33.6
Ultuna experiment				
Fallow	1.9	9.4	11.2	55.4
Calcium nitrate	0.9	4.7	8.8	48.7
Ammonium sulfate	0.3	1.7	5.5	34.9
Green manure	1.8	9.3	10.5	54.2
Peat	1.4	8.5	11.4	67.8
Farmyard manure	2.3	8.3	14.0	51.1
Sewage sludge	0.2	4.4	2.7	23.8

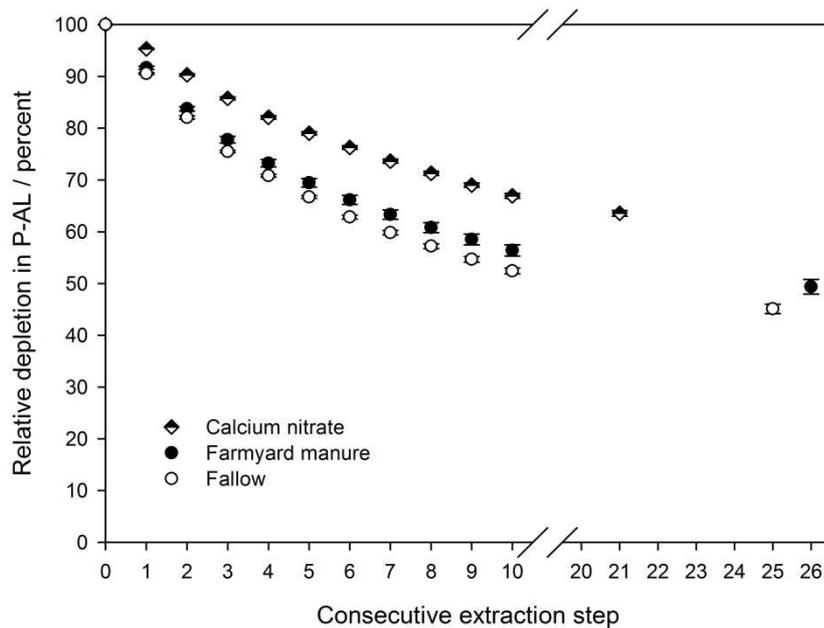


Figure 5 Depletion of P-AL in three soil treatments through ten consecutive extraction steps with water, with standard error, $n=4$. Values after the break are based on the minimum water soluble phosphorus concentration ($P_{\min-H_2O}$) obtained by the function given in Table 3.

3.3 Comparison of water-soluble P with P fractions in soil

In 1991, the soil P status in the Ultuna experiment was determined applying the Hedley P fractionation method (Otabbong et al., 1997; Hedley et al., 1982). In principle, a stronger reagent was used at each extraction step to release more strongly bound P fractions. In figure 6, these fractions were stapled on top of each other for four treatments. Comparing the data sets of the Hedley P fraction with those from consecutive P extraction allowed to estimate which fractions of P are desorbed by consecutive P extraction: All resin P is removed by the consecutive water extraction. Besides of that, 12, 20, 38 and 0.02 per cent of the sodium bicarbonate fraction are removed from the fallow, green manure, farmyard manure and sewage sludge treatment, respectively. Resin-P is regarded weakly bound to soil surfaces. Thus, the idea of being easily removable with water is rational. The bicarbonate fraction, on the other hand, entails more strongly bound P on surfaces, as well as phospholipids and nucleic acids.

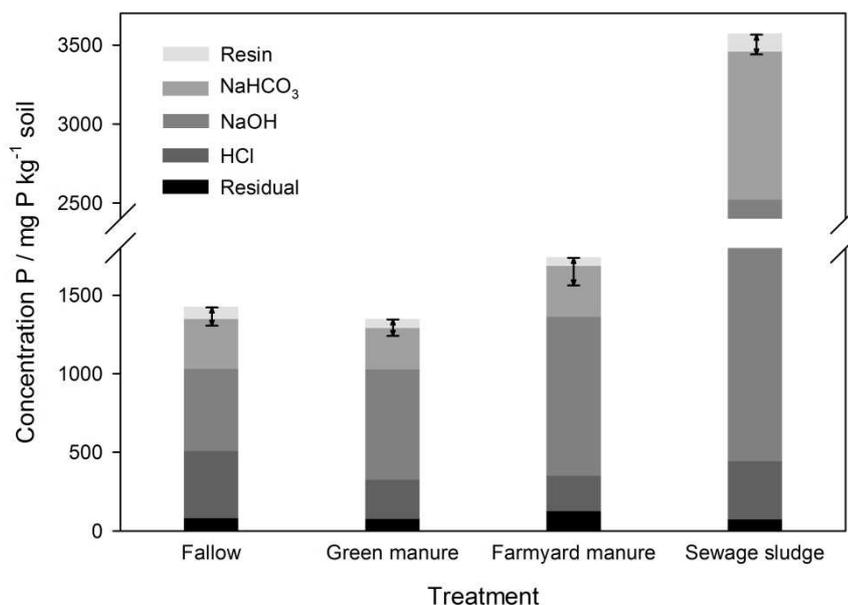


Figure 6 Four treatments of the Ultuna experiment, fractionated in 1991, shown in the columns (Ottabong et al., 1997) compared to P removed with consecutive water extractions ($P_{\text{rel-H}_2\text{O}}$) shown with arrows. Described fractions correspond to the following P-binding: Resin: chloride anion resin - weakly surface adsorbed P; NaHCO_3 : 0.5 M sodium bicarbonate - Al-bound P, phospholipids and nucleic acids; NaOH: 0.1 M sodium hydroxide - Fe-bound P and P encapsulated by Fe and Al compounds; HCl: 1M hydrochloric acid - Ca-bound P; Residual: concentrated H_2SO_4 + 25 per cent H_2O_2 - residual-P in mineral matrices and very stable humic substances.

3.4 Correlation of water-soluble P with total P and P-AL

P-AL is positively correlated with $P_{\text{rel-H}_2\text{O}}$ contents, correlation of total P with $P_{\text{rel-H}_2\text{O}}$ is slightly weaker (see figure 7). Sewage sludge was excluded due to high loads of total P and Al-P (P bound to aluminium oxides which serve as a binding site). Common understanding of P fertilization is that large amounts of P applied do not remain plant available, but become adsorbed to soil particles and total P contents in soil tend to increase when fertilized regularly (Stevenson and Cole, 1999; Bundy et al., 2005). This is also proven by the slope of the graph. For each 100 mg in total P increase, $P_{\text{rel-H}_2\text{O}}$ only increases by 23.5 mg in average for all soils. Thus, more than 75 per cent of P added to the system as fertilizer remain or are turned into non-water soluble P. For P-AL this ratio is naturally not as strong, with 58 mg in $P_{\text{rel-H}_2\text{O}}$ increase per 100 mg P-AL. A multiple linear regression performed showed that the model could potentially be further improved if pH would be added as predictor for $P_{\text{rel-H}_2\text{O}}$. However, too few data points are available to apply this method reliably. The measured pH values of different extraction steps can be found in appendix II.

