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An assessment of soil P extractability and plant P availability for a resin-based (DGT) and batch extraction methods

Master Thesis

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Abstract

The use of mineral phosphate fertilizers constitutes an integral part of modern agriculture. Due to the limited availability of mineral phosphate resources (“peak phosphorus”), it becomes evident that a more sustainable use of phosphorus (P) fertilizers is urgently needed. Furthermore, economical and especially the ecological implications of its excessive use are profound. Optimization of P fertilization is largely depending on the accurate determination of plant-available phosphorus in the soil. Nowadays numerous measurement techniques are in use in different countries and it is challenging to compare them to each other. The basic principle of these techniques is the extraction of soil P with the help of chemical reagents. The amount of P extracted with these techniques often showed a weak correlation with plant P concentrations and/or yield.

To improve the determination of plant available phosphorus in agricultural soils and the derived fertilizer recommendations, the establishment of a new sampling method is highly required. This work attempted to clarify if the diffusive gradients in thin films technique (DGT) is a suitable means to predict plant available P in a wide range of soils. Another aim was to unravel the influence of certain soil characteristics on the extractability of P with the use of the DGT technique in comparison to conventional extraction techniques.

Therefore, 41 Austrian soil samples covering a large variety of soil types and P concentrations were investigated with the following methods: Diffusive gradients in thin films (DGT), Soil porewater concentration, Calcium acetate lactate (CAL), Water extraction (H_2O), Sodium bicarbonate (Olsen), Mehlich 3, Electro-Ultrafiltration (EUF), Acid Ammonium Oxalate in Darkness (AAO), Sodium Citrate-Bicarbonate-Dithionite (CBD), Total P (*aqua regia).* While it was not productive to compare the amount of phosphorus extracted by the various methods, correlations of varying degrees were found between them. DGT displayed a strong relatedness not only with the other weak extraction methods but also with some of the stronger ones. Significant correlations with all soil P tests except CAL could be observed for the soil parameters pH, texture and Total P content which indicated a strong influence of these factors on the general P extractability.

A plant experiment was conducted in order to compare the correlation between the established methods and DGT with regard to plant P uptake. The results did not indicate a clear superiority of DGT over the other techniques as had been reported previously by various other authors. The performance of DGT appeared to be strongly dependent on certain soil characteristics, especially on the P sorption capacity. However, it proved to be a solid method that can be used over a wide range of soils.

Zusammenfassung

Die Verwendung von mineralischen Phosphat-Düngern stellt einen integralen Teil moderner Landwirtschaft dar. Aufgrund der beschränkten Verfügbarkeit von mineralischen Phosphat-Ressourcen („peak-Phosphor“) ist eine nachhaltigere Nutzung dringend erforderlich. Außerdem sind die ökonomischen und vor allem die ökologischen Auswirkungen bei exzessiver Anwendung schwerwiegend. Um die Optimierung der P-Düngung zu gewährleisten, ist eine korrekte Bestimmung des pflanzenverfügbaren Phosphors im Boden unerlässlich. Heutzutage kommen in verschiedenen Ländern zahlreiche unterschiedliche Bestimmungsmethoden zum Einsatz und ein direkter Vergleich ist schwierig. Das Grundprinzip dieser Techniken ist die Extraktion von Boden-Phosphor mittels chemischen Reagenzien. Der damit extrahierte Phosphor zeigt oft nur eine geringe Korrelation mit den P-Konzentrationen in der Pflanze und/oder mit dem Ertrag.

Um die Bestimmung von pflanzenverfügbarem Phosphor in landwirtschaftlich genutzten Böden und die daraus ableitbaren Düngeempfehlungen zu verbessern, ist die Etablierung einer neuen Testmethode dringend erforderlich. Das Ziel dieser Arbeit war die Feststellung ob die “Diffusive gradients in thin films“ (DGT) Technik geeignet ist, um den pflanzenverfügbaren Phosphor in einer großen Anzahl von Böden mit unterschiedlichen Eigenschaften vorherzusagen. Ein weiteres Ziel war die Ermittlung des Einflusses, den bestimmte Bodeneigenschaften auf die Phosphor-Extrahierbarkeit mit DGT im Vergleich zu konventionellen Extraktionsmethoden ausüben.

Zu diesem Zweck wurden 41 Bodenproben aus Österreich, die eine große Vielfalt an Bodenarten und Phosphor-Gehalten abdeckten, mit den folgenden Methoden untersucht: Diffusive gradients in thin films (DGT), Bodenporenwasser-Konzentration, Calcium Azetat-Laktat (CAL), Wasser-Extraktion (H_2O), Natriumhydrogencarbonat (Olsen), Mehlich 3, Elektro-Ultrafiltration (EUF), Ammonium Oxalat (AAO), Natrium Citrat-Bicarbonat-Dithionit (CBD), Gesamt-P (*aqua regia*). Ein direkter Vergleich der extrahierten P-Mengen der verschiedenen Methoden erwies sich als nicht zielführend, dafür wurden Korrelationen unterschiedlichen Ausmaßes zwischen den Methoden festgestellt. DGT zeigte nicht nur enge Beziehungen mit den anderen schwachen Extraktionsmethoden, sondern auch mit einigen der starken. Signifikante Korrelationen zwischen den Bodenparametern pH, Textur und Gesamt-P mit allen Boden-Phosphortests ausser CAL wurden festgestellt, was auf einen starken Einfluss dieser Faktoren auf die generelle P-Extrahierbarkeit hindeutete.

Um die Korrelation zwischen DGT und den etablierten Methoden in Hinblick auf die P Aufnahme durch Pflanzen zu vergleichen, wurde ein Pflanzenversuch durchgeführt. Die Ergebnisse deuteten auf keine klare Überlegenheit der DGT-Technik im Vergleich zu den anderen Methoden hin; eine solche war zuvor von mehreren Autoren berichtet worden. Das Abschneiden von DGT schien stark von bestimmten Bodeneigenschaften abhängig zu sein, ganz besonders von der P-Sorptionsfähigkeit. Dennoch erwies sich DGT als eine solide Methode, die für Böden mit unterschiedlichsten Eigenschaften eingesetzt werden kann.

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1. Introduction

1.1. Phosphorus - A crucial element

Phosphorus (P) is considered to be the second most important soil nutrient after nitrogen for the growth and development of plants (Zheng and Zhang 2012). P is involved in central physiological processes such as energy transformation, plant metabolism and structure (Zheng and Zhang 2012) and has a distinct importance in the development of young tissues. Within those, phosphorus promotes root growth, flowering, fruiting and seed formation (Smil 2000). Inside the plant, it exists in various forms such as phosphate ester within phospholipids, nucleic acids and phytin. If a plant lacks P, the symptoms will include inhibition of growth of shoot, leaves and roots. On older leaves, often a reddish discoloration and to some extent chlorosis and necrosis can be observed (Blume et al. 2010).

On planet earth, P occurs in different geological fractions. For example, the earth´s upper crust contains about 0.1 % (Cordell and White 2011). Rocks are the biggest global storage with 10^{13} Tg, followed by soils and seas (2 respectively 1.10^{5} Tg) and the terrestrial biomass (3.10^{3} Tg). The phosphorus contained in rocks consists of phosphates which predominantly occur as apatites. These minerals are the parent material of the soil phosphates as well as the source material for phosphate fertilizers (Blume et al. 2010). Apatite contains about 95 % of all the P in the earth´s crust and is poorly soluble. Within the soil also more soluble forms of P are usually quickly transformed into insoluble forms and thus become fixed (Smil 2000). Phosphorus in soils exists exclusively as orthophosphate and by far the largest portion occurs in ligated form. The part which can be taken up by plants exists in the soil solution and comprises only less than 0.1 % of Total P (Blume et al. 2010). Due to its nature, it is one of the most limiting essential nutrients in agricultural crop production (Ziadi et al. 2013; Cordell and White 2011).

For that reason, agricultural production relies heavily on the use of P fertilizer. Smil (2000) reports that now the applications of inorganic P fertilizers derived from phosphate rock amount to an estimated 13 - 16 Mt per year. There are several major issues concerning the dependency of agricultural production on fertilizer-derived P. Some of the most important involve the increasing costs of mining and processing of phosphate rock while the demand for P fertilizer is rising every year. The quality of the mined phosphate rock is constantly decreasing and causes problems due to its ever higher impurities caused by cadmium and uranium. Another major issue of a geopolitical dimension is the highly skewed occurence of phosphorus reserves in the world. Approximately 95 % of the remaining reserves are under the control of only 5 countries; Morocco, USA, China, South Africa and Jordan. This tilt is outperformed by the fact that Morocco alone controls about 85 % (Cordell and White 2011). Another important issue that needs attention is the mismanagment of this critical resource; according to Cordell and White (2015) there are huge losses and inefficiencies in the chain that spans from the mines to the fields and finally to the plates of the human population. They claim that only about one-fifth of the phosphorus mined specifically for food production reaches its final destination. Additional to these quality, economical, geopolitical and management issues the environmental implications of fertilizer P use in agriculture have to be considered. The most obvious and critical one is the eutrophication of water bodies such as lakes, rivers and seas. It can be caused, amongst other reasons, by an inefficient application of P fertilzer and the followed runoff into water bodies (Cordell and White 2015). Eutrophication causes a severe oxygen deficiency in water bodies due to a boosted growth of aquatic life-forms and can lead to dead zones (Liu et al. 2008; Cordell and White 2011).

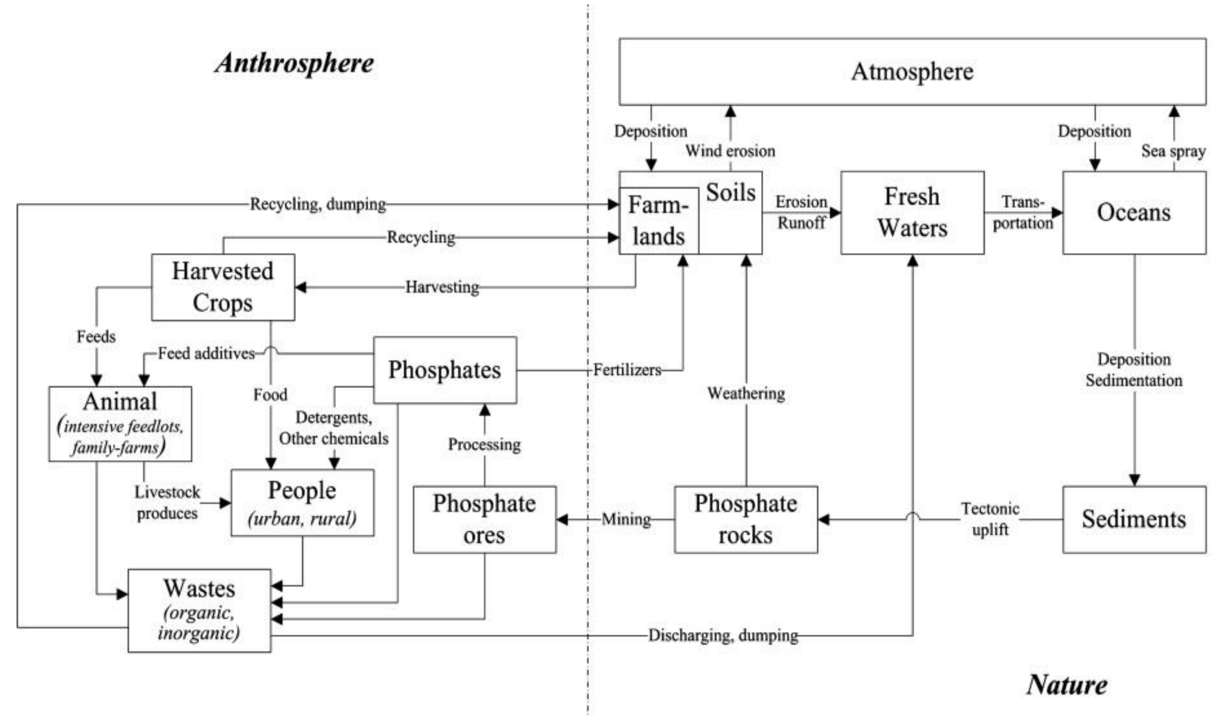


Figure 1: The human-intensified global phosphorus cycle (Liu et al. 2008).

As pointed out above, the agricultural production is heavily dependent on inputs from P fertilizer and therefore on the availability of phosphate rock. The central question to consider is: how long will the reserves last? There is an intense debate concerning this issue. According to Cordell and White (2011) the estimates range between 30 - 300 years. This broad range is because of a lack of publicly available data and substantial uncertainty about various factors that impact this subject. In context to remaining reserves the concept of Peak Phosphorus has gained attention during the last decades. The basis of this concept lies in the idea that growing demand for phosphate rock will reach a point where it outstrips the economically available supply. Advances in technology and efficiency are also included in the evaluation (Cordell and White 2011). This implies that shortages in supply will occur long before this ressource is completely depleted. Cordell and White (2011) state that one of the models most authors refer to and which was fed with data derived from the US Geological Survey and industry predicts the peak for around the year 2033. While it may be impossible to predict the exact year because of too many unpredictable factors, it is clear that decisive actions need to be taken in order to allow for a smooth transition through that phase. One of these actions needs to include the enhancement of agronomical soil P testing methods in order to allow for a better prediction of plant needs and hence for a much more efficient use of P ressources.

1.2. Agronomical P-testing

The purpose of agronomical testing is to obtain knowledge about the soil P status of a soil and subsequently provide recommendations to the farmer whether it is advisable to add nutrients in the form of various fertilizers. Adding fertilizers only makes sense if a crop response will be generated. The procedure involves the extraction of a certain amount of P by a particular technique. The extracted amount of P is proportional to that which can be taken up by the crop during the growing season. As today there are many different methods in use, it is absolutely essential that any such method is calibrated for certain regions with similar soils, cropping systems and climatic conditions. When this principle is being followed, recommendations can be given using crop response data that has been generated empirically (Pierzynski 2000). Furthermore, it is crucial to attain knowledge about how particular soil tests relate to the different soil P pools and processes of P supply. Then it appears feasible to apply this knowledge in order to understand which factors are responsible for P availability in particular soil - crop - climate situations (Moody et al. 2013).

1.2.1. Different P-pools

In the soil, P typically exists as orthophosphate and as such mostly in a ligated form. The P content of a soil usually increases from the sand- to the clay-fraction and with rising humus-content. The P which becomes released from the apatitic bedrock over time through the pedogenic process can take different pathways. It can either get fixed by constituents of the clay-fraction (oxids, clay-minerals), it may precipitate as newly formed (secondary) mineral or it becomes incorporated into humic matter. Hence, phosphorus in soils can be found in many different fractions which can be assigned to different “pools“. Four main pools can be distinguished: mineral P, sorbed P, organic P and solution P (Figure 2); (Blume et al. 2010; Moody et al. 2013).