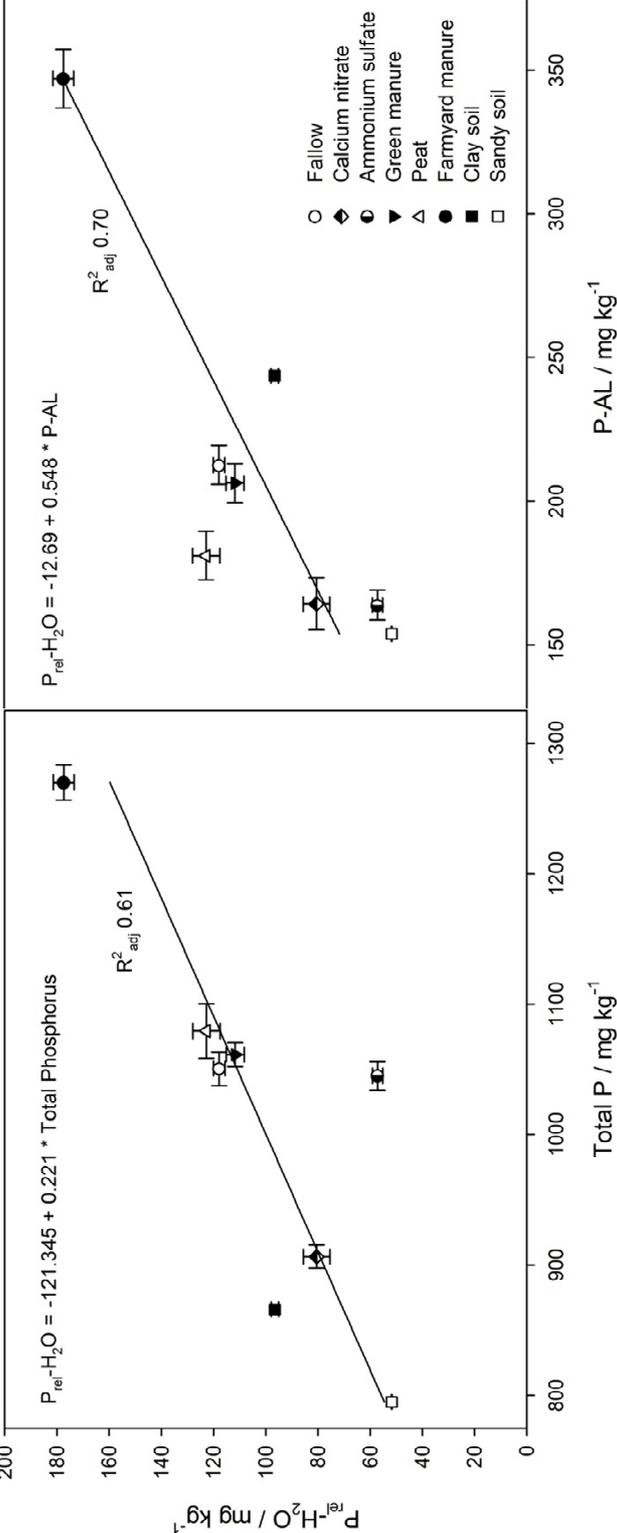


Figure 7 Correlation between potentially releasable phosphorus (P_{rel}-H₂O) and total phosphorus (left) and P-AL (right), respectively. Data include 8 soils in total, with standard error, n=4. Sewage sludge is excluded due to extremely high total P and P-AL contents (4,900 and 446 mg P kg⁻¹ soil, respectively).

3.5 Comparing water soluble P with P fractions of the initial soil

The sequential fractionation method after Hedley et al. (1982) was also applied to the original soil of the Ultuna experiment before the treatments were started in 1956.

As shown in figure 8, initial fractions of P from start of the field experiment were analysed (stacked column). The size of the different fractions was related to data derived from consecutive water extractions whereby the plot of the relation of total P to $P_{\text{rel-H}_2\text{O}}$ was added in figure 8. Combining the two types of information and assuming a hypothetical $P_{\text{rel-H}_2\text{O}}$ value of zero, the regression line intersects the fractionation column below the resin and the bicarbonate fractions. This is the range where commonly the start of the more labile pool in soils is assumed to be located at (Stevenson and Cole, 1999).

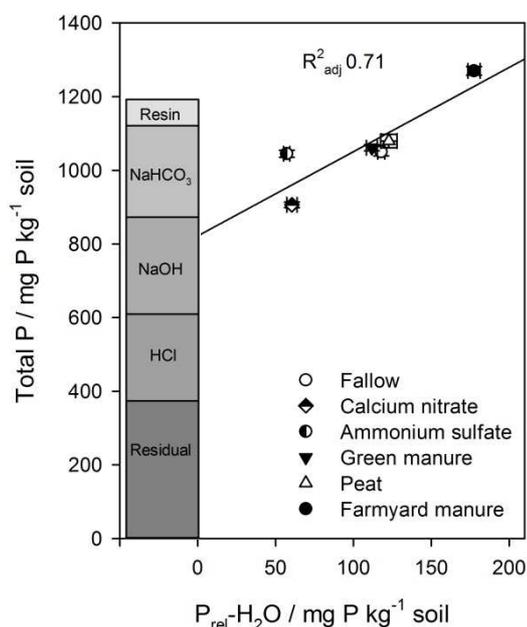


Figure 8 Comparison of P-fractions from the study of Otabbong et al. (1997), showing the initial fractions (year 1956) in the Ultuna long term soil organic matter experiment in the left column with the correlation figure showing of accumulated water soluble P ($P_{\text{rel-H}_2\text{O}}$) to total P. The treatment sewage sludge was excluded from this figure due to very high total P content (~4,900 mg kg⁻¹ soil). Total P content in soil in 1956 was 1,190 mg kg⁻¹ soil.

3.6 Quantity-Intensity relation and desorption coefficient b

Relative changes in supply potential during continuous desorption of P is shown by the buffer capacity. This is approximated by the ratio of total P (or P-AL) which represents the quantity factor and $P_{\max}\text{-H}_2\text{O}$, representing the intensity factor. For both approaches of estimating the quantity-intensity ratio, the fallow, green manure and farmyard manure have the lowest buffer capacity, whereas ammonium sulfate and sewage sludge show the highest. The clay soil has a lower buffer capacity than the sandy soil when the quantity is approximated with total P but a higher when approximated with P-AL.

The release coefficient (b value) is an indicator on how readily P is desorbed from soil, where higher values indicate a steeper decline in (consecutive) desorption than lower values. As shown in figure 9, does a lower desorption coefficient coincide with a wide ratio of capacity to intensity, i.e. a higher buffer capacity. In this case, the P supply is high compared to the P in soil solution, thus effectively buffering against P removal. A steep release curve, i.e. a large difference in concentration between the first and the last extraction step (and a high release coefficient), is accompanied by a narrow ratio between capacity and intensity indicating low P buffering.