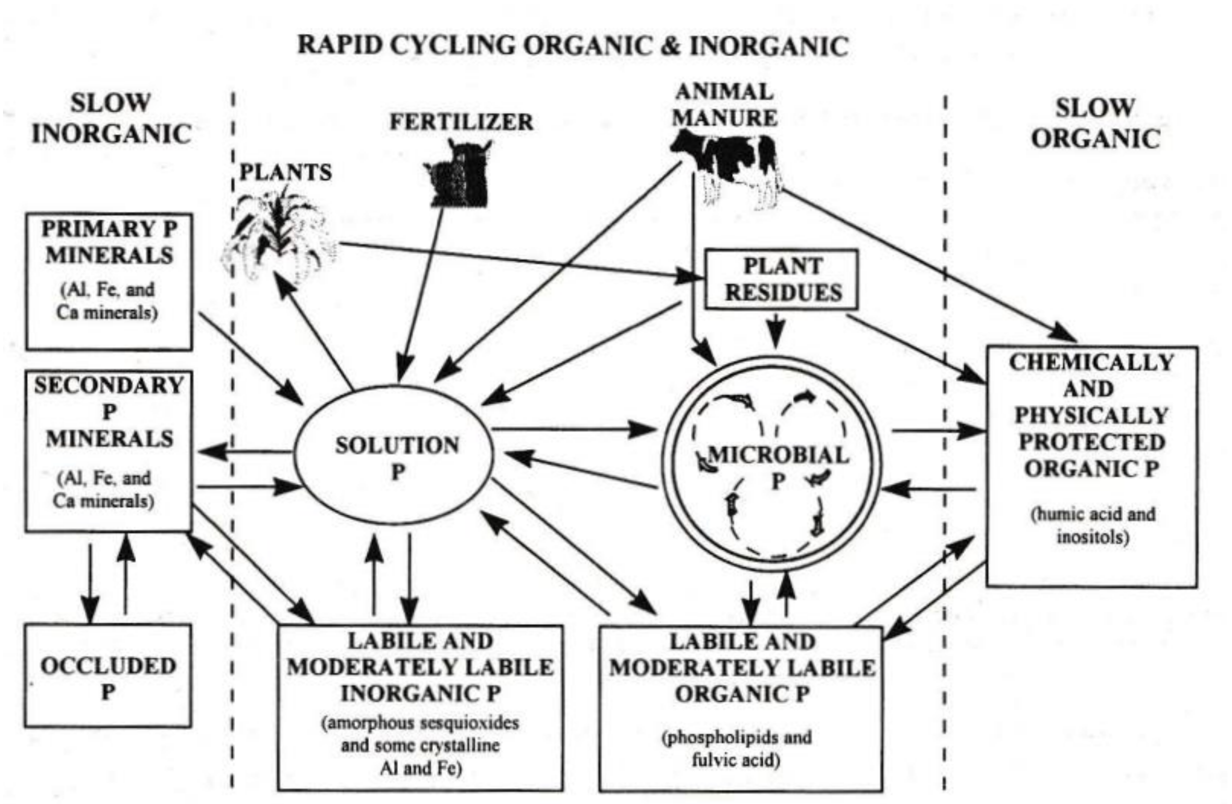


Figure 2: The soil P cycle: its components, forms and flows (Sumner 1999).

Mineral P forms include constituents of diverse specific surface areas and structural organisations, which can range from amorphous to crystalline. The anions of orthophosphoric acid (H_3PO_4) possess a high affinity towards Ca_2^+-, Al_3^+-, Fe_3^+-, and Fe_2^+-ions. With these, they form hardly soluble phosphates in soils. As mentioned above, apatite, being a tri-calciumphosphate, is one of the most important P-sources. The cause of the formation of these mineral P forms may be pedological processes or the addition of P fertilizers to the soil, which then form sparsely soluble reaction products with various soil constituents (Pierzynski 2000; Blume et al. 2010; Moody et al. 2013).

Sorbed P includes the P in soils that is adsorbed onto surfaces of other soil components by electrostatic and covalent bonding. The most important sorbents are Fe- and Aloxyhydroxides, Ca-carbonates (Moody et al. 2013) and clay minerals (Wuenscher et al. 2015). Furthermore, Fe and Al that is ligated in humic matter can act as a sorbent. In central-european soils the constituents most decisive for P sorption are Fe-oxides and Fe- and Al-organic compounds. In tropical soils, the P contained in apatitic bedrock with the help of weathering became transformed completely into P bound to Fe- and Al-oxides. The content of those is high and therefore a high capacity of P sorption and fixation in tropical soils is prevalent. This often results in a lack of (available) P in these kind of soils. The sorption of P is highly pH-dependent; it rises multiple times when the pH decreases from 9 to 2. One main reason for this pH-dependancy can be explained by the sorption characteristics of Fe- and Al-oxides. Moreover, the availability of readily soluble P derived from fertilizers can be substantially lowered due to its sorption on oxide-surfaces (Blume et al. 2010).

The organic P fractions in soil are diverse; they comprise phospholipids, nucleic acids, phosphoproteins, inositol phosphates, different sugar phosphates, phytates and various compounds that have not been identified. The aforementioned fractions are part of microorganisms (microbial biomass), plant residues and soil organic matter. Phytates (occuring in plant residues and soil organic matter) account for more than 50 %, providing the most important form of organic P in soils. The percentage of organic P in fertilized A-horizons of inorganic soils typically lies between 5 - 35 %. This percentage decreases correspondingly to humus content in deeper soil layers (Pierzynski 2000; Blume et al. 2010; Moody et al. 2013).

Solution P denominates the share of phosphorus that is present in the soil solution. The predominant species are $H_2PO_4^-$ and HPO_4^{2-} ions and these are also the P forms that get taken up by plant roots most easily. The dominance of either of those two forms is dependent on the pH; from 2.1 - 7.2 $H_2PO_3^-$ prevails and from 7.2 - 12.0 it is HPO_4^{2-}. Another part of the dissolved phosphate usually consists of organically bound P. In topsoils rich in humus this fraction can constitute 20 - 70 % of total dissolved P. The phosphate concentration of the soil solution usually varies from 0.001 - 0.1 mg P L^{-1} in unfertilized soils and from 0.1 - 5 mg P L^{-1} in fertilized A-horizons. The percentage of the P in soil solution when compared to Total P usually amounts to less than 0.1 %. For optimal plant yields a concentration of 0.3 - 0.8 mg P L^{-1} is considered essential (Blume et al. 2010).

1.2.2. Plant-available P (bioavailability)

The total amount of mineral elements in soil stands in no direct context with their availability for plants (Degryse et al. 2009). Therefore, with the help of applied soil P tests, plant available P needs to be determined. As stated above, the requirements for P differ, depending on crop and availability in soil. Plant available P is a theoretical concept which focuses on the P reserves in a certain soil that should be utilizable by a plant during its life cycle and it usually constitutes only a very small part of the total P. (Schnug and De Kok 2016). Adding to this, Tandy et al. (2011) stated that plant available P was a combination of intensity, quantity and buffering capacity of soil. This concept can be refered to as the I-Q concept and often gets applied in context to plant available P.

Intensity (I) is related to the concentration or rather activity, which denominates the effective concentration of the chemical species of a nutrient in the soil solution. (Blume et al. 2010). Therefore, intensity describes the concentration of P in the pool that is directly available to plants (Nawara et al. 2017). Quantity (Q) refers to the reserve of a nutrient contained in the particulate matter in the root zone which can be mobilised during a growing season (Figure 3); (Blume et al. 2010). More specific, quantity denotes the P which is reversibly adsorbed on the solid phase and that which exists in labile precipitates. The concentration of P in the soil solution is directly linked to the P in the solid phase; in particular to the phosphate adsorbed to Al- and Fe-(hydro)oxides and fresh precipitates of Ca-phosphate. P from these compounds of the solid phase is released when plants take up P from the soil solution. The Q-pool therefore exerts control over the P concentration in the soil solution and acts as a buffer for the P in soil solution (Schnug and De Kok 2016).

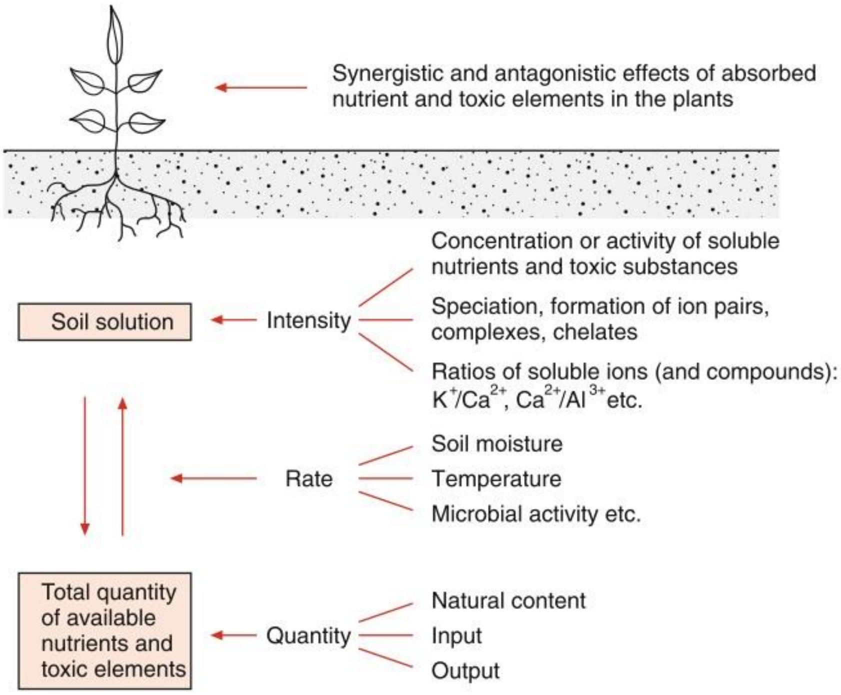


Figure 3: Factors influencing nutrient and pollutant availability in soils (Blume et al. 2015).

An equilibrium exists between the orthophosphate in the soil solution (I) and the other pools (Q). When this balance gets disturbed due to processes like e.g. plant P uptake or P fertilization, the different pools get affected by various reactions. For the mineral P pool in the soil these reactions consist of dissolution and precipitation, for sorbed P there are desorption- and sorption-reactions and P connected to the organic pool can be mineralized or immobilized (Moody et al. 2013). When the plant root takes up P from the surrounding soil solution it provokes a diffusion reaction (Schnug and De Kok 2016). The extent of this diffusion depends on parameters like the concentration gradient between the root surface and the bulk soil solution and furthermore on the volumetric soil water content. Tortuosity is also decisive for diffusion rates; it takes into account the connectivity of water films in soil pores and the P buffer capacity (Moody et al. 2013). Subsequently, diffusion causes the desorption or dissolution of P from the solid phase (Schnug and De Kok 2016). It was reported by Nawara et al. (2017) that diffusion holds a key role in P uptake of plants while (Mollier et al. 2008) put forward that it was found out through model-based investigations that also *mass flow* is involved. Nawara et al. (2017) further claimed that therefore the P uptake is influenced more strongly by the parameter intensity (I) than quantity (Q). However, also the buffer capacity of a soil exerts a big influence on the bioavailability as it represents the capacity to mobilize P that is not immediately available. Crucial for this parameter are the number of sorption sites and the energy of adsorption as these determine the amount of P that can be adsorbed or desorbed from surfaces (Moody et al. 2013).

The I-Q concept allows for a good predicition of plant available P but also holds some flaws. For instance, it does not take into account the processes that take place in the rhizosphere or the role of P mineralization for P bioavailability (Nawara et al. 2017). It is well known that plant roots influence their surroundings through the release of HCO_3^-, CO_2 and other organic compounds (Schnug and De Kok 2016).

Many different methods are being used for the determination of plant available P. In Europe alone, over 10 different extraction techniques are applied in routine soil testing for P (Nawara et al. 2017). The main purpose of those techniques is to transfer the P from the soil to the liquid solution in order to subsequently measure the comprised amounts. Each method accesses different P-pools and therefore the amounts of P extracted from one and the same soil differ depending on the method implemented. The vast majority is based on the principle of mixing a defined amount of soil with a standard amount of extractant and the intermixture of those two components through shaking for a certain period. As mentioned above, it is decisive that the generated data are calibrated against the actual uptake of plants under specific conditions in order to allow for later interpretations of the results and derived recommendations out of it (Schnug and De Kok 2016).

1.3. Widely applied P-testing-methods

Since no universally applicable soil P test exists, (Schnug and De Kok 2016; Moody et al. 2013; Tandy et al. 2011) many different methods are applied in routine soil testing. Hence it is not surprising that depending on the country, different tests are defined as “standard P test“. For instance, the CAL method depicts the standard in Austria and Germany, Mehlich 3 is the routine method in parts of the USA and Estonia while Olsen is the standard technique applied in the UK, Denmark, Israel and parts of the USA. Usually the particular method in use was chosen due to its suitability for the majority of the soils in a country (Schnug and De Kok 2016). Nevertheless, other reasons like costs or the convenience of a method might also play a role.

According to Sibbesen (1983), most of the solution based P testing methods in use today rely on one of the following four mechanisms or their combinations:

1) Disturbance of the equilibrium between solution P and soil P; this is achieved by an increase of the solution-to-soil ratio (in order to decrease the concentration of P in the solution)

2) Acid dissolution (through the use of dilute acids, ph-buffers or dilute bases)

3) Cation complexation and cation hydrolysis

4) Anion exchange; phosphate becomes desorbed and/or the re-adsorption of already desorbed phosphate becomes limited

All of those methods are based on the concept of dissolving a part of P from the solid phase with the help of a defined extractant. It is well established that in general acidic extractants are more suitable for acidic soils and basic extractants for calcareaous soils (Schnug and De Kok 2016). Two of the main advantages of the available methods is that a wide range of different soils can be covered and many different elements can be measured and their amounts determined (Jordan-Meille et al. 2012). The solution based techniques differ in their mechanisms of extraction but tend to correlate even despite the fact that the extracted P amounts vary considerably (Schnug and De Kok 2016).

Besides the advantages of the solution based extraction methods there are also various associated problems. For instance, usually there is a weak correlation between the extracted P by a method and the plant uptake. Even more so, if a wide range of different soil types are analyzed (Tandy et al. 2011). Due to the strong nature of some extractants, P which would not be acessible to plants gets dissolved. Contrary with weak extractants; less P may be dissolved than a plant could access. Therefore, depending on the method used, it may come to an over- or underestimation of effectively plant available P. Furthermore, the methods in use exhibit a poor theoretical background and often lack a scientific rationale (Jordan-Meille et al. 2012).

It is not only the results of the individual methods that diverge from each other. According to Jordan-Meille et al. (2012) there is also much variation in the interpretation of results of one method between different laboratories and the fertilizer recommendation schemes between countries. Their study displayed more than 3-fold differences in terms of fertilizer recommendations for one and the same soil-crop system tested. Hence, a comparison between countries and a european standardization of P fertilizer recommendation currently appears to be impossible.

The lack of accuracy of the methods in use today may oftentimes lead to over- or underfertilization and hence causes unsatisfactory crop yields, unnecessary negative environmental effects or additional costs (Tandy et al. 2011). This is because the established techniques do not mimic the entire process of P uptake by plants in agricultural soils (Nawara et al. 2017). In the field of research, for reasons of comparability and for the purpose of practical, accurate fertilizer recommendations it would therefore be necessary to establish a new standard method for P soil testing. One method that could meet these demands is the so called DGT technique (diffusive gradients in thin films). The DGT method acts on the principle of an infinite sink and imitates a plant root. It incorporates the central parameters for the determination of plant-availaible P in soils; as stated above these are intensity, quantity and buffering capacity (Tandy et al. 2011).

1.4. Diffusive gradients in thin films (DGT) as agronomical P-test

1.4.1. What is DGT

DGT stands for „Diffusive gradients in thin films“. It is based on hydrogels attached to a plastic device that can be used in numerous ways, e.g. for the measurement and evaluation of certain chemical elements in sediments, waters or soils. Within the standard DGT sampling unit, three different layers are arranged: a binding gel (resin) layer, in which the elements under investigation accumulate; a diffusive gel layer, through which the elements diffuse and a membrane filter (Figure 4). There are various versions of binding gel layers which are being fabricated according to the requirements of the analytes, be it organic compounds, metals or nutrients. One feature of the DGT is that it can be used to predict bioavailability (Degryse et al. 2009). For investigations about P availability in soils a ferryhydrite gel-binding layer is applied, which acts as an inifnite sink (Schnug and De Kok 2016).

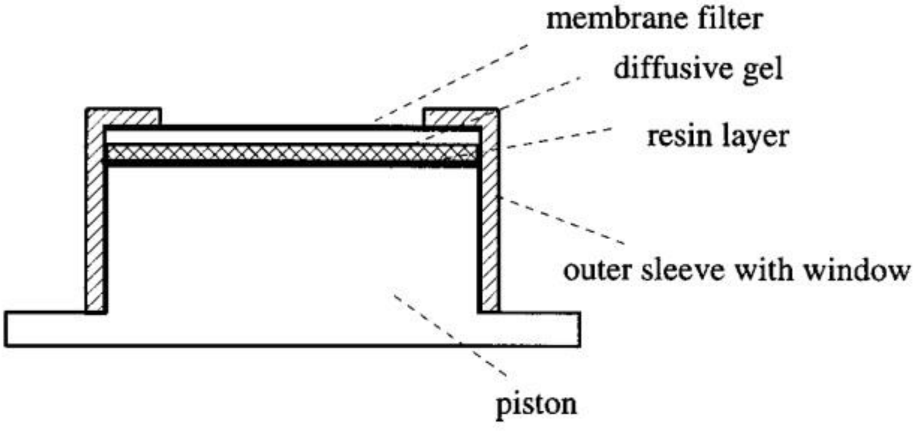


Figure 4: Schematic representation of a section through the DGT holder (Zhang et al. 1998).

1.4.2. Underlying principles

With DGT, it is possible to measure the content of various elements in soils. When the soils are wetted in order to allow for diffusion it becomes possible to quantitatively measure labile element-species in these soil-solution-systems.

The underlying principle of the DGT-method refers to Fick´s first law of diffusion. It can be applied if the capacity of the resin is not exceeded and the concentration of ions on the surface of the binding layer is maintained at effectively zero throughout the time of measurement. In this way, a steep concentration gradient (Figure 5) gets established, which drives the DGT-system. If these conditions remain constant during testing, the flux (F) of ions diffusing first through the diffusive boundary layer (DBL) and then through the diffusive gel into the resin can be calculated using Eq1:

(1) $F = DC_{DGT} / \Delta g$

where D stands for the molecular diffusion coefficient, C_{DGT} for the investigated element in bulk-solution and Δg for the thickness of the the ion-permeable hydrogel membrane (diffusive gel).

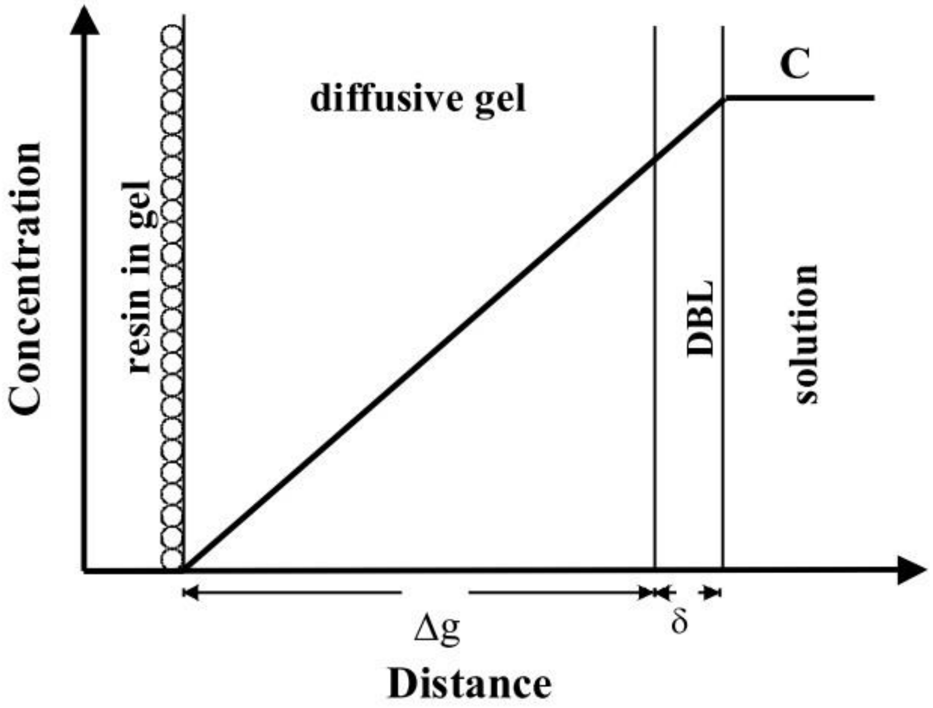


Figure 5: Schematic cross-section through a DGT device in contact with solution, showing the steady-state concentration gradient. The diffusive layer is shown as a single layer of gel, but it can include a gel layer and filter. The thickness of the diffusive boundary layer (DBL) in solution depends on the rate of water movement.

The design of the DGT with its different layers makes sure that ions reach the binding layer solely by free diffusion. The concentration of the element under investigation in the bulk-solution, C_{DGT}, can be determined with the help of a calculation. When the thickness of the ion-permeable hydrogel membrane Δg is known, the following formula (2) can be applied:

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

where M is the measured mass of the element accumulated within the resin of the binding layer, D is the molecular diffusion coefficient, t stands for the deployment time and A is the exposure surface area of the membrane.

M can be determined with the use of various techniques, one of these being ICP-OES. D can be identified with the help of a table referring to the investigated element and the prevalent temperature during deployment. A is a fixed constant on all DGT-devices (Zhang and Davison 1995; Zhang et al. 1998).

1.5. Research questions and objectives of the thesis

As stated above, no universally applicable soil test for phosphorus exists and there is no method that shows a clear superiority above others. Nawara et al. (2017) argued that various studies performed on tropical soils showed a clear superiority of DGT compared to conventional methods with reference to the prediction of plant growth. They performed a study with 218 soils, compared 5 different soil tests and found out that on european soils DGT was not superior to the other methods in predicting plant-available P. It was concluded that the strongly differing soil properties of tropical and european soils were responsible for this result.

Currently no study about the performance of DGT in comparison to other extraction methods and in relation with plant-uptake of P exists for austrian soils. The aim of this investigation was to identify wether DGT is a suitable method for determining the plant-available P over a wide range of austrian agricultural crop land and its performance in comparison to established standard extractions. Furthermore, we wanted to find out which soil parameters exhibit the strongest influence with reference to the P extractability by the various methods and if DGT performs better than the other methods over a wide range of different soils. Therefore, the main research questions of this study were:

1. How does DGT-extractable P compare to P-extractability of conventional soil tests?
2. How do soil characteristics affect the extractability of P by soil P-tests?
3. How well do the evaluated soil P tests predict plant biomass and plant P uptake in early dry matter of *Secale cereale* L.?

2. Materials & Methods

2.1. Investigated soils – 41 Austrian soil samples

In order to evaluate the suitability of the DGT method as a standard technique, we investigated 41 soil samples that were taken from Austrian cropland. Importance was given to the fact that the samples covered a wide range of different soil types with diverse characteristics. The samples were taken predominantly from the eastern parts of Austria (Lower Austria, Carinthia) and Salzburg in the year 2016.

2.2. General soil characteristics

The measurement of pH was done with 0.01 M $CaCl_2$ according to OENORM L1083-89, the carbonate content ($CaCO_3$) was determined using the so-called “Scheibler method“ following the protocoll of OENORM L1084. Total nitrogen (N) and total carbon (C) were determined by dry combustion according to OENORM L1080. With that data at hand, it was possible to calculate the C/N-ratio. Organic carbon (C_{org}) was calculated as $C_{org} = C_{total} - C_{carbonate}$. The CEC was determined following the protocoll of OENORM L1086-89, using 0.1 M $BaCl_2$ solution. Amorphous fractions of Fe- and Al-oxides were determined with the method referred to as “Acid Ammonium Oxalate in Darkness (Tamm´s reagent)“ as described in *Methods of soil analysis. Part 3. Chemical methods* (Sparks 1996). In order to predominantly extract the cristalline fractions of Fe- and Al-oxides, the Sodium Citrate-Bicarbonate-Dithonite method as described in *Methods of soil analysis. Part 3. Chemical methods* (Sparks 1996) was conducted. Total phosphorus (P) content was determined with the help of *aqua regia* as described in 2.4.10. The textural class of each soil was classified corresponding to the *Guidelines for soil description* (FAO 2006) after the division in sand - silt - clay fractions had been conducted beforehand by the Institute of Soil Research, University of Natural Resources and Life Sciences in Vienna.

2.3. Chemical analysis

We used two different measurement methods for the analysis of our extraction-solutions; photometer in combination with the molybdenum blue method and ICP-OES. These two vary in the kind of P-species they are able to determine. The ICP-OES measures the total amount of P in the solution, whereas the colorimetric method determines only the ortho-phosphate amount in the solution (OENORM L 1087, 2006). This difference was taken into account by a conversion factor between the two different methods.

2.3.1. Photometer in combination with the molybdenum blue method

A Varian UV visible spectrophotometer DMS 200 in combination with the molybdenum blue method as proposed by Murphy and Riley (1962) was used for phosphorus-analysis from a number of extraction-methods.

Before measurement in the Photometer the molybdate blue staining method had to be applied. It is based on various chemical reactions, one of the main being the reaction of orthophosphate with molybdate upon which a complex is formed (the so called molybdenum blue complex). When reduced by ascorbic acid, the solution turns blue (Divrikli et al. 2009).

For each measurement the spectrophotometer had to be calibrated with the help of a number of standards containing known P concentrations. Then the measurement was performed and the P contained within the soils could be determined depending on the level of light-absorbance of each sample in comparison to the standards (Barrett 2002). The used wavelength was 881 nm.

2.3.2. ICP-OES and ICP-MS

ICP-OES stands for inductively coupled plasma-optical emission spectrometry while ICP-MS stands for inductively coupled plasma-mass spectometry. Both methods can be used in a wide array of elemental analysis, including soil-research. For analysis of the soil samples, two instruments were used: Perkin Elmer Optima 8300 ICP-OES and Perkin Elmer Elan DRCe 9000 ICP-MS (Figure 6). With both devices it is possible to measure many different elements at the same time. The measurements were performed according to the ISO 22036 (2008) standard procedure.

For analysis of soil-samples with ICP-OES it is necessary to perform the desired extraction beforehand, so as to have a liquid solution. Samples find their way into the center of the plasma as aerols. The contained elements get charged with very high energy from the plasma, causing one of their electrons to leave the ground state and get excited. In this way, the elements become ionized. When they change back to their ground state, a photon is being emitted, which is measured by the detector unit. For these kind of techniques, most frequently the electromagnetic spectrum of 160 – 800 nm (UV/visible region) is used (Charles and Fredeen 1997; Olesik 1996).

As the electrons in each element have their specific energy-levels and therefore their own characteristic emission wavelengths, they can be distinguished from each other and in this way qualitative and quantitative information about a sample can be obtained. Qualitative information (which elements are present within the sample) are connected to the wavelengths recorded by the mass spectrometer. Quantitative information (how much of each element is present) is associated with the amount/intensity of electromagnetic radiation (photons) that is emitted (Charles and Fredeen 1997; Olesik 1996).

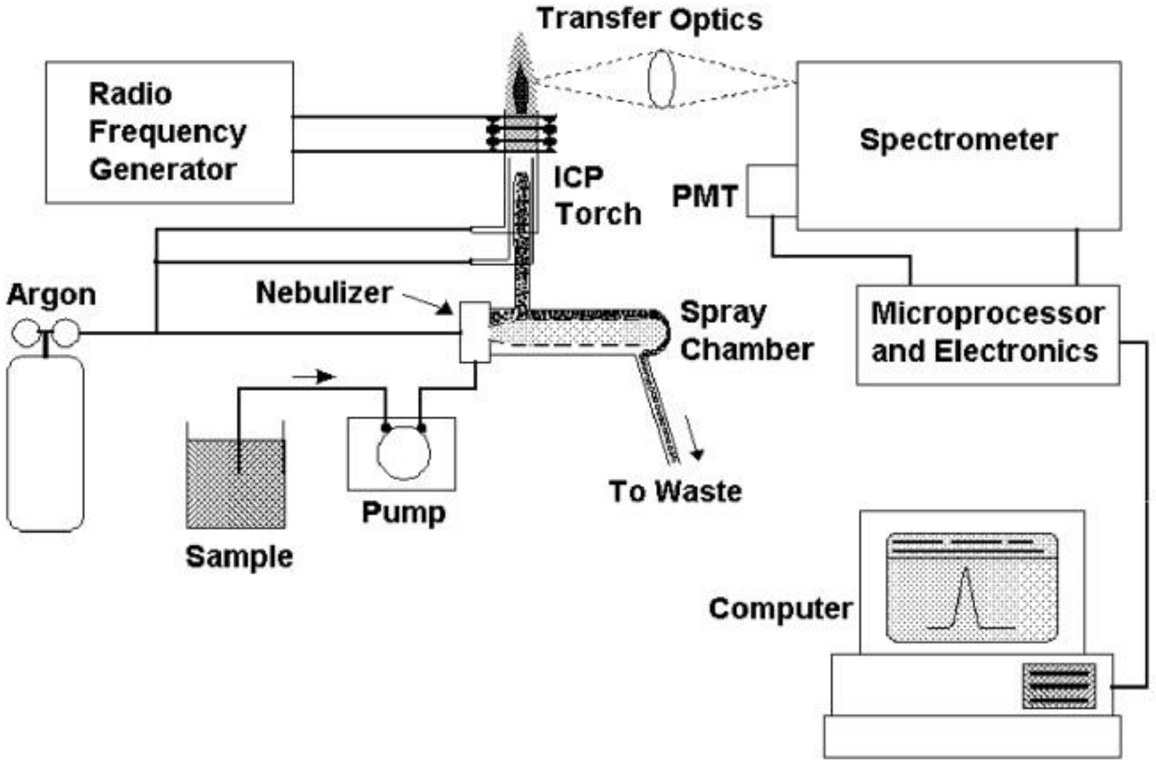


Figure 6: Major components and layout of a typical ICP-OES instrument (Charles and Fredeen 1997).