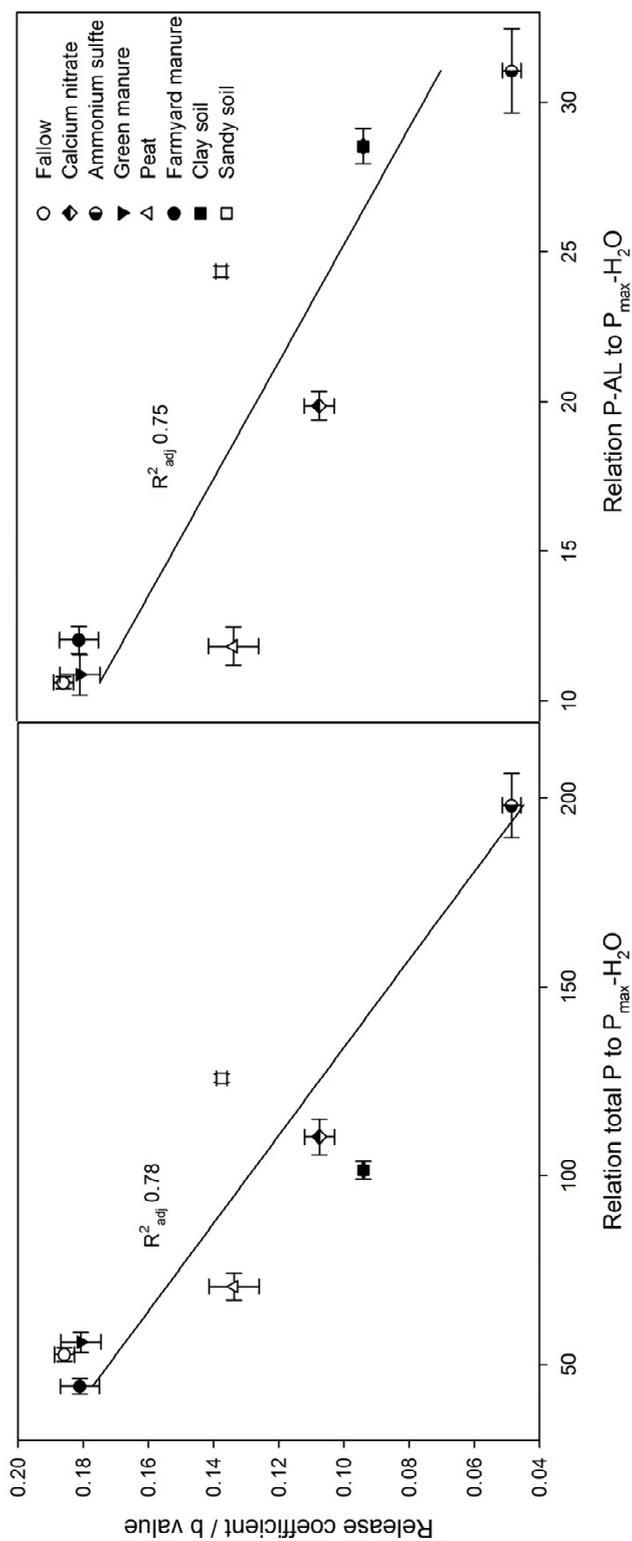


Figure 9 Relationship between P release coefficient b, describing the slope of P release curve over 10 extraction steps and the ratio of phosphorus quantity over phosphorus intensity. Phosphorus quantity is approximated by total P and P-AL, phosphorus intensity by maximum water soluble phosphorus during consecutive extraction (P_{max}-H₂O). Ammonium sulfate and sewage sludge show maximum water soluble P concentration at extraction step 3, all other treatments at extraction step 1. Sewage sludge was excluded for total P due to very high total P content (4,900 mg P kg⁻¹ soil). Error bars represent standard error, n=4.

4. DISCUSSION

4.1 Amount of phosphorus releasable by water in the short term

Compared with total P content, relatively high quantities of water soluble P are releasable into soil solution within about 30 consecutive extraction steps of one hour each (compare table 3). This highlights the fact that large amounts of P from soil are potentially available within a short time scale for entering soil solution. This is naturally dependant on soil properties, as discussed below. In more general terms, researchers suggest the model of different pools of P in soil (Syers et al., 2008):

Four different pools are defined by the accessibility, extractability and availability of P in soil to plant. Each pool shows interactions with the neighbouring pool. If the first pool, which represents immediately accessible P that is ready for plant uptake in soil solution, is exhausted, it will be partly replenished by the second pool (readily available P). The same is true for the other pools, where the third one represents less extractable P more strongly bound to soil and the fourth pool equals the P, deeply locked within the soil matrix. However, not only replenishment of emptied pools takes place, but more commonly, the reverse process occurs. When easily plant available P is applied on soils, a large part is quickly transformed in less available forms of P (Havlin et al., 1999). In natural systems the size of the different pools differs considerably, with the immediately accessible pool being the smallest and the pool of very low accessibility the biggest (Mengel and Kirkby, 1982). In highly weathered soils, where most of easily available forms of P are depleted, only strongly bound forms on iron and aluminium oxides remain (Walker and Syers, 1976).

Relating the P-pool model to the applied method, the results show that the first and the second pool of P are approximated and emptied by consecutive extraction (compare figure 6). By using water for continual removal of P, both P in solution and easily available P is affected. Potentially, more water soluble P is present in soil than extracted with 10 steps. Weathering and dissolution processes, as well as mineralization of organic P compounds are time dependent processes. Therefore, if more time would be given between extractions, the amount of water soluble P would increase, presumably, again.

In soil P is mainly transported via diffusion (Kovar and Claassen, 2005). Differences in concentration are the main driver for diffusion. The diffusion rate is very slow, 10^{-12} to 10^{-15} $\text{m}^2 \text{s}^{-1}$. Once the rhizosphere is depleted of P, it requires a considerable time until additional P is diffused close enough to the root for uptake via plants roots (Schachtman et al., 1998). Blume et al. (2010) defined the rate of diffusion as the main determinant factor with regard to P uptake by plants. However, since all soil aggregates are destroyed in the applied consecutive extraction method, diffusion processes were excluded. Thereby, maximum potential of P release to the soil solution was measured.

The finding suggests that it may be more the physical limitation of slow diffusion of P to replenish soil solution quickly, rather than the strong chemical binding of P into the soil matrix, that limits P availability in soil. If diffusion would proceed faster, more water soluble P would be instantly available. This finding is two sided. On the one side, the limit is shown on how plants can have access to P in soil. It is common understanding that only a very small fraction of total P content is actually available in soil solution. On the other hand, it gives an explanation on the rather low tendency of P leaching, compared with other nutrients, in the soil – water system. The importance of soil aggregates for keeping P “in place” is demonstrated here. This effect appears even stronger when relative availability is compared with actual plant uptake, as discussed below.

4.2 Differences between soil treatments

The amounts of $P_{\text{rel-H}_2\text{O}}$ differ strongly among treatments from the Ultuna experiment, being threefold higher for the farmyard manure than for the ammonium sulphate treatment, see table 3. Here, possible explanations on individual treatments and soils are discussed.

The amount of organic amendments applied to the Ultuna experiment is based on their carbon content. Phosphorus contents of the amendments differ considerably. As a mean value for the years 1956 to 1991, 73 kg P ha⁻¹ yr⁻¹ were applied through farmyard manure (Kirchmann et al., 1994). This is in addition to the 20 kg P ha⁻¹ yr⁻¹ superphosphate added (compare table 7 in appendix II). Bremer et al. (2008) made an analysis upon the water solubility of feedlot cattle manure, which is comparable to the manure applied in the Ultuna experiment. Their results show that up to 24 per cent of P in manure is water soluble (0.5g dry matter shaken for 1h with 100 ml double distilled water). The high water solubility of P in farmyard manure may thus be an explanation for the high release of $P_{\text{rel-H}_2\text{O}}$ amounting to 177 mg P kg⁻¹ soil. Besides of that, also relative high pH values of the farmyard manure treatment could explain the higher release of P, compared to other treatments (see table 9, appendix II). The average pH value of the extracts of the farmyard manure treatment is 6.3, which is within the ideal pH range for plant uptake. Orthophosphate ions are most labile in this pH since no precipitation of P with aluminium, iron or calcium ions take place (Havlin et al., 1999). All other soils of the Ultuna experiment show average pH values below 6, thus, precipitation with aluminium minerals can be expected.

Sewage sludge released 130 mg P kg⁻¹ soil being the second highest concentration of the soil treatments. Still, the solubility of P was low compared to total P present in soil. This is due to the strong binding of P with ferric chloride when precipitated during waste water treatment and ending up in sewage sludge in insoluble forms (Deneux-Mustin et al., 2001). This is also obvious from the comparison of P fractions (compare figure 6), where a large fraction of hydroxide P was determined. Hydroxide P is regarded to represent inorganic and organic P compounds, particularly bound on Fe and Al soil surfaces. In addition, reactive iron compounds are added with sewage sludge compared to other treatments. Although a higher desorption of water soluble P was expected, the relative availability of P from sewage sludge treated soils is actually low. This has implications on future management of P through sewage sludge, since it is regarded as an option to recycle P to arable land (Cohen et al., 2011)

The treatments green manure, peat and fallow show rather similar values of $P_{\text{rel-H}_2\text{O}}$, being 112, 123 and 118 mg P kg⁻¹ soil, respectively. Likewise to the farmyard manure treatment, not only superphosphate serves as a constant P source in all treatments, but P contents differ in the added amendments being, on average, 27 and 4 kg P ha⁻¹ yr⁻¹ for green manure and peat, respectively (compare table 7 in appendix II). Surprisingly, green manure has the lowest P release even though it receives more P than peat and fallow.

The mineral fertilizer amendments calcium nitrate and ammonium sulfate release the least P, amounting to 61 and 80 mg $P_{\text{rel-H}_2\text{O}}$ kg⁻¹ soil, respectively. In comparison with the other treatments of the Ultuna experiment, no additional P other than superphosphate was added to the plots, which might explain the rather low release. For ammonium sulfate, also the change in pH might affect the P release, as discussed below.

4.3 Releasable phosphorus and phosphorus removal by plants

The quantity of P incorporated in plants differs among species, ranging for the most important crops from 0.04 to 0.7 per cent of dry weight (Troeh and Thompson, 2005). The amounts of P removed by plants in one cropping period range as well greatly among various crops (Pierzynski et al., 2000), e.g.:

- Corn: 26 kg ha⁻¹
- Wheat: 10 kg ha⁻¹
- Alfalfa: 34 kg ha⁻¹
- Corn silage: 39 kg ha⁻¹
- Potatoes: 17 kg ha⁻¹

As shown in table 3, the calculated $P_{\text{rel-H}_2\text{O}}$ values exceed common P removal on a hectare basis at least fivefold for corn, grown at the Ultuna experiment site. This suggests that actually enough P is available in water soluble form, but it is rather physical constraints that hinder plant roots to access this sources. Jungk (1984) estimated that roots only explore 25 per cent of top soil in each growing season, which leaves a large part of soil “untouched” for direct root uptake. Several decades ago, P use efficiency of plants was aimed to be increased by altering the architecture of the root system. However, this approach lost favour after it was found that roots rather grow towards areas with high P content than exploring the whole soil volume (Syers et al., 2008).

Please note that at the Ultuna experiment site a delayed cultivation of crops takes place due to management reasons. Therefore it can be assumed, that lower amounts of P than normally expected are removed by plants, since no focus is put on maximal plant yield during cropping period.

4.4 General factors affecting release of water soluble phosphorus

4.4.1 pH

Soil pH seems to affect P availability even in the short term. This was observed in the ammonium sulfate treatment, where a shift in pH for almost one unit, from 3.9 to 4.7 was measured during ten extraction steps (see table 9, appendix II). Very low pH values in this treatment are the result of the nitrification process, where ammonium is turned into nitrate, two protons are released and thus pH is decreased. At the low pH values observed, P potentially precipitates with Al- and Fe-minerals (Havlin et al., 1999). The sharp increase in pH during extraction coincided with higher amounts of P released. Using a buffered solution as extracting agent might have mitigated that problem. However, using water (non-buffered solution) approximates natural condition.

Note that pH values measured from annual soil analysis (table 7, appendix II) differ from pH values measured from extracts of individual extraction steps (table 9, appendix II). Different ratios of soil to water were used for the pH analysis, which can explain this discrepancy. Besides of that, also natural fluctuations of pH occur in soil depending on the stage of the growing season (Blume et al., 2010).

4.4.2 Soil texture

Soil P released is also affected by soil texture. Coarser soils are expected to release less P, since the relative number of binding sites is lower, compared to finer textured soils. In this study, the amounts of P released was not analysed in regard of soil texture. However, a clear difference was obvious between the soils from the horse paddock, where the $P_{\text{rel-H}_2\text{O}}$ value of clay soil is almost double as high as the one for sandy soil (see Table 3).

4.4.3 Total phosphorus content

Total P was found to be a relatively good predictor for $P_{\text{rel-H}_2\text{O}}$ and adding pH would improve the prediction potentially even more (see figure 7). More data are, however, required for allowing a reliable multiple regression analysis. An increase of a certain amount of total P is accompanied by an increase of 25 per cent as $P_{\text{rel-H}_2\text{O}}$. This is in accordance with current knowledge on P fertilizer application. Large portions of P applied turn quickly into less plant available or insoluble form, which led to the term “fertilizer efficiency”, describing the extent of how much P is available for plants (Withers et al., 2005). At the Rothamsted experiment site, Olsen P (NaHCO_3) increased only for 13 per cent of total P. Also other studies indicated only a little increase of extractable P in relation to total P increase (Syers et al., 2008). For the Ultuna soils, even though different treatments were performed for more than 50 years, the soils still show very similar properties. The type of fertilizer, applied on this site to all plots is superphosphate ($20 \text{ kg ha}^{-1} \text{ yr}^{-1}$) which guarantees practically no limitation of P for plant growth on the site. Superphosphate is regarded as an excellent fertilizer for plants, since most of it is water soluble and therefore easily available for plant uptake. This is in contrast to other fertilizers where larger amounts can be initially in a not plant available state such as apatite rock. The high correlation of soluble P with total P might therefore be probably a specific result caused by the type of fertilizer applied. If not superphosphate but pure phosphate rock would be used, total P might increase to some extent but a lower fraction of P would be soluble in water. However, more analysis would be required to make general statements on this finding.

4.4.4 Other tested correlations

No correlations between P characteristics and total organic carbon content of the Ultuna experiment were found.