The principle of ICP-MS is closely related to the ICP-OES as it uses the same type of ICP source. In mass spectrometry (MS) the ions find their way into a mass spectrometer where they are separated according to their mass/charge ratios. This way the ions of interest can then qualitatively and/or quantitatively be measured (Charles and Fredeen 1997).

One of the main differences between the two described methods is that the ICP-MS has a higher sensitivity and is able to detect even lower quantities of the various elements than ICP-OES (Charles and Fredeen 1997).

2.4. Extraction methods

The power of an extraction method depends on the extractant used, on the soil:solution ratio and on the duration and strength of the shaking-procedure (Schick et al. 2013). A soil P test on its own can only provide a rough idea about the P status of a particular soil and hence also only a rough estimate about the ability of it to provide an adequate P supply to plants (Sibbesen 1983).

2.4.1. Overview and general lab procedures

Before analysis, all soil samples were air-dried and sieved with a 2 mm standard-sieve. All containers that were used for the various extractions were put into an acid bath with 5 % HNO_3 for at least 5 hours, rinsed at least 3 times with 18.2 MΩ cm^{-1} high quality water (HQ) provided by a TKA GenPure water purification system and subsequently air-dried. Along with each extraction procedure samples with reference soil and blanks were carried out; the same background solution as for the extractions was used for those. In order to filter the soil/extraction-solution mixtures Munktell filter papers (grade 14/N) were applied. For ICP-OES measurements internal standards with Yttrium were used; these were also made with the background solution that acted as the extractant. If the measurement could not be conducted immediately after extraction, the solution containing the extracted nutrients was mixed with 2 % HNO_3 at a ratio of 1:9 for the purpose of conservation.

Table 1: Overview of the soil P extraction methods used in the investigation

Full name	Abbreviation	Extraction solution	Extraction (shaking) time	Method of measurement
Diffusive gradients in thin films	DGT	Ferryhydrite based binding gel	24 hours	photometer
Porewater concentration	-		Centrifugation	ICP-OES
Calcium acetate lactate	CAL	0.3 M CH_3COOH, 0.05 M $C_6H_{10}CaO_6 . 5 H_2O$ 0.05 M $(CH_3COO)_2 . H_2O$	2 hours	photometer
Water extraction	H_2O	H_2O (High Quality water)	ca. 16 hours saturation time; 1 hour on shaker	photometer
Sodium bicarbonate	Olsen	1 M $NaHCO_3$; pH 8.5	30 min	photometer
Mehlich 3	Mehlich 3	0.2 M CH3COOH, 0.25 M NH4NO3, 0.015 M NH4F, 0.013 M HNO3, 0.001 M EDTA	5 min	photometer
Electro Ultrafiltration	EUF	H_2O (De-ionized water)	5 & 30 min (2 fractions)	external
Acid Ammonium Oxalate in Darkness	AAO	0.175 M $(NH_4)2C_2O_4 . H_2O$, 0.1 M $H_2C_2O_4$, 1.0 M $CH_3CO_2 NH_4$	2 hours	ICP-OES
Sodium Citrate-Bicarbonate-Dithionite	CBD	0.3 M $C_6H_5Na_3O_4 . 2H_2O$, 1.0 M $NaHCO_3$, $Na_2S_2O_4$	ca. 1 hour in 80° C water bath; centrifugation	ICP-OES
aqua regia		HCL, HNO_3	30 mins in microwave	photometer

2.4.2. Diffusive gradients in thin films (DGT)

2.4.2.1. Fabrication of the gels

For the preparation of the gels the following constituents, following the standard protocoll of Zhang and Davison (1995), were used: gel solution containing 15 % acrylamide, 0.3 % cross-linker (DGT-research Ltd, Lanchester, UK), diluted with HQ-water. Ammonium persulphate (APS) solution (10 % w/w) had to be made daily.

Two different kinds of gels were made: thin gels (binding layer) with a thickness of 0.4 mm and thick gels (diffusive layer) with a thickness of 0.8 mm. For producing one binding layer with the size of approximately 7 x 22 cm we used 2 ml gel solution, 14 µl APS and 5 µl N,N,N',N' tetramethylethylenediamine (TEMED). These ingredients were mixed together and cast with a pipette in between two glass-plates, which were separated by a spacer with a width of 0.25 mm. For the fabrication of one diffusive layer with the same size the amount of each constituent was doubled and spacers with a width of 0.5 mm were used.

After casting the gels, the appliance was put into the oven at 44° C for 1 hour for polymerization. Following this, the appliance was disasembled and the gels carefully dispensed from the glass-plates. They were put into 2 L vials filled with HQ-water for 24 hours. During that time the HQ-water was changed 3 times and the gels sweled to their stable size.

The Ferryhydrite-gels (binding layer) were prepared following the method described by Santner et al. (2010). First, 2.7 g $FeCl_3 \cdot 6H_2O$ were poured into a 120 ml vial and afterwards dissolved by adding 40 ml HQ-water. 3 thin diffusive gels (binding layer) were put into each vial and filled up to 100 ml with HQ-water. For 2 hours they were allowed to sit in the water, in order to ensure an even distribution of Fe in the gels (Figure 7).

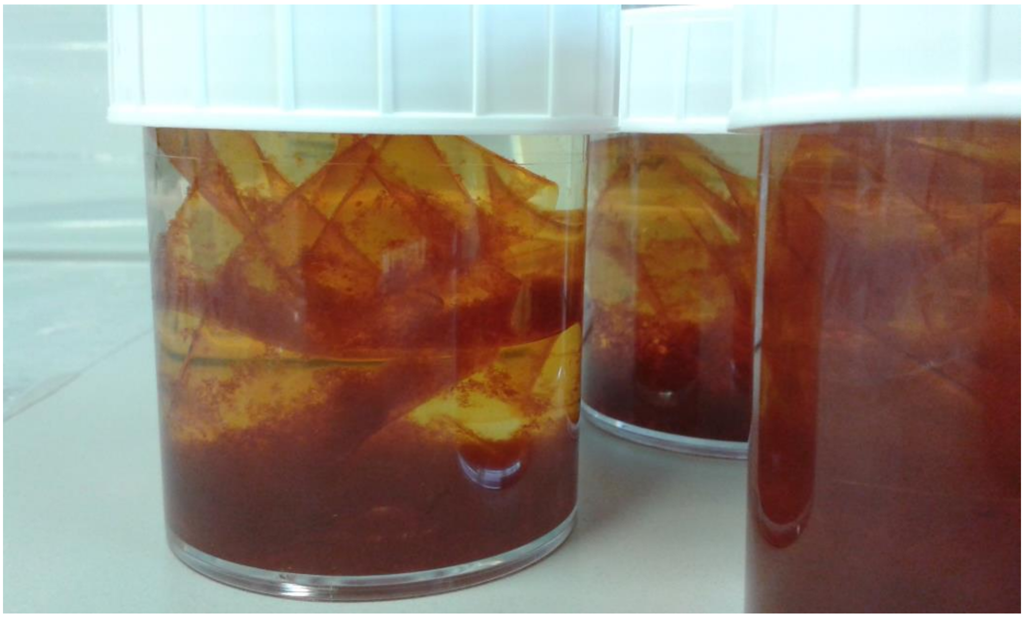


Figure 7: Precipitation of the Ferryhydrite-gels (binding layers)

Then each gel was transferred into 100 ml 0.05 mol L^{-1} 2-(N-morpholino)-ethanesulfonic acid (MES) to precipitate. The MES had before been adjusted to pH 6.7 with 1 mol L^{-1} NaOH. During the first 60 seconds we used a tweezer for stiring the gels in order to guarantee a homogenous precipitation. Afterwards they were put on a plate shaker for 30 min and shaken gently. Subsequently, each gel was rinsed and put into a vial containing HQ-water for washing periods of at least 2 hours, changing the water at least 3 times. At the end of the procedure we stored the gels in 0.03 M $NaNO_3$ at around 4° C in the refrigerator.

2.4.2.2. DGT set-up

The rectangular gels were die-cut with a standard round cutting die, so as to fit into the DGT-device perfectly. Before the DGT set-up, these round gels were stored in 0.03 M $NaNO_3$ at around 4° C in the refrigerator once again.

The device was set-up as shown in chapter 1.4.1. First we put the Ferryhydrite binding gel directly onto the piston. Secondly, a thin filter membrane (0.2 µm pore size, Whatman Nuclepore, Sigma-Aldrich, St. Louis, USA) was added, followed by the thick diffusive gel. On top we put a thick membrane (PES, 0.2 µm pore size, Sartorius, Göttingen, GER). When everything was in place, the outer sleeve with window was carefully adjoined. Blanks were included. For storage, the DGT´s were put into plastic-bags, which had been moistened before and then put into the fridge at approximately 4° C.

2.4.2.3. Conducting the DGT-measurement

All soil-samples to be tested (3 replicates) were moistened to 75 % of their water holding capacity with HQ-water, which had been calculated before, according to their characteristics. The samples were put into an incubator at 20° C and allowed to soak up the water for 24 hours. A short time before applying the soils to the DGT´s, they were brought to their saturation point through the addition of the corresponding amount of HQ-water. The soil-water mix was stirred intensely with a spatula until a glossy appearance was reached.

Figure 8: (I) Soils smeared onto DGTs with the help of connected plastic frames; (II) DGTs inside a box during incubation; (III) DGTs in the process of disassembly

We took the DGT-devices out of the fridge and allowed them to warm up to room-temperature. Plastic-frames were connected to the DGT´s in order to facilitate the application of the soils. Then we smeared the soils onto the appliance and wrote down the exact time each time we did so, in order to make sure that each soil would be in contact with the device for exactly 24 hours. The DGT´s with the soils were put into containers with wetted tissue papers on the bottom so as to not let the samples dry out during the incubation-period. The lid was loosely attached onto the box and it was put into the incubator again at 20° C.

Each soil was carefully removed from the DGT after exactly 24 hours with a spatula and the remaining visible soil-particles were wiped off gently from the membrane-surface with a tissue-paper. Afterwards, the devices were disassembled and each binding layer put into a vial filled with 5 ml 0.25 M H_2SO_4 for elution which was then put on a plate shaker (GFL

3017) for 2 hours. Analysis of the eluates was done with reference to the protocoll *DGT - for measurements in waters, soils and sediments* proposed by Zhang (2003).

2.4.3. Porewater concentration

In the course of performing the DGT measurements we also gained our basic material for the determination of the soil porewater concentration. Therefore, we prepared more soil than would be needed to conduct the DGT measurements and brought it all to the water saturation point. The spare soil was apportioned into vials and centrifuged, which gave us a small but adequate amount of soil porewater for analysis.

2.4.4. Calcium acetate lactate (CAL)

The CAL-method (Calcium Acetate Lactate-method) is the standard method being used by the Austrian agency AGES (Agentur für Gesundheit und Ernährungssicherheit). The Austrian “guidelines for an appropriate fertilization“ refer to this method for the determination of phosphorus and potassium in agriculturally used soils.

In this work, the CAL-method was performed as described in the OENORM L 1087 (2012). Therefore, 5 g of soil were weighed into a 250 ml container and the extraction-solution was added. For the extraction to take place the containers were shaken on a rotating shaker (GFL 3040) with a frequency of 160 revolutions/min for 2 hours. Afterwards, the soil-solution-mix was filtrated with Munktell filter-papers and additionally with a 0.20 µm syringe filter.

The filtrate was then prepared by applying the molybdenum-blue method and shortly after analyzed with the spectrophotometer.

The underlying principle of the CAL-method is the extraction of readily soluble, exchangeable phosphates. The P of apatitic phosphates does not get dissolved, in contrast to the Ca-phosphates from fertilizers, which are easily dissolved. In soils with a pH below 6 this technique might not work properly as the extracted phosphate can be re-adsorbed by Al- and Fe-oxides (Wuenscher et al., 2015).

2.4.5. Water extraction (H_2O)

A saturation extract with water based on OENORM L 1092 (2013) was conducted. For this, 5 g of soil were weighed into a 100 ml plastic vial. Then, water was added until the saturation-point of each individual soil was reached. The amount of added water was determined through differential measurement of weight. The extraction-solution with the soil was left overnight and shaken on a horizontal shaker with 160 revolutions/min for 1 hour the next day. The filtration took place with the use of Munktell filter-paper and additionally with a 0,20µm syringe filter. 0.25M H_2SO_4 was added to the filtrate for stabilization. Analysis was done utilizing the spectrophotometer.

With the water extraction only readily availble P fractions like e.g. easily soluble Fe- and Al-compounds are being measured (Schnug and De Kok 2016).

2.4.6. Sodium bicarbonate (Olsen)

The Olsen-method has been introduced by Olsen (1954). Since then, it has gotten well established around the world and became the standard method for soil-nutrient-determination in many countries. It can be applied in both acidic and calcareous soils (Schoenau and O´Halloran 2007; Jordan-Meille et al. 2012).

$NaHCO_3$ acts as the extracting-agent and various chemical reactions come into play. The main mode of action constitutes of a pH and an anion exchange effect. Inorganic P in solution, some of the labile P within the solid phase and also labile organic forms become dissolved and can be measured with this procedure (Schoenau and O´Halloran 2007).

In this work, the procedure was conducted according to Schoenau and O´Halloran (2007). 2,5 g of soil were weighed into 100 ml plastic containers. 50 ml of $NaHCO_3$ extraction-solution with a pH of 8.5 and afterwards 0,4 ml of charcoal suspension were added. In order to remove the P which is contained within, the charcoal was washed with 3% HCL and afterwards washed with bi-distilled water for several times.

The samples then were shaken on a horizontal shaker with 160 revolutions/min for 30 min. Stabilisation before analysis was achieved through addition of H_2SO_4. The samples were analyzed according to the molybdenum blue method on the spectrophotometer.

The Olsen-method is based on the principle of increasing the solubility of Ca-phosphates. This is achieved through the decreasing of the activity of Ca_2^+ in solution by the precipitation as $CaCO_3$ and the formation of oxyhydroxydes out of soluble Al_3^+- and Fe_3^+-species. Furthermore, the high pH-levels imply fewer sorption-sites on Fe- and Al-oxide surfaces and therefore also enhance desorption of P into soil solution (Pierzynski 2000; Schnug and De Kok 2016).