4.5 Estimation of labile P pool

Based on the fractionation method of Hedley et al. (1982), an estimation was made upon which fractions of P are involved in the consecutive water soluble P release. For four treatments it was possible to test, since P fractionations were done earlier. As for fallow, green manure and sewage sludge, the fractions from which P was released were resin P and sodium bicarbonate P (see figure 6). Only in the farmyard manure treatment more than one third of the sodium bicarbonate fraction was released through consecutive water extraction. In the fractionation scheme, sodium bicarbonate is expected to release Al-P, P more strongly bound than resin P, phospholipids and nucleic acids (Otabbong, 1996). In comparison to fallow or green manure, a higher amount of Al-oxides cannot be expected in the soil. Phospholipids and nucleic acids are organic P compounds. The procedure of consecutive water soluble P extraction detects only inorganic P, unable to measure organic P fraction with the ascorbic acid sulfomolybdo-phosphate blue colour analysis, as it was applied in this study. However, organic P was estimated with the difference method (Stevenson and Cole, 1999) by determination of total P and subtraction of inorganic P for the treatments fallow and sewage sludge. The finding was that only in the extracts of the first and second extraction steps very little organic P was present. The possibility that organic P was mineralized into inorganic P during the actual analysis can be excluded due to too short extraction intervals. Thus, only the aforementioned high water solubility of farmyard manure seems to be a rational explanation for the relatively high amount of sodium bicarbonate fraction release. The definition as “P more strongly bound than resin P” remains unsatisfactory, but appears to be linked with farmyard manure properties.

4.6 Quantity – Intensity relation

For the Ultuna experiment, the farmyard manure, fallow and green manure treatments showed the lowest P buffer capacity. These are also the treatments with highest P input by fertilization (20 kg P ha⁻¹ yr⁻¹ as superphosphate) in combination with an organic amendment P supply; table 7, appendix II). The peat treatment, with lowest P addition by organic amendment, had a slightly higher buffer capacity. Treatments with only mineral fertilization showed the widest quantity to intensity ratio since their P input are lower compared to the other treatments. Hence, treatments with low P inputs resulted in low P release, but maintained this P release more constantly than treatments with higher P inputs (and release), where substantial declines occurred during the initial extraction steps (see figure 3, table 2). This finding is in accordance with previous studies, where long-term application of animal manure or inorganic P fertilization coincides with relatively low buffer power (Kovar and Claassen, 2005).

The P supplying potential during continuous desorption (figure 9) showed a very similar pattern as discussed in van Rotterdam-Los (2010). Also here, P buffering was more effective when the quantity to intensity ratio was high. The lower this ratio, the steeper was the decline in P release during consecutive extractions.

4.7 Minimum concentration of phosphorus in soil

The estimation of P_{min-H₂O} was based on the extrapolation of two curves (estimation of P_{rel-H₂O} and thus by knowing the hypothetical amount of required extraction steps consequently estimation of P_{min-H₂O}). For both the sewage sludge and the ammonium sulfate, the values are well above 1 mg P kg⁻¹ soil. For both treatments, not ten but only eight data points were available for estimating P_{min-H₂O}, since calculation started always from P_{max-H₂O}, which was delayed compared to other treatments (see table 2). Therefore, the results of modelling for these two treatments have to be treated with care, when compared to others, where more data points were available for curve extrapolation. For the other treatments, values ranged from 0.06-0.36 mg P kg⁻¹ soil. In literature, a concentration of 0.2 mg P L⁻¹ soil solution or higher is desired for optimal plant growth (Pierzynski et al., 2005). The conversion from a “mg kg⁻¹ soil” to “mg L⁻¹ soil solution” unit requires, however, soil physical data such as pore size distribution etc., which were not assessed in this study. Besides that, also the ratio of water to soil (5:1) used in the consecutive extraction method is very different from field conditions, which is a potential source of strong distortions in the resulting data. That is why a conversion of P_{min-H₂O} values from mg kg⁻¹ soil to mg L⁻¹ soil solution was not performed.

However, potentially this value may be used for modelling purposes of constant P replenishment of soil solution with P. Various models in this respect have been already developed. For the EPIC model (Environmental Policy Integrated Climate) Jones et al. (1984) designed a simple add in for a soil – plant P model. The P pools used for soil were stable, active and labile inorganic and fresh organic and stable organic P. More recently, Kreuzeder (2011) developed a dynamic P model for soil, which is based on an initial ratio of 500 : 250 : 1 between inorganic P, organic P and P in solution, respectively. Inorganic and organic P can be determined by standard soil tests (Tan, 2005), whereas P in soil solution could be approximated by using the introduced P_{min-H₂O} value.

4.8 Possible pitfalls of the developed method

The particle sizes of the soil material used at the first extraction step has to be considered. Probably, it is not sufficient to grind and sieve the dried soil until mesh size of 2 mm, since micro pores might not be reached within the first two extraction steps. This could be an explanation for the delayed maximum P concentration from the ammonium sulfate and sewage sludge treatment.

Also, water repellent properties of the organic matter, especially in the treatment with sewage sludge, but also peat, could decrease P release in the first extraction steps. Only after the entire soil matrix is wetted, P release reaches maximum.

Throughout all extraction steps, the samples were shaken 1 hour each time. It was found that about 30 hours of continuous P removal with water would be required to deplete the soil system. However, it should be mentioned that the process could be performed even more quickly. In appendix I, the choice of a 1h extraction period is explained. However, one hour is the upper limit; most probably even shorter times would be sufficient for certain soils.

The effect of drying soil has a known impact on P release. Bartlett and James (1980) analysed water soluble P release using moist, dried and re-wetted soil. In their finding, moist soil released approximately 30 mg P L⁻¹, whereas dried soil only released 5 mg P L⁻¹. Rewetted soil had a release of approximately 20 mg P L⁻¹. The authors conclude that, in any case, soil should be stored in wet condition, at 4°C if storage is necessary for longer time. This finding may have an impact on the analysis of soil samples. A dried soil was chosen for this experiment mainly since homogenization of soil samples is easier when soil is dry, especially in soil with high clay content. However, other studies have shown that the effect of drying may not be as severe (Parvage et al., 2011).

5. CONCLUSION

The method developed using consecutive extraction of water soluble P allows insight into the desorbability of P in dependence of soil properties. Large fractions of soil P are potentially water soluble within a short time. A comparison of P fractions revealed that all resin P and partly sodium bicarbonate P is released by the method, although data are limited. Current understanding on limited P access to plants is based on the assumption, that only a small fraction of soil P is dissolved and available in soil solution. Although this is the case, this study showed a high capacity to remove soil P from exchange sites into soil solution. This result puts plant availability of P into a different perspective. It seems rational to assume, that it is rather diffusion and the physical ability of roots to gain access to P found in soil solution than the release of P to the soil solution, determining plant uptake of P.

6. APPENDIX I, METHOD DEVELOPMENT

The procedure for consecutive extraction of water soluble phosphorus is described in chapter “materials and methods”. Here, the development of the method, as well as the calculation of potentially releasable water soluble P ($P_{\text{rel-H}_2\text{O}}$) and the minimum water soluble P concentration ($P_{\text{min-H}_2\text{O}}$) is outlined.