2.4.7. Soil extraction with weak acids (Mehlich-3)

The Mehlich-3 method has been introduced by Mehlich in 1984. Multiple elements can be extracted and it is being used for a wide range of soils. Due to that versatility this extraction technique is in use for routine-soil-analysis in many countries (Carter and Gregorich 2008).

The Mehlich-3 procedure was conducted as described by Ziadi and Tran in Carter and Gregorich (2008). In the extraction solution a combination of ammonium fluoride (NH_4F), ammonium nitrate (NH_4NO_3), acetic acid (CH_3COOH), nitric acid (HNO_3) and the chelating agent ethylene diamine tetraacetic acid (EDTA) takes effect. With this method it is possible to extract the following elements: P, K, Ca, Mg, Na, Cu, Zn, Mn, B, Al and Fe. In the present work, only 3 elements were relevant: P, Fe and Al.

For the procedure, 3 g of soil were weighed into 100 ml plastic vials. The soil:solution ratio for the extraction was 1:10 and thus 30 ml of extracting solution were added to each vial with the help of a dispenser. On a rotary shaker the soil-liquid-mix was shaken for 5 min. After shaking, the filtration through Munktell filter paper into 50 ml storage vials took place.

The P extraction is promoted by the acetic acid (CH_3COOH), because of which the dissolution of Ca-phosphates takes place. More P is released from Al-phosphates through the addition of Ammonium fluoride (NH_4F) as Al-F-complexes are being formed in solution (Wuenscher et al. 2015).

2.4.8. Electro-Ultrafiltration (EUF)

The Electro-Ultrafiltration (EUF) analysis was conducted at the laboratory of the Bodengesundheitsdienst GmbH in Rain am Lech, Germany. The EUF method uses deionized water as a means of extraction. In combination with adjustable temperature and an electric field induced by platinum electrodes and furthermore vacuum, this method is able to predict the short- and longterm availability of nutrients in soils. A big advantage of this technique is that all macronutrients as well as many micronutrients can be determined by only one extraction from one soil sample, which can be conducted in a very short time. If DTPA is added in another step, even more micronutrients and also heavy metals can be determined (Schnug and De Kok 2016).

In the process, the nutrients from the soil-suspension have to pass through an ultra-membrane filter. Depending on their charge they are then moving towards either the anode or the cathode and are collected in an aqueous eluate, from which an analysis with standard analytical methods can be performed afterwards. As the process continues, there is a constant resupply from the solid soil taking place. Depending on the variation of extraction time, water temperature and voltage, fractions of different availabilty can be measured (Schnug and De Kok 2016).

2.4.9. Acid Ammonium Oxalate in Darkness

The Acid Ammonium Oxalate in Darkness extraction was performed in accordance with the method described in *Methods of soil analysis. Part 3. Chemical methods* (Sparks 1996). All soil samples were finely ground before the extraction in order to be as homogeneous as possible. Before the extraction could take place, it was necessary to identify the samples which contained $CaCO_3$. The $CaCO_3$-content had been evaluated beforehand and 26 out of 41 samples were calcareous.

In treatment of the 26 calcareous soils with 1.0 M ammonium acetate some variations to the method were made. 90 ml were added to 1.5 g of soil. After a constant pH of approximately 5.5 had been established, the ammonium acetate was poured away. Then, water was added to the soil and it was put into a centrifuge for 5 min at 3,000 revolutions/min. The water was discharged, new water added and the centrifuge-process was repeated with the same settings as before. All the 26 samples were put into an oven and were dried without the lids on the containers for 5 days at 35° C.

3 replicates were made out of each soil sample that had been treated this way. Deviating from the planned 500 mg of soil samples, we had to establish 3 more sets of sample-amounts: 450, 400 and 350 mg. This was due to the soil which got lost during the process of expelling the $CaCO_3$. In order to preserve the extraction-ratio of the acidified ammonium oxalate, the amount of extraction-solution was adapted accordingly to 27 ml for 450 mg, 24 ml for 400 mg and 21 ml for 350 mg.

The acidified ammonium oxalate extraction was performed for all 41 soil-samples. Immediately after adding the extraction-solution, aluminium-foil was wrapped around the container so as to make it light-proof. After that, the soil-liquid mixes were put into a rotary-shaker and shaken for 2 hours. Afterwards, the suspension was filtered through a Munktell filter paper into 50 ml vials. The measurement took place on the ICP-OES device.

Fe-oxides play an important role in relation with phosphorus, as they are able to bind phosphate firmly onto their surface and hence reduce its mobility in soils (Blume et al. 2010). Wuenscher et al. (2015) state that this method is not a conventional P-extraction method, meaning that here Fe-bonds of various kinds get disintegrated and accompanied by that also P becomes available. This holds true for poorly cristaline Fe, ferryhydrite and also for soil organic matter which comprises Fe.

Courchesne and Turmel (2007) put forward that crystalline Fe- and Al-oxides become only slightly dissolved by Oxalate. In contrast to that, amorphous inorganic forms should be dissolved by this method. However, the Fe-oxides extracted by this method cannot be viewed as the quantifiable amount contained within the sample. It rather should be viewed as measure of the „activity“ of the Fe-oxides (Schwertmann 1973). McKeague and Day (1966) stated that the values gained from this method can be used as approximation of the degree of weathering of a soil because during that process amorphous compounds accumulate.

2.4.10. Sodium Citrate-Bicarbonate-Dithionite

The Sodium Citrate-Bicarbonate-Dithionite extraction is not a conventional method for the extraction of P. Rather, it is being used for determining the various forms of Fe in a soil sample (Wuenscher et al. 2015). Due to its specific functionality, various P-fractions become solubilized. The procedure was undertaken as described in *Methods of soil analysis. Part 3. Chemical methods* (Sparks 1996).

0.4 g of soil were weighed into plastic vials and to each sample 18 ml of 0.3 M sodium citrate ($C_6H_5Na_3O_4$. $2H_2O$) and afterwards 2.8 ml of 1 M sodium bicarbonate ($NaHCO_3$) were added. 12 samples at a time were put into a 80° C water bath until they reached approximately that temperature. Then 0.4 g of Sodium dithionite ($Na_2S_2O_4$) were added to each replicate and the whole soil-solution-mixture stirred intensely. After about 25 mins again

0.4 g of Sodium dithionite were added and intense stirring took place. Deviating from the protocol, the samples sat in the water bath after adding the first portion for approximately 45 mins instead of 25 mins.

After cooling off, the samples were put into a centrifuge for 15 mins at a speed of 1200 revolutions/min. After that, the supernatant was filtered through Munktell filter paper into vials for storage. For analysis on the ICP-OES, 0.1 ml of each sample was diluted with 9.9 ml of 4 % HNO_3.

Wuenscher et al. (2015) stated that Sodium-Dithionite acts as a reducing agent and the Citrate as a chelating agent. As a result, the total amount of secondary free Fe, with the exception of magnetite, gets solubilized. McKeague and Day (1966) argued that the values of this method approximate the amount both of amorphous forms of Fe and crystalline Fe-oxides.

2.4.11. Aqua Regia

Aqua regia is an established and widely used alternative to other methods that determine total soil P like sodium carbonate fusion or perchloric acid digestion. It is a rapid and safe method that is easy to use and can be applied over a wide range of soils (Crosland et al. 1995). The extraction with *aqua regia* was performed according to the protocol as described in *Methods of soil analysis. Part 3. Chemical methods* (Sparks 1996) with a few modifications.

The standard soil samples were ground with the help of a ball mill before the extraction took place. This was done in order to make it fine and homogenous. Then, 0.5 g of soil was put into a 20 ml container and 6 ml of *aqua regia* was added. This mixture was put into a microwave in order to initiate the solubilization. The ramp time was 10 min and the hold time was 20 min at 1.200 W and a temperature of 200° C.

2.5. Plant experiment

The plant experiment was carried out in the indoor growth chamber of the Department of Crop Sciences, University of Natural Resources and Life Sciences in Tulln from the 8th of June – 19th of July 2017 (6 weeks). The ambient conditions were regulated. Light periods of 14 hours alternated with darkness periods of 10 hours. Temperature was held at 20° C during the whole experiment.

2.5.1. Set-up of the pots

For the plant-experiment a subset of 30 soils out of our total of 41 soils was used. For each soil, 4 replicates were included, which meant a total of 120 pots. The same soil (sieved > 2 mm) was used as for all the other extractions. Every pot was filled with 1.000 g of soil, corrected by the correspondening water content.

2.5.2. Nutrient solution

The nutrient solution was prepared on the basis of the protocol of Middleton and Toxopeus (1973) but with slight variations. Each week, 7 liters of working solution were made anew. The working solution was prepared from 3 stock solutions. They were (for an amount of 7 liters of working solution): macronutrients (1.4 L), micronutrients (1.4 L) and Fe-EDDHA (0.28 L). To reach the 7 L, the rest was filled up with HQ-water. The composition of the stock solutions is shown in Table 2.

Table 2: Itemized composition of the nutrient solution used in the plant experiment.

Macronutrients (2 L)	**Micronutrients (2 L)**	**Fe-EDDHA (1 L)**
For 2 L of macronutrient-solution (stock 1), the following constituents had to be mixed together and filled up with HQ-water.	For 2 L of micronutrient-solution (stock 2), 20 ml of each of the following constituents had to be mixed and filled up with HQ-water.	For 1 L of this solution, 60 mg of Fe-EDDHA had to be dissolved in 1,000 ml HQ-water.
40 g NH_4NO_3	30 mg H_3BO_3 in 100 ml	60 mg Fe-EDDHA in 1,000 ml
14.70 g K_2SO_4	20 mg $CuCl_2$ in 250 ml	
4.44 g $MgSO_4$	275 mg $MnCl_2 . 4 H_2O$ in 100 ml	
3.60 g $CaCO_3$, anhydr.	10 mg $(NH_4)Mo_7O_{24} . 4 H_2O$ in 250 ml	
72 ml HCL 37% (1M)	15 mg $ZnCl_2$ in 100 ml	

2.5.3. Conducting the plant experiment

The chosen species of plants for the experiment was rye (*Secale cereale).* For a good and uniform germination, the seeds were placed in special wetted paper-sheets and put into an incubator for 48 hours at 22° C.

Figure 9: (I) Seeds of *Secale cereale* after 48 hours of incubation; (II) Seedlings after 1 week; (III) plants 5 days before harvest; (IV) selection of pots after the harvest

One day before the planting took place, the pots were watered with 50 mL H_2O and 50 ml nutrient solution. 7 seedlings were evenly distributed into each pot and planted in a depth of 2 – 3 cm. Care was taken to choose the most vigorous ones. After planting, 50 mL of H_2O was given to each pot.

During the experiment, nutrient solution was given every week, except the week before harvest. Water was given according to requirement, which was evaluated visually and by touch of the soil. Care was taken to always keep the soils moist. Furthermore, every pot was moved to a different position randomly once every week.

Table 3: Main measures taken during the plant experiment.

Date	Task
7th of June	50 mL H_2O; 50 mL nutrient solution
8th of June	Planting; 50 mL H_2O
12th of June	100 mL H_2O
14th of June	50 mL H_2O; 50 mL nutrient solution; randomization
19th of June	Thinning out: Of the 7 plants growing in the pot always the tallest and the shortest were taken out, so that in each pot 5 plants would remain. 50 mL H_2O; weeding
22nd of June	50 mL H_2O; 50 mL nutrient solution; weeding; randomization
27th of June	Installation of 4 wooden sticks and a rubber band per pot for ensuring the upright growth of the plants
29th of June	50 mL nutrient solution; randomization
3rd of July	30 mL H_2O only for very dessicated pots
5th of July	50 mL nutrient solution; randomization
10th of July	50 mL H_2O; randomization
18th of July	50 mL H_2O
19th of July	Harvest

After harvest, all plants of a pot were put into one paper bag. All the paper bags with the plants inside were put into an oven at 65° C for 5 days. The bag was loosely open in order to allow for a proper drying process.

2.5.4. Plant analysis

After drying, the plants were weighed immediately. Subsequently, the plant digestion was conducted on the basis of the protocol “Microwave digestion“ which is the guideline for the standard procedure at the Department of Crop Sciences, University of Natural Resources and Life Sciences, Vienna.

The samples were finely ground in order to homogenize the material. Before the weighing in of the samples took place, they were all put into the incubator for 4 hours at 80° C. Then, 200 mg of the dried sample material was put into each Xpress vessel. Afterwards 8 mL HNO_3 were added to each sample and everything was left to settle overnight. The following morning 2 mL of H_2O_2 were added. The microwave digestion was performed with the settings as described in the protocol. Ramp time was 20 min and hold time was also 20 min at a temperature of 200° C and 1,080 W. The following analysis was done by spectrophotometer.

2.6. Statistical Analysis

The data presented are the arithmetical mean values of the correspondent number of replicates (soil samples n=3; plant experiment n=4). The statistical calculations were done using Excel 2010 (Microsoft) and SPSS 24.0 (IBM) for Windows.

3. Results & Discussion

3.1. Basic soil characteristics

In Table 4 the most important soil characteristics are depicted. The 41 investigated soils had a wide range of properties. The pH values ranged from 5.0 to 7.7. The organic carbon displayed a span from 7.5 to 35.6 g kg^{-1}. The soil with the lowest carbon to nitrogen ratio was measured with 8, the highest with 21.7. Out of the 41 soils tested, 18 were completely free of $CaCO_3$, whereas in 23 Calcium Carbonate was present. Of the soils containing $CaCO_3$, the one with the lowest amount had 2.5 g kg^{-1}, while the highest amount measured was 251.8 g kg^{-1}.

Texture also showed a large variability. The minimum sand content measured was only 73 g kg^{-1}, the highest 802 g kg^{-1}. The silt fraction ranged from 103 to 759 g kg^{-1}, the clay fraction from 74 to 501 g kg^{-1}. The soil with the lowest cation exchange capacity displayed 57 mmol$_c$ kg^{-1}, the one with the highest 413 mmol$_c$ kg^{-1}. The total P concentrations were measured in *aqua regia* digests. The lowest P concentration was 471 mg kg^{-1}, the highest 3270 mg kg^{-1}.

The extractions performed with Acid Ammonium Oxalate (AAO) and Sodium Citrate-Bicarbonate-Dithionite (CBD) can be used to draw conclusions about the P content indirectly. Therefore, the Al and Fe contents were quantified. With AAO, the lowest Al content was 330, the highest 3540 mg kg^{-1}, the soil with the lowest Fe had 745, the one with the highest 7900 mg kg^{-1}. The range of Al and Fe extracted with CBD was equally broad. Al spanned from 484 to 3853 mg kg^{-1}, Fe from 4240 to 18,990 mg kg^{-1}.

Table 4: Summary of basic soil characteristics; n=41

	pH	Corg	C/N	$CaCO_3$	Sand	Silt	Clay	KAK	*aqua regia*	AAO Al	AAO Fe	CBD Al	CBD Fe
		g kg^{-1}		g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	mmol$_c$ kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Mean	6.7	20.6	10.9	57	330	490	180	167	1160	1370	2900	1510	10710
Median	6.9	18.5	10.3	13	288	488	146	165	1030	1220	1760	1220	10700
St. Dev.	0.9	6.9	2.3	78	189	140	97	69	530	710	2190	910	4060
Min	5.0	7.5	8.0	0	73	103	74	57	470	330	750	480	4240
Max	7.7	35.6	21.7	252	802	759	501	413	3270	3550	7910	3850	18990

3.2. Comparison of applied soil phosphorus extraction methods

In this chapter, we display the data we gained from comparing the performed P extraction techniques with each other. In Table 5 the mean, median, standard deviation and the minimum and maximum concentrations of phosphorus extracted from the 41 investigated soil samples are presented. For the direct comparison of the methods, a Pearson correlation was performed. The results are shown in Table 6.

Table 5: Comparison of the extractable P by the 9 extraction methods in depicted in mg kg^{-1} except cDGT and porewater concentration, µg L^{-1}; n=41

	H_2O	Olsen	EUF	CAL	Mehlich 3	AAO	*a. regia*	cDGT	porew.
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	µg L^{-1}	µg L^{-1}
Mean	11.9	25.1	27.3	69	77	600	1160	89	242
Median	7.0	21.3	23.6	54	47	470	1030	74	177
St. Dev.	12.3	21.6	16.9	53	72	360	530	79	248
Min	0.1	3.1	6.7	8	6	170	470	6	14
Max	46.3	114	86.4	280	260	1510	3270	310	900

Various authors have stated that in many cases a direct comparison between one kind of soil test performed in different laboratories can be limited due to local modifications of the techniques, especially extracting times or the ratio of soil to extracting solution. Furthermore, the soil properties of the soils tested in our study can differ greatly from those evaluated in other studies and therefore yield very different results when tested with extraction techniques that are e.g. not suitable for the spectrum of soils in one of the studies (Neyroud and Lischer 2003; Wuenscher et al. 2015; Schnug and De Kok 2016). There are simply too many factors of uncertainty and therefore a direct comparison, if at all, only makes sense if the exact protocol of the procedure is available.

For some extraction techniques soil classification values exist, which should be taken into account. Soil classification values also should be looked upon with caution though, as they vary by country. Schick et al. (2013) found in a study involving 257 soil samples from Germany, Estonia, Finland and Poland that depending on the country and method used, the result achieved could lead to the classification of a site being low in P (meaning deficient) in one country, as sufficient in another and as high in yet another.

In our study, as expected, Ammonium Oxalate and *aqua regia* were able to extract the highest P amounts, on average 604 mg kg^{-1} and 1160 mg kg^{-1}, respectively. Mehlich 3 with a mean of 77 mg kg^{-1} and CAL with 69 mg kg^{-1} extracted on average almost similar amounts of P from the tested soils. The average EUF (27.3 mg kg-1) and Olsen (25.1 mg kg^{-1}) P concentrations were also moderate and relatively similar. The smallest P concentration was extracted with water (11.9 mg kg^{-1}). Not directly comparable to the results referred to before because of the different units, the DGT mean for 41 soils was 89 µg L^{-1}, for the porewater extraction 242 µg L^{-1}. The mean values, except for DGT and porewater concentration, are depicted graphically in Figure 10.

For the determination of total P in soils various methods exist. Generally, very strong chemicals are used to dissolve all P bonds, be it organic matter or soil minerals. A*qua regia* is slighly weaker and therefore can be referred to as “semi-total P” (Schnug and De Kok 2016). A*qua regia* is not being used for determining plant available P and thus is not relevant for agricultural fertilizer recommendations. Therefore, no meaningful reference values could be obtained. Neyroud and Lischer (2003) performed an extensive comparative study including 12 P extraction techniques applied on 135 European soils. Their mean result for *aqua regia* was 683 mg kg^{-1}, compared to a mean of 1163.4 mg kg^{-1} in our study.

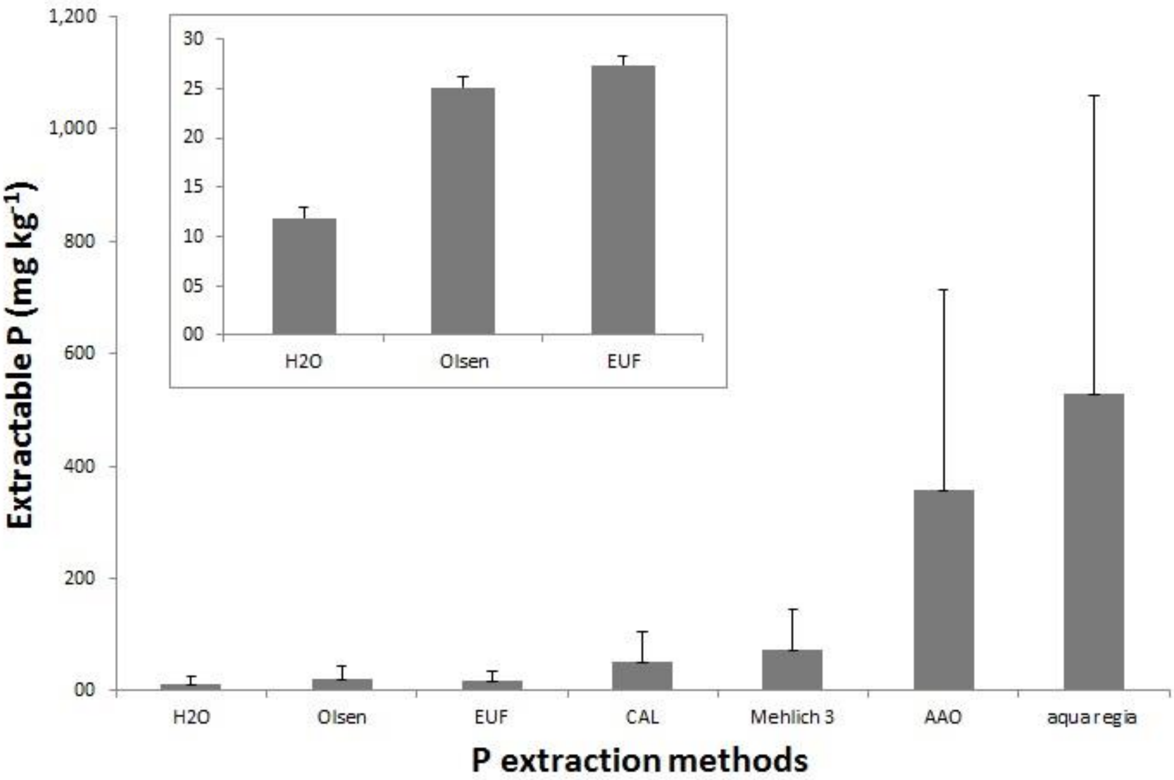


Figure 10: Comparison of the performed P extraction methods (except DGT and porewater concentration because of different measurement units) by the average amount of extracted P and standard deviation

Acid Ammonium Oxalate (AAO) mean was 604 mg P kg^{-1} in our study, and thus higher than the 328 mg kg^{-1} reported by Neyroud and Lischer. AAO is not used as a standard extraction technique for investigation of soil P. It rather serves as a tool to gather additional information about a soil, namely the amounts of the various Fe- and Al-fractions (chapter 2.4.9.). Therefore, there are no availalbe reference P values with reference to agricultural soil testing. Nawara et al. (2017) state that the P extracted by Ammonium Oxalate makes up about 50, 70 or even 90 % of total soil P, depending on the investigated soils. This variation can be attributed to the dependency of AAO extraction on the amount of amorphous Fe- and Al-oxides in the soils investigated and their dissolution. Our measured values are in line with this; the mean of AAO was about 50 % that of the mean P amount extracted by *aqua regia*.

For the Mehlich 3 extraction the previously cited study of Neyroud and Lischer (2003) reported a mean of 62 mg P kg^{-1} compared to 77 mg P kg^{-1} average for our measurements. Our mean value was higher than the 45-50 mg kg^{-1} which is supposed to be the optimum for an adequate plant supply suggested by Pierzynski (2000). In our study, the mean of extracted P with the CAL method was quite similar to that of Mehlich 3; 69 mg kg^{-1}. This was substantially higher than the 41 - 45 mg kg^{-1} compiled by Neyroud and Lischer (2003). The CAL method is used as a standard method in Austria and can therefore be found in the Austrian guidelines for fertilization. Here, the mean of our 41 soils lies within the C-range (47 - 111 mg kg^{-1}), which signifies a sufficient supply with P (BMNT, 2017). In Figure 11 a more detailed itemization can be found.

In order to find out how well our tested soils are supplied with P, we made an exemplary classification according to two different protocols. One was done for the CAL method with reference to the Austrian guidelines for Fertilization, the other one for the Olsen method, according to the British DEFRA Fertilizer Manual. The results for the CAL categorization showed that the majority of soils fell into the categories “Low” with 40 % or “Adequate” with 45 %. The category “High” displayed 10 % and “Very high” 5 %; none of our soils was classified as “Very low”. The application of the DEFRA Fertilizer Manual for the Olsen method showed a different picture. In this classification system 34 % of the tested soils were categorized as “Very low” and 7 % as “Low”. 10 % of the soils fell into the category “Adequate”, 34 % “High” and 15 % were recognized as “Very high”. Overall, these observations presented a very mixed picture, depending on the method used and it confirmes the statement made previously about the difficulty of comparing results over a range of soils, methods and countries.

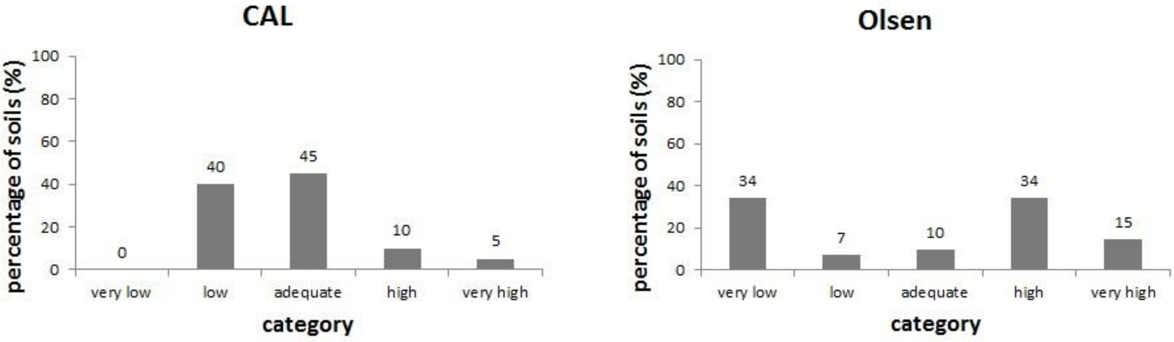


Figure 11: Classification of the 41 tested soils according to the Austrian guidelines for Fertilization (CAL) and the British DEFRA Fertilizer Manual (Olsen); numbers above the columns display the percentage of the tested soils that can be attributed into the particular category

The EUF method is part of the Austrian fertilization guidelines as well. Our mean of 27.3 mg kg^{-1} corresponds with the C-category (21 - 48 mg kg^{-1}) which stands for a sufficient supply with P. For Olsen, a guideline value would be 10 mg kg^{-1} (Pierzynski 2000) which is viewed as an optimum for plant growth; the average in our 41 soils amounted to 25.1 mg kg^{-1}. Neyroud and Lischer (2003) found Olsen values between 15 - 30 mg kg^{-1} wherein our result of an average of 25.1 mg kg^{-1} is located.

H_2O extraction yielded an average of 11.9 mg kg^{-1}, which is higher than the average of 4.7 - 7.3 mg kg^{-1} that Neyroud and Lischer (2003) found in their comparative study including 12 P extraction techniques applied on 135 european soils.

Soil solution P is a P fraction that is usually not investigated, due to the laborious procedure and possible analytical issues for soils with low P contents. Also, it stands only for a very small fraction of the P that the plant takes out of the soil (Schnug and De Kok 2016). Blume et al. (2010) suggested that in fertilized soils the P concentration of the soil solution usually lies between 0.1 - 5 mg L^{-1} and for optimum plant yields 0.3 - 0.8 mg L^{-1} are required. The porewater concentration mean of our tested soils was 0.24 mg L^{-1} which appears to be on the lower end of the typical concentration range.

For DGT-P, we found a mean value of 89 µg L^{-1}, which is in accordance with Mesmer (2017) who reported a range of 56 - 105 µg L^{-1} for 4 different soils, but differs strongly from Nawara et al. (2017) who measured a mean of 200 µg L^{-1} for a number of 218 European soil samples. 66 µg L^{-1} was suggested as a P deficiency threshold for wheat tested on 20 Australian soils (Mason et al. 2010). To the knowledge of the author, other meaningful reference values for the DGT technique do not exist yet.

While it is not constructive to compare the values of different experiments directly to each other due to the multiple reasons mentioned above and reliable reference values are not available for all methods applied, it is possible to compare the general trend of our results with those of other studies. When checking the amount of extracted P of the conducted extraction techniques against each other, the order, starting with the lowest, was as follows:

H_2O < Olsen < EUF < CAL < Mehlich 3 < AAO < *aqua regia*. Porewater concentration and cDGT cannot be compared directly, as the unit of measurement was not mg kg^{-1} but µg L^{-1}.

Our findings are in accordance with those of other studies (Wuenscher et al. 2015; Schick et al. 2013; Kulhánek et al. 2009; Neyroud and Lischer 2003; Jordan-Meille et al. 2012) and reflect the different extraction mechanisms of the methods and their efficiencies in dissolving P species from various pools (Schnug and De Kok 2016). Please see chapter 2.4. for detailed information about the underlying extraction mechanisms of the methods applied.

When a method extracts low amounts of P, this can be related to the P which is immediately available to plants. Elsewise if a method extracts high amounts of P, it can be related to the P fractions which are not easily available and may become dissolved over longer timespans. With this knowledge, depending on the method used, immediate and long-term fertilizer strategies may be deducted (Neyroud and Lischer 2003). As Schnug and De Kok (2016) pointed out, even though the amounts of P extracted by the various methods vary considerably, the results determined are generally correlated. For assessing the relation between the tested extraction methods a correlation matrix was established (Table 6).