6.1 Method principle

The consecutive P-method extraction aims to measure the potential amount of water soluble P that can be released by an unlimited number of shakings with water and was defined as $P_{\text{rel-H}_2\text{O}}$. For that purpose, 10-12 consecutive extractions with water were performed. The amounts released were added and plotted as cumulative curve. In addition, repeated extractions provide a measure for minimum concentration of P in the soil solution upon finite replenishment of the soil solution defined as $P_{\text{min-H}_2\text{O}}$, presupposing that the change is non-linear.

6.2 Soils used

Two soils differing in texture, a clay soil from Uppsala and loamy sand from Krusenberg, located in Uppsala County in Sweden were used. Both soils were taken from pastures used for horse grazing for more than 15 years (grazing density of 3.2 and 7.3 animal units ha^{-1} at Uppsala and Krusenberg, respectively). Chemical characteristics of the soils are given in Table 5.

6.3 Questions addressed

- i) Which extraction time is required to ensure that P in soil solution is in equilibrium with P in the solid phase? It was aimed to determine the shortest shaking time, which means that any prolonged shaking time would not result in an increase of P in solution.
- ii) Through which function can the cumulative release of P from soil into soil solution upon water extractions be described?
- iii) How many times does the consecutive P extraction with water have to be repeated to gain valuable information?

6.3.1 Determination of a suitable extraction time

It was assumed that equilibrium between P released from soil solids and P in soil solution can be reached within hours. This assumption was based on a calculation showing that soil solution needs to be replenished up to 20 times per day, when plants' need of P is highest (Syers et al., 2008). Experiments with isotopic dilution where soluble P was added in form of ^{33}P -isotopes showed that equilibrium was established even within minutes, defined as the mean residence time of inorganic P in solution (Frossard et al., 2011; Sinaj et al., 2002). However, in this study it was aimed to determine a maximum shaking time required to achieve a constant P concentration in soil solution. For that purpose, a clay soil and a sandy soil were shaken with water for 0.5h, 1h, 2h and 4h, respectively.

The results of the sandy soil show that shaking time longer than one hour does not result in higher P concentrations in the extract. For clay soil the finding was that, the longer the clay soil is shaken, the less P is released into soil solution (figure 10). This was not expected, since commonly a longer shaking interval is assumed to lead to more P in solution or no further change. Presumably re-adsorption of P released to new surfaces that became available during shaking is responsible for this observation.

Table 5 Chemical characteristics of the two soils used for method development; clay soil (Uppsala clay) and sandy soil (Krusenberg loamy sand): water soluble phosphorus (WSP); phosphorus in ammonium acetate lactate extract (P-AL); nitric acid digested P (total P); degree of P saturation (DPS); organic carbon (C); and total nitrogen (N); soil texture in classes clay (<0.002mm), silt (0.002-0.02mm) and sand (0.02-2mm).

Soils	WSP	P-AL	Total-P	pH (H ₂ O)	DPS	Organic C	Total N	Texture		
								(mg kg ⁻¹)		
Clay soil	5.27	244	866	7.4	54	2.99	0.27	46.6	24.1	30.3
Sandy soil	5.49	154	795	6.4	26	2.36	0.22	9.2	6.9	83.9

To define an adequate time until P concentrations in the extract are not influenced by the shaking time, the data were statistically evaluated. For the clay soil, an ANOVA analysis revealed that there is no significant difference in P concentration at each extraction step whether samples were shaken 0.5 or 1h (p=0.05). However, the concentration of P in the extract differed significantly, whether the samples were shaken 1h or 2h.

In contrast to the clay soil, the sandy soil showed a significant difference of P concentration in extracts when shaken for 0.5 or 1h and no significant difference when shaken for more than 1h until the third extraction step. Aiming to develop a method applicable for a wide range of soils, it was concluded that a 1h extraction step seemed to be ideal.

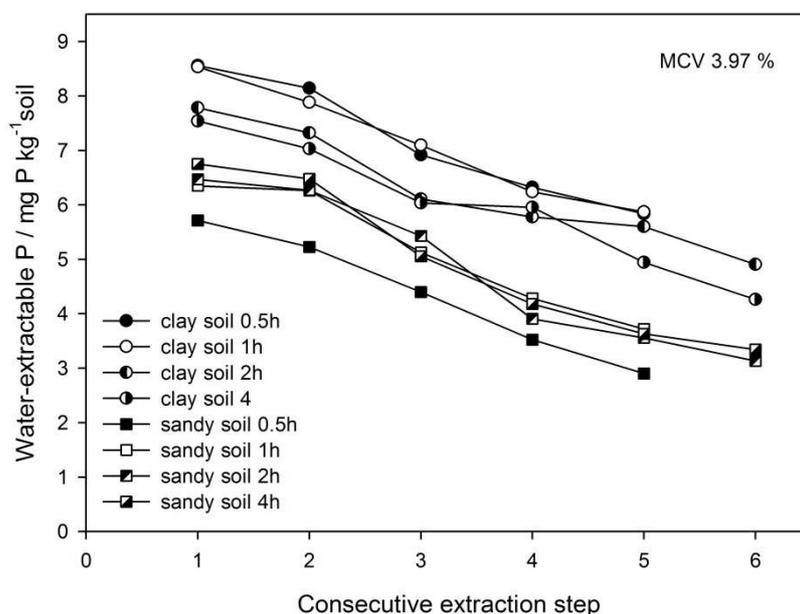


Figure 10 Content of water-extractable P after 0.5, 1, 2 and 4 hours of extraction. The variance is displayed as mean coefficient of variance (MCV) of all data, n=4 for each data point. The four upper curves represent the clay soil whereas the four lower curves represent the sandy soil, with different shaking times. Values of data can be found in table 8 in the appendix II.

6.3.2.1 Determination of total accumulated water soluble phosphorus

Once the extraction time of 1 hour was found to be suitable twelve extraction steps were run, each of them lasting one hour, see figure 11.

Data gained from the extraction procedure were treated in the following way: Firstly, the concentrations of each extraction step were added up and a curve for the incremental increase of P extracted at each consecutive extraction step was plotted (Figure 12, part 1 and 2). An exponential rise to maximum function [$f = a*(1-e^{(-b*x)})$] was fitted to the curve derived where parameter a represents the total amount of water extractable P at an infinite number of consecutive extractions. The value b is the release coefficient and x the number of extraction steps performed. Weathering processes of minerals and microbial decay of organically bound P is ignored by this method. In practice such depletion would be difficult to reach, since an infinite number of consecutive extractions is required excluding other flows and processes in soil. That is why P_{rel-H_2O} was defined as 99 per cent of the “a” value. After reaching this point, only marginal amounts of P will additionally be released with each following extractions step, compared to P release in the first extraction period. Defining P_{rel-H_2O} as 99 per cent of the a value derived from curve fitting, allows to gain an insight about how much water soluble P can be released within an realistic amount of extraction steps. Note that the amount of extraction steps required to reach P_{rel-H_2O} can be calculated easily with the formula of exponential rise to maximum.