Table 6: Pearson correlation coefficients between the P extraction methods; significance in * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; I=Intensity-based method, Q=Quantity-based method

	DGT	**Olsen**	**CAL**	**H_2O**	**porew.**	**Mehl. 3**	**AAO**	**EUF**
	(I)	(Q)	(Q)	(I)	(I)	(Q)	(Q)	(I)
Olsen	0.87***							
CAL	0.629***	0.67***						
H_2O	0.928***	0.816***	0.578***					
porew.	0.96***	0.797***	0.624***	0.915***				
Mehl. 3	0.82***	0.838***	0.553***	0.821***	0.778***			
AAO	0.582***	0.730***	0.310	0.572***	0.497***	0.759***		
EUF	0.884***	0.899***	0.652***	0.805***	0.806***	0.811***	0.68***	
a. regia	0.459**	0.613***	0.300	0.441**	0.415**	0.632***	0.856***	0.629**

The extraction methods overall showed a moderate to high correlation. Other authors also found medium to high correlations amongst different methods (Schnug and De Kok 2016; Wuenscher et al. 2015). As described earlier (chapter 1.2.1.) there are different P pools in the soil and depending on the method used, different P fractions are extracted. The methods applied can be grouped into two different groups: intensity-based methods (I-methods) and quantity-based methods (Q-methods). The I-methods include those which measure P that is easily available, i.e. P in soil solution and a small quantity of labile P from the solid phase, which serves as a buffer when the equilibrium between soil solution and solid phase gets disturbed. The Q-methods measure P from the potentially available pool, which the plant cannot directly access (Nawara et al. 2017; Six et al. 2013a, b). In our case, the weak extractants such as DGT, soil porewater, H_2O and EUF can be allocated to the I-methods whereas the strong extractants *aqua regia* and AAO, Olsen and Mehlich 3 can be considered Q-methods. CAL extraction is located in between the weak and the strong extractants but is also related to the Q-methods as it mainly extracts P that is not immediately plant available (Blume et al. 2010).

Our data reflects the aforementioned concept of classification very well. High to very high correlations can be found mainly within the cluster of I-methods and within the cluster of Q-methods. Olsen and Mehlich 3, despite being classified as Q-methods, also exhibited high correlations with all of the I-methods and also amongst each other while the relation with CAL and *aqua regia* was less pronounced. Other authors also reported medium to high correlations between Olsen and Mehlich (Moody et al. 2013; Neyroud and Lischer 2003; Pierzynski 2000). This is on the one hand surprising, as the two methods are based on very different principles. On the other hand these two methods are generally viewed as applicable over a wide range of soils (chapter 2.4.6; 2.4.7.) which applies to our set of tested soils.

When focusing on DGT, as expected, it had the highest correlation rates with the water based extractions, namely soil porewater, H_2O and EUF. Menzies et al. (2005) likewise found a high correlation between DGT and soil porewater. Even though not water based, the P extracted by Olsen was also highly correlated with DGT. The same was observed for the Mehlich 3-extractable P, which also showed a rather high correlation, despite the fact that this method is based on using a weak acid for extraction. The strong extractants AAO and *aqua regia* were also correlated but on a lower level. In contrast to these results, Wuenscher et al. (2015) found only weak to no correlations between AAO and weak extraction methods.

It is unclear why the CAL method generally showed weak relationships with the other extraction techniques. With two methods, AAO and *aqua regia*, it displayed no significant correlation at all. With all the other extractions, even though significant, it was on the lower end of the spectrum, compared to the correlations within the I- and Q-methods.

As stated above, there are many factors influencing the results of the specific extraction techniques and their correlation between each other. One factor of central importance comprises the soil chemical characteristics. The soil characteristics play a big role in the performance of each method, as the P availability depends very much on key factors such as e.g. pH, Corg and contents of Fe and Al (Moody et al. 2013; Schick et al. 2013).

3.3. Influence of basic soil characteristics on extracted P

The P solubility, (plant-)availability and extractability strongly depends on key soil parameters like pH, soil texture, $CaCO_3$ content, mineralogical composition, organic matter content and the presence of Al and Fe (hydr-)oxides (Abdu 2006). Therefore, we conducted a Pearson correlation test, comparing the amount of extracted phosphorus by each extraction method with the soil properties of all the tested soils (Table 7).

There are some general trends within the data set that could be observed. Soil properties which had the strongest influence on the extractable P were soil texture and pH. All performed extraction methods except CAL were affected by these parameters. Overall, the texture displayed a stronger correlation with the extractions than the pH. In another study of Zheng and Zhang (2012) it was concluded that soil texture and particle size distribution had a strong effect on the P fractions in soils and hence on their extractability. One reason for this may be the huge difference in sorption capacity of sand particles which differs substantially from that of silt or especially clay particles. As stated by O'halloran and Stewart (1987) the dependence of P solubility on texture may be due to the increase or decrease of the sites for P adsorption. The difference in sorption capability of the three texture fractions with reference to their specific surface explains the dramatic impact of this parameter. The specific surface of sand amounts to less than 0.1 $m^2\ g^{-1}$, silt to 0.1 - 1 $m^2\ g^{-1}$ and clay to 5 - 500 $m^2\ g^{-1}$ (Blume et al. 2010).

Table 7: Pearson correlation coefficients between soil extraction methods and soil properties; significance in * P > 0.05; ** P > 0.01; *** P > 0.001

	DGT	porew.	EUF	H_2O	CAL	Olsen	Mehl. 3	AAO	*a. regia*
	(I)	(I)	(I)	(I)	(Q)	(Q)	(Q)	(Q)	(Q)
pH	**-0.394***	**-0.329***	**-0.421****	**-0.400****	0.208	**-0.407****	**-0.526*****	**-0.588*****	**-0.453****
C_{org}	0.039	0.061	-0.013	0.032	0.040	0.151	0.085	**0.368***	0.195
C/N ratio	-0.065	-0.026	-0.184	0.064	0.218	-0.060	-0.033	-0.240	-0.169
$CaCO_3$	-0.300	-0.226	**-0.427****	-0.188	0.195	**-0.324***	-0.257	**-0.377***	**-0.352***
sand	**0.508*****	**0.398****	**0.517*****	**0.514*****	0.093	**0.466****	**0.616*****	**0.482****	**0.452****
silt	**-0.427****	**-0.347***	**-0.428****	**-0.436****	-0.078	**-0.381***	**-0.502*****	**-0.378***	**-0.351***
clay	**-0.376***	-0.276	**-0.392***	**-0.375***	-0.069	**-0.360***	**-0.464****	**-0.397***	**-0.376***
CEC	-0.276	-0.210	-0.227	**-0.325***	0.040	-0.202	**-0.395***	-0.239	-0.174
a. regia	**0.459****	**0.415****	**0.629*****	**0.441****	0.302	**0.613*****	**0.632*****	**0.856*****	1
N_{tot}	0.017	0.015	0.031	-0.051	-0.113	0.107	0.045	**0.395***	0.219
Me. 3_Al	0.132	0.057	0.276	0.133	-0.302	0.314	**0.384***	**0.647*****	**0.469****
Me. 3_Fe	**0.411***	0.303	**0.540*****	**0.354***	0.088	**0.605*****	**0.461****	**0.707*****	**0.691*****
AAO_Al	0.114	0.037	0.171	0.122	-0.105	0.279	**0.346***	**0.699*****	**0.440****
AAO_Fe	**0.356***	0.270	**0.458****	0.300	-0.063	**0.471****	**0.383***	**0.749*****	**0.669*****
CBD_Al	0.161	0.061	0.228	0.122	-0.207	0.284	0.290	**0.594*****	**0.347***
CBD_Fe	0.250	0.171	**0.309***	0.110	-0.119	0.302	0.112	**0.429****	**0.318***

It is notable that the sand content of our soils correlated positively with extractable P, while the silt and clay contents correlated negatively in all cases. This indicates a trend which would mean the higher the sand content of a soil, the more P could be extracted. Wuenscher et al. (2015) found a similar behaviour in their investigation of 14 P extraction methods applied to 50 agricultural soils from Central Europe. In their study, it was most of the weaker extractions that were influenced to a certain degree by texture. This differs somewhat from our results as we found medium to high correlations of texture with all methods except CAL. In our study the sand content asserted the highest influence on P extractability within the texture. All methods except CAL displayed medium correlations with this parameter while Mehlich3 stands out somewhat with an $r^2=0.616$. The silt- and clay fractions were correlated negatively on a low to medium basis with all extractions except CAL. Also, the results indicate that porewater P was not influenced by clay content. It is important to note that during analysis of a certain soil type, which depends on parameters of central importance such as clay mineralogy, clay content and pH, particular pools (and the buffering processes between them and soil solution P) may exhibit stronger influence on the P availability than in another soil type (Moody et al. 2013).

The ratio of sand, silt and clay fractions exhibits a strong influence on complex chemical, mineralogical and biological processes and hence the characteristics of a soil. Soil texture is a key soil characteristic and is closely linked to the pH value of a soil (Blume et al. 2010). For instance, one effect of a high sand content is the very limited availability of sorption sites for H^+ and other molecules, which lower the pH when they are not bound but remain in solution. While depending on various factors, a high sand content can be generally connected to a low pH which was confirmed by our data. We could observe a strong negative correlation between sand and pH and conversely positive ones with silt and especially clay.

In our study, as with the silt and clay contents, the pH showed a negative correlation with the extraction methods; if a negative correlation exists, this means the higher the pH, the less P can be extracted. The investigation of the influence of pH on extractable P showed the highest correlations with Mehlich 3 and AAO, *aqua regia* being slightly lower. This is rather surprising as it was expected that the stronger methods would extract P independently of a pH value because of their ability to dissolve non-labile fractions. The other methods, except CAL, correlated on a low to medium level with this central parameter. The I-methods such as DGT, H_2O, soil porewater and EUF are not very dependent on the pH level because they generally extract easily accessible fractions of P (Schick et al. 2013). In the study of Wuenscher et al. (2015) it was found that about half of their performed extractions (weak and strong ones) correlated negatively with pH and therefore seemed to be influenced by it. They attributed it to the connection between pH and $CaCO_3$ content, where they observed a strong relation; these parameters also displayed a negative correlation. However, in our data we could only observe significant connections with $CaCO_3$ content for EUF, Olsen, AAO and *aqua regia* which also showed negative correlations. This could be explained by the differing extraction mechanisms as the correlation displayed between pH and $CaCO_3$ was r^2=0.709.

The CEC displayed the strongest connection with the soil texture (data shown in Appendix), in particular with the clay and sand content; it was also pH dependent. Blume et al. (2010) mentioned that CEC is influenced primarily by pH, the amount and kind of clay and the amount of humic organic matter. The CEC is an indicator for the sorption-capacity of the soil for cations such as Fe, Al and Ca and hence also for the ability of the adsorption of the various fractions of P anions. The fact that for all methods except water extraction and Mehlich 3 (which showed a weak correlation) there was no correlation with this parameter, indicates that the P availability in our tested soils was not determined by desorption mechanisms.

For the extractable Fe- and Al-contents the highest correlations were achieved by the strongest extraction methods, namely AAO and *aqua regia*. This was expected, as these methods are able to dissolve P that is strongly bound to both inorganic and organic complexes. Mehlich 3 also displayed correlations with both Fe and Al but on a much lower scale. The amount of extracted P of Olsen showed a relatively high correlation with the Fe-content even though this method is usually more closely associated with Ca. Al did not seem to have any effect on the weaker extraction techniques. It appeared that also the weak extractions DGT and EUF were affected by Fe contents as they showed low to medium correlations respectively. Fe-contents showed strong correlations with Total P which may indicate that a big fraction of P is either weakly associated or strongly adsorbed to or occluded by varying iron-species.

One rather surprising result were the medium to high correlations between Pseudo Total P (*aqua regia*) and the I-methods. This could be viewed as an indication for the big influence the Total P status of a soil asserts on the easily available fractions in our tested soils. The higher the total P in a soil, the more P should proportionally be stored in labile fractions. The fertilization history might be responsible for the high total P concentrations and therefore for the occupancy of a high number of the sorption sites. As stated by Schnug and De Kok (2016) due to intensive P fertilizer applications over decades a build-up has taken place in many agriculturally used soils. It appears that this has led to the occupation of many of the potential sites for P sorption and hence to lower abilities of the soils to strongly fix added P. As there was no available fertilization history of our examined soils it is unclear which amount and which type of fertilizers were applied over the years and how much of that P got conveyed off field. Wuenscher et al. (2015) reported that the results of their cited study were likely to have been influenced to a considerable amount by the fertilizer residues and their different solubility. These may have partly masked the influence of soil properties and thus produced weaker correlations. As we only examined soils from agricultural fields it is very probable that the same effects also influenced our results in a certain way.

It is noteworthy that the CAL method did not show any significant relations to any of the investigated soil parameters. Schüller (1969), who developed this method, put forward that the CAL method was only suitable for soils with a pH higher than 6 and a content of free and reactive $CaCO_3$ below 15 %. When we splitted the soils into 2 groups with one above and one below pH 6 there was still no relation to be observed; with one exception: *aqua regia* in the soils above pH 6 showed a very significant correlation. In our set of 41 soil samples there were 10 with a pH below 6 and 8 with a $CaCO_3$ content of more than 150 g kg^{-1}. While it is not clear how much of the Calcium Carbonate was reactive, it is likely that we could not observe any significant correlations due to the use of this method for soils it is not suitable for.

One important fraction of P in soil, i.e. the organic P pool, was not considered in this study. According to Eriksson et al. (2009) various forms of organic matter like Fe-, Al-, and Ca complexes and amorphous molecules of Fe and Al are involved in binding and sorption processes of P. The relative amounts of inorganic P in soils are usually 50 - 75 % but can vary from 10 - 90 % (Sumner 1999). Our results may have been influenced by the organic P to certain extents.

The amount of P extracted by a soil P test is only partly reflecting the amount of P that can actually be taken up by a specific plant on a certain soil. To evaluate the predictability of a soil P test, a correlation of the extracted P with plant parameters such as yield or P content within the tissue (Schnug et al. 2016) can provide a valuable basis. It was suggested by Sibbesen (1983) that such evaluations should include many different soils and be made under well controlled conditions with soil P as the only limiting growth factor. He stated that pot experiments are better suited for soil test evaluation than field experiments because in the field many uncontrollable growth factors occur. Therefore, we performed a 6 week pot experiment with rye plants (*Secale cereale*) and afterwards determined plant biomass and shoot P.

3.4. Plant experiment

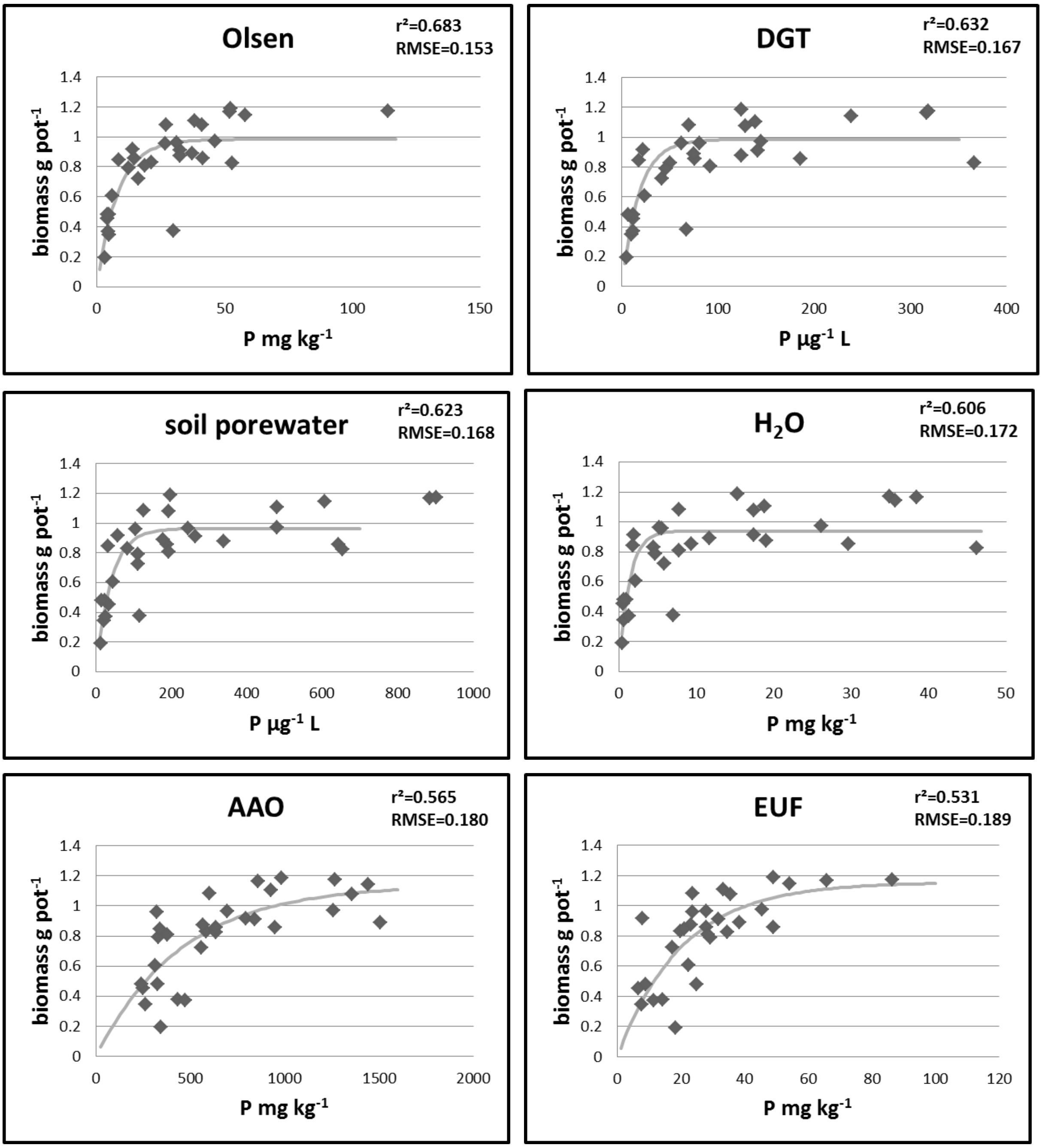
A detailed description of the plant experiment was given in chapter 2.5. The results showed a wide range of values for the dry weight of the biomass and shoot P concentration depending on the soil the plants were grown on. The 4 pots of the soil that produced the lowest biomass had a mean of 0.19 g dry weight, the ones with the heighest biomass weighed on average 1.19 g. The mean of the biomass for the subset of the 30 soils used in the plant experiment was 0.81 g with a standard deviation of ± 0.28 g. The soil which gave rise to the minimum shoot P concentration (calculated over 4 replicates) exhibited an average of 1110 mg kg^{-1} and a maximum of 4390 mg kg^{-1}. The mean of the shoot P concentration for the subset of the 30 soils added up to 2480 mg kg^{-1} with a standard deviation of ± 1010 mg kg^{-1}.

Table 8: Biomass (g) and Shoot P concentration (mg kg^{-1}) of early dry matter of *Secale cereale* (rye) after 6 weeks of growth; DW, dry weight; conc., concentration; Stdv, standard deviation; values shown are the mean of n=4 pots for each soil

	biomass			**shoot P conc.**		
Soil	**DW (g)**		**Stdv**	**conc. (mg kg^{-1})**		**Stdv**
1	0.38	±	0.04	1608	±	148
2	0.96	±	0.15	2553	±	319
3	1.08	±	0.12	2646	±	168
4	0.83	±	0.11	2207	±	181
5	0.92	±	0.14	1782	±	147
6	1.08	±	0.09	3767	±	173
7	1.17	±	0.09	4386	±	311
8	0.91	±	0.10	4337	±	359
9	1.17	±	0.05	3945	±	288
10	1.14	±	0.16	3642	±	204
11	1.19	±	0.14	3685	±	99
12	0.61	±	0.11	1428	±	114
13	0.79	±	0.15	1676	±	158
16	0.86	±	0.16	3260	±	124
17	0.19	±	0.03	1553	±	149
18	0.35	±	0.06	1114	±	51
19	0.45	±	0.07	1290	±	193
20	0.48	±	0.06	1404	±	76
21	0.37	±	0.04	1269	±	347
22	0.88	±	0.14	2244	±	104
23	0.83	±	0.06	3298	±	256
24	0.86	±	0.11	1999	±	183
25	0.81	±	0.05	2130	±	124
26	0.48	±	0.03	1254	±	63
27	0.85	±	0.10	1725	±	136
28	0.96	±	0.14	2411	±	161
29	0.89	±	0.08	3349	±	336
30	0.97	±	0.06	2939	±	176
31	0.72	±	0.22	1930	±	148
32	1.11	±	0.12	3531	±	250
mean	0.81	±	0.28	2480	±	1010
median	0.86			2230		
min	0.19			1110		
max	1.19			4390		

3.5. Correlation of plant biomass and plant P values with extracted P

We compared the extracted P by the various methods with the biomass P in rye (*Secale cereale*) after 6 weeks of growth in a growth chamber with controlled conditions (Figure 12) and fitted the data using the Mitscherlich model ($y=a*(1-e^{-bx})$). The different soil P extractions displayed the ability to predict the crop response (biomass) and shoot P to various degrees; the only exception in both cases was CAL which was not suitable to predict the biomass with the applied model and showed only a weak correlation with shoot P. The performance of a Pearson correlation of shoot P with biomass displayed an $r^2=0.807$ (data not shown).



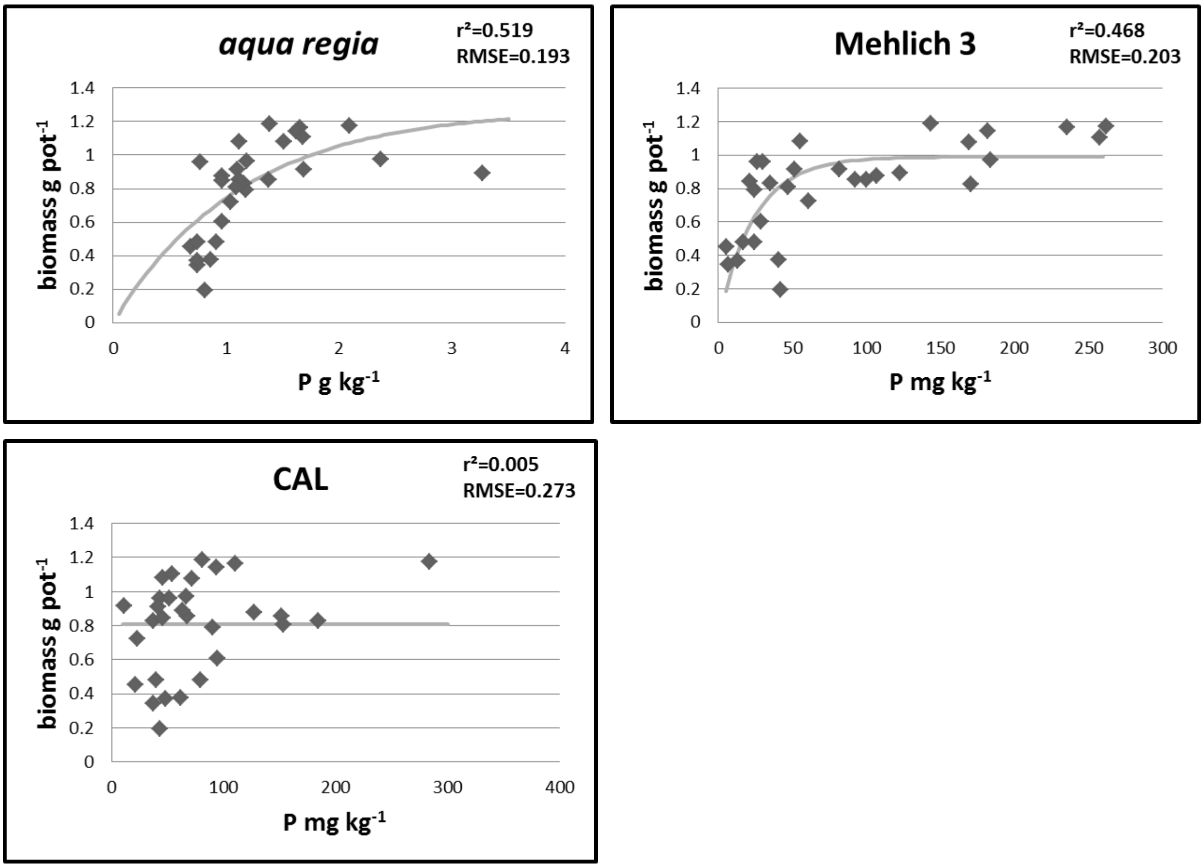


Figure 12: Biomass as a function of soil P values extracted by the different methods; the points represent the mean of n=3 soil replicates correlated with n=4 pot replicates; lines are predicted with the Mitscherlich model

With regard to the relation of extractable soil P with shoot biomass, the Olsen method performed best with an r^2=0.683 and outperformed DGT (r^2=0.632) and the other intensity based methods soil porewater (r^2= 0.623), water extraction (r^2=0.606) and EUF (r^2=0.531). The quantity based methods like AAO (r^2=0.565), *aqua regia* (r^2=0.519) and Mehlich 3 (r^2=0.468) showed in general lower predictive power than the aforementioned methods.

However, the correlation between shoot P (mg/kg) and the extractable soil P showed a different picture. Generally, correlations were very high and here DGT performed best with an r^2=0.852. Six out of eight of the other methods positioned in the same range: Olsen (r^2=0.808), EUF (r^2=0.809), H_2O (r^2=0.799), Mehlich3 (r^2=0.793), AAO (r^2=0.792) and soil porewater (r^2=0.765). *Aqua regia* with r^2=0.680 performed markedly worse and CAL showed only a weak correlation with r^2=0.362.

Table 9: Pearson Correlation of the Shoot P with the P extracted by the various extraction methods; significance in * P > 0.05; ** P > 0.01; *** P > 0.001

	DGT	porewater	H_2O	Olsen	EUF	CAL	Mehlich 3	AAO	*a. regia*
Shoot P	**0.852*****	**0.765*****	**0.799*****	**0.808*****	**0.809*****	0.362*	**0.793*****	**0.792*****	**0.680*****

Comparing the predictive power of extraction methods for plant P uptake in a review of 29 papers, Sibbesen (1983) divided them into three groups, with anion-exchange resins being the best, water and sodium bicarbonate (Olsen) intermediate and all acid based methods performing the worst. Also Fixen and Grove (1990) reported a superiority of resin methods against all the others in a multitude of studies they reviewed and stated that they were suitable better over a wide range of soils (Schnug and De Kok 2016). Our results match these declarations insofar as DGT ranked highest and second highest accordingly. The observation of the rather similar performance of I- and Q-methods in respect to shoot P diverged from the cited studies. In the investigation of various other authors it was found that DGT performed superior on soils were P supply was limited by diffusion (Six et al. 2013; Tandy et al. 2011; Mason et al. 2010; Menzies et al. 2005) while Nawara et al. (2017) found that DGT was not superior to other extraction methods on European soils. Their conclusion was that DGT was not a better predictor of plant available P when the soils are more affected by the quantity than the intensity of P which is usually the case in European soils. They also put forward that I-methods should have an advantage on soils that sorb P strongly which would be the case for highly weathered tropical soils. As our results did not suggest a clear superiority of DGT, it seems that generally in our soils a big part of the present P was only weakly adsorbed in comparison to tropical soils and hence the quantity of P appeared to be the critical factor in terms of P availability.

The European soils are much younger, not as highly weathered as tropical soils and do not contain as many Fe-/Al-oxides (Blume et al. 2010) that have the ability to strongly bind P. The P in our tested soils seemed to be more easily accessible than on highly weathered tropical soils. This is one factor that might partly explain the overall relationship between our tested extraction techniques and the shoot P and to a certain degree also the correlations with biomass. If there are high quantities of easily available P which are not diffusion-limited, the various methods all correlate well with plant P and biomass, even if used on soils with very different characteristics. An indication for this is the rather unexpected high correlation between *aqua regia* and shoot P. Furthermore, the strong extractions AAO and *aqua regia* and also Mehlich 3 all showed a medium correlation with biomass. Like stated earlier, the accumulation of P in the soil as potentially available fractions due to the (over-)fertilization over decades may also have had an impact on our results.

Our result for H_2O extraction corresponds well with that of Kulhánek et al. (2009) who found a correlation of $r^2=0.66$ between water extraction and P uptake of barley plants. They found the relationships for other methods like Mehlich 3 and anion exchange membranes to be significantly lower. Wünscher (2013) found in her investigation with wheat plants that the weaker extraction methods achieved better correlations with the total plant P uptake which was determined in mg m^{-2}. In her study the highest correlation was also achieved by H_2O extraction, though with a much lower $r^2=0.337$. In stark contrast to our results, the rest of the investigated methods like e.g. Mehlich 3, AAO, Total P, Olsen did not show any correlation and failed to predict P uptake by wheat plants. The reasons for the strong divergence from our results may be manifold: differing P contents and buffer capacities of the examined soils (chapter 3.3.), variations in the execution of the various extractions (chapter 3.2.) and also different traits of the plants used for the experiment.

A crucial factor for the predictive performance of an extraction method may be the kind of plant which is used and its respective uptake mechanisms. As Six et al. (2013) pointed out, reactions in the rhizosphere and/or root mobilisation may influence P uptake to a certain extent. These mechanisms are not taken into account by the extraction methods. In their experimental comparison between P uptake of rice and maize on tropical soils, the predictive power of various extraction techniques including DGT depended on the plant species used. For maize, DGT showed the highest predictive power but for rice it was methods like Olsen and Mehlich 3. In our study, it is unclear which role these mechanisms might have played.

Another central factor for the predictive quality of a method is the moment of sampling of the plant. Different plants showed to have different time frames in which they aquire P (Moody et al. 2013). Mundus et al. (2017) found in a field experiment in northern Europe with barley plants a high $r^2=0.83$ for DGT when the P concentrations in the youngest fully emerged leave of unfertilized plants were measured 30 days after plant establishment. When they took measurements again later in the growth season the correlations decreased and at harvest had completely disappeared. They attributed this phenomenon to a number of external factors like climatic conditions which cannot be reflected by soil tests. A conclusion was that these inherent factors greatly limit the validity of soil tests in predicting