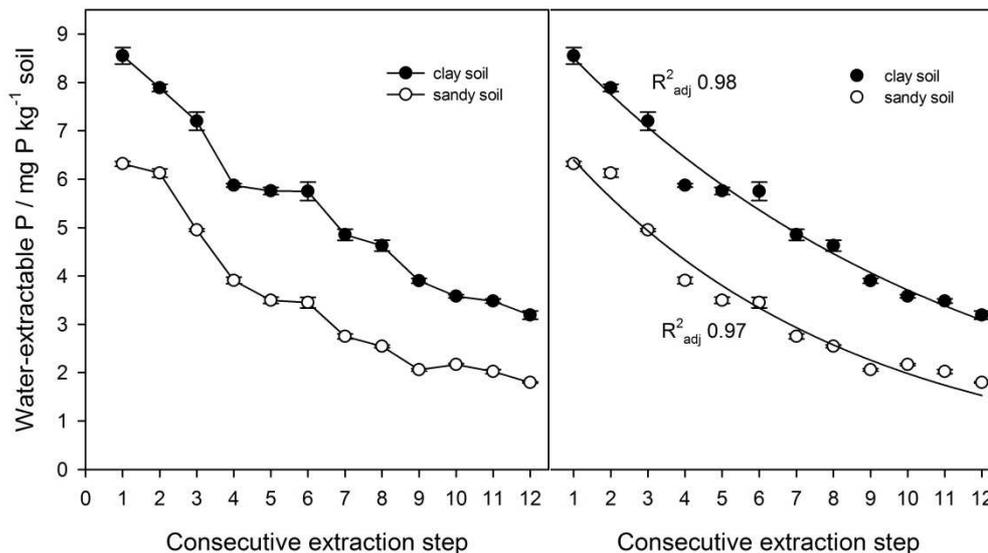


Figure 11 Concentration of water-soluble P in extracts from the clay soil and sandy soil upon twelve consecutive extraction steps of one hour each (Standard error, n=4). The left figure shows data of individual extractions whereas the right figure shows the same data with the added trend line from a curve fitting procedure, applying an exponential decay function ($f = d*e^{(-b*x)}$).

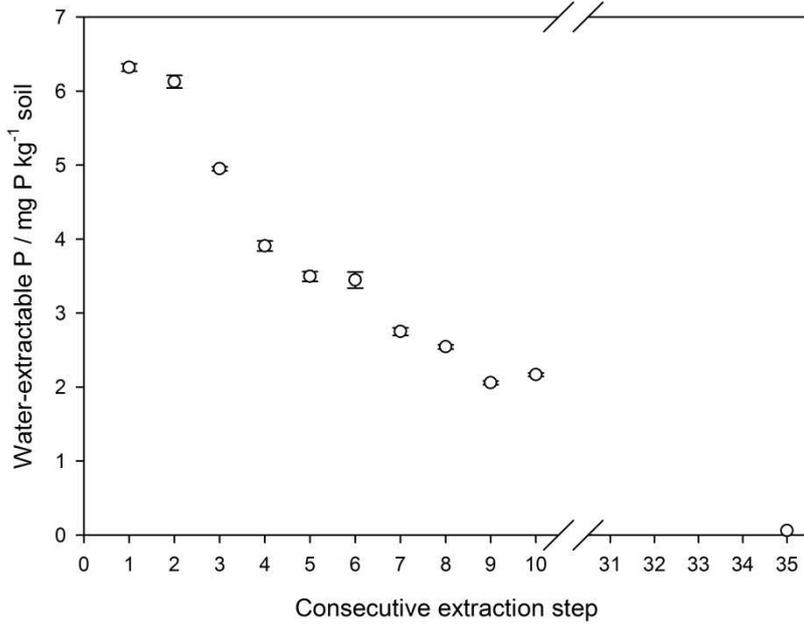
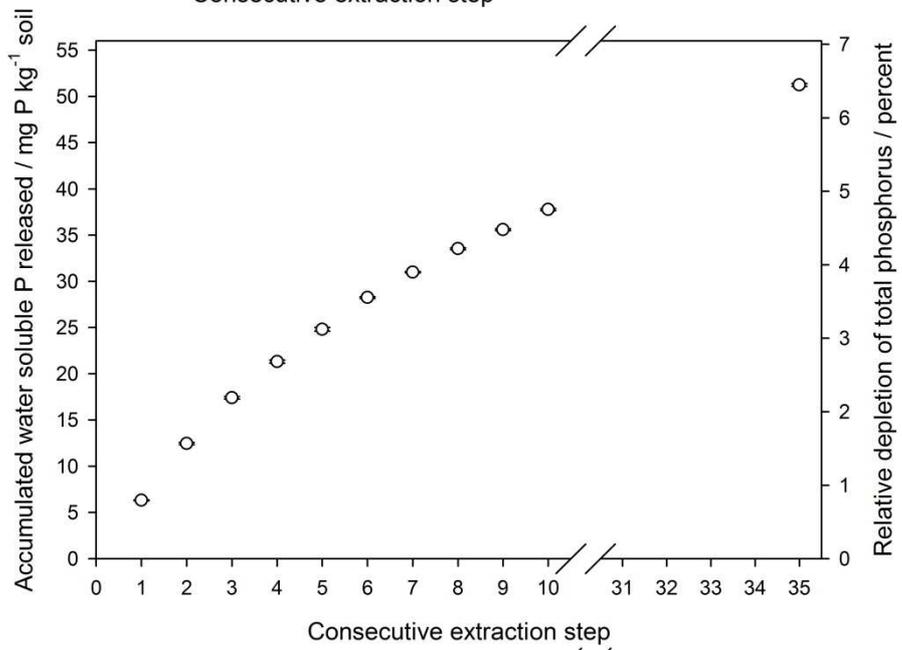
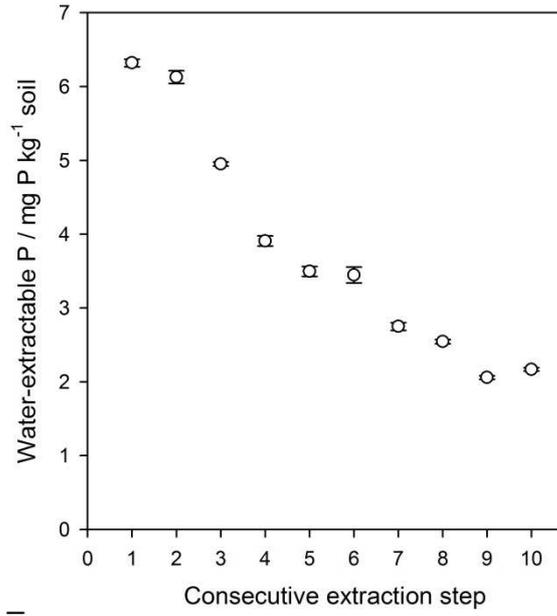
6.3.2.2 Determination of minimum concentration of water soluble phosphorus

It is also possible to estimate the concentration of constant P replenishment from soil, $P_{\min-H_2O}$. For that the concentrations measured at each extraction step are fitted with a curve, using an exponential decay function:

$$(f = d * e^{(-b * x)})$$

where d represents the hypothetical starting point or concentration of P that is potentially releasable before the first extraction step. The value b is the release coefficient and x the number of extraction steps. Applying this formula means that an infinite amount of extractions would result in total depletion of the system with P. However, this stage was not assumed to be reached in the experiment. On the other hand, it was aimed to determine a level of a low concentration of P that can be maintained in soil for a long period, even if P would be constantly removed. For that the required amount of extraction steps needed to be found. It was defined that the extraction steps needed to reach P_{rel-H_2O} are equal to the amount of extraction steps to reach the area of minimal P delivery ($P_{\min-H_2O}$), see figure 12, part 3.

Figure 12 (next page) Consecutive water-extractable P in the sandy soil, ten consecutive extraction steps for one hour each (Standard error, n=4). Part 1 shows concentrations of water-extractable P for ten consecutive extraction steps. The number of extractions steps required to reach $P_{\min-H_2O}$ is not known at this stage. Part 2 shows the accumulated amounts of P extracted at each consecutive extraction step. The value after the break ($=P_{rel-H_2O}$, or 99 per cent of “a” value; at extraction step 35) was extrapolated by curve fitting from the data using the function of exponential rise to maximum ($f = a * (1 - e^{(-b * x)})$). Part 3 shows the concentrations of water-extractable P over consecutive extraction steps as in part 1. After knowing the amount of extraction steps required to reach P_{rel-H_2O} , also $P_{\min-H_2O}$ can be determined.



6.3.3 Number of consecutive extraction steps

The number of consecutive extraction steps required to make a consistent analysis of the data was tested. It was assumed that after a certain number of extractions, release of water soluble P from soil through water extraction will become rather constant, after a declining release initially. As shown in figure 11, P release levels off and after nine or ten extraction steps, a rather stable release of P can be observed, which is most adequately described by an exponential decay function. The more extraction steps, the better the curve can be described. Data were fitted using different numbers of extraction steps. As shown in table 6, the parameters of the curve fitting procedure changed marginally with the number of used consecutive extraction steps and ten extraction steps were acceptable.

Table 6 Method development, change in R^2_{adj} values for the exponential decay function ($f = d * e^{(-b * x)}$) over the amount of consecutive extraction steps of clay soil and sandy soil used for curve fitting. The more consecutive extraction steps are made, the better is the reached R^2_{adj} value. With ten consecutive extraction steps an acceptably high R^2_{adj} value is achieved. Besides of that, also a and b values do not differ strongly. Tukey test with 95 per cent simultaneous confidence interval is given for clay soil and sandy soil where different letters indicate significant differences in the means of released P at the corresponding extraction step, n=4.

Consecutive extraction step	Clay soil				Sandy soil			
	R^2_{adj}	tukey	a value	b value	R^2_{adj}	tukey	a value	b value
12	0.980	a	9.323	0.092	0.968	e	7.288	0.130
11	0.977	ab	9.348	0.093	0.967	f	7.352	0.133
10	0.973	bc	9.379	0.094	0.969	g	7.439	0.137
9	0.965	c	9.353	0.093	0.969	fg	7.526	0.142
8	0.954	d	9.300	0.091	0.960	h	7.512	0.141

7. APPENDIX II, ADDITIONAL DATA

Here, various data gained during the analysis process, as well as data from other sources used for correlation analysis are presented.

Table 7 Chemical properties of soils used (analysis 2011); pH (soil to water ratio 1:2.5), total organic carbon (TOC), total P, P-AL (year 2011) and P input. Basic fertilization according to Kirchmann et al. (1994) of 20 kg P ha⁻¹ yr⁻¹ at all soils from Ultuna experiment, plus averaged P input by organic soil amendments, bulk density from Kätterer et al. (2011), with standard error, n=4.

Soil	pH (H ₂ O)	TOC (%)	Total P (mg kg ⁻¹)	P-AL	P input (kg ha ⁻¹ yr ⁻¹)	Bulk density (kg L ⁻¹)
Horse paddock						
Clay soil	7.4	2.99	866	244		
Sandy soil	6.4	2.36	795	154		
Ultuna experiment						
Fallow	6.4	1.03	1,050±11.2	213±6.7	20	1.43
Calcium nitrate	6.5	1.41	903±7.6	164±9.1	20	1.28
Ammonium sulfate	4.0	1.34	1,045±9.4	164±5.2	20	1.21
Green manure	5.9	1.67	1,061±8.2	206±6.8	20+27	1.34
Peat	5.4	2.79	1,079±18.2	181±8.4	20+4	1.12
Farmyard manure	6.5	2.04	1,270±11.6	347±10.1	20+73	1.24
Sewage sludge	4.8	2.80	4,899±97.4	547±12.9	20+348	1.02

Table 8 Method development, finding of adequate shaking time. Clay soil and sandy soil were shaken for various lengths of 0.5, 1, 2 and 4 h. Values represent mg P kg⁻¹ soil, with standard error, n=4. Data are shown in figure 10 in appendix I.

	Consecutive extraction step					
	1	2	3	4	5	6
	0.5h	1.0h	1.5h	2h	2.5h	
Clay 0.5h	8.56±0.14	8.14±0.05	6.92±0.08	6.32±0.11	5.83±0.13	
Sand 0.5h	5.71±0.03	5.23±0.13	4.40±0.06	3.52±0.05	2.90±0.07	
	1h	2h	3h	4h	5h	
Clay 1h	8.53±0.11	7.88±0.21	7.10±0.07	6.24±0.23	5.87±0.11	
Sand 1h	6.35±0.06	6.26±0.10	5.12±0.13	4.28±0.04	3.72±0.04	
	2h	4h	6h	8h	10h	12h
Clay 2h	7.78±0.18	7.32±0.08	6.11±0.37	5.78±0.15	5.60±0.14	4.91±0.04
Sand 2h	6.47±0.04	6.27±0.06	5.43±0.36	5.90±0.05	3.55±0.04	3.13±0.05
	4h	8h	12h	16h	20h	24h
Clay 4h	7.54±0.15	7.03±0.08	6.03±0.09	5.95±0.27	4.94±0.11	4.26±0.11
Sand 4h	6.75±0.20	6.48±0.11	5.06±0.14	4.18±0.11	3.63±0.07	3.34±0.07

Table 9 Average pH values (soil to water ratio = 1:5) of treatments of the Ultuna experiment at the corresponding extraction step, with standard error, n=4. At empty cells values were not measured.

Soil	Extraction step									
	1	2	3	4	5	6	7	8	9	10
Fallow	5.68±0.06	5.71±0.06			5.56±0.05	5.48±0.03			5.37±0.09	5.47±0.1
Calcium nitrate	5.92±0.07	6.05±0.04			5.99±0.03	5.94±0.02			5.73±0.07	5.81±0.08
Ammonium sulfate	3.95±0.03	4.38±0.03			4.66±0.03	4.78±0.04			4.83±0.05	4.73±0.04
Green manure	5.47±0.06	5.62±0.06			5.71±0.03	5.73±0.02			5.49±0.05	5.46±0.04
Peat	4.98±0.04	4.12±0.07	5.08±0.03	5.42±0.06	5.53±0.08	5.21±0.09			5.25±0.09	5.36±0.14
Farmyard manure	6.44±0.19	6.51±0.14			6.45±0.13	6.34±0.07			6.11±0.13	6.08±0.07
Sewage sludge	4.47±0.08	4.62±0.04	4.73±0.03			4.78±0.01	4.84±0.03	4.82±0.02	4.82±0.02	4.80±0.03

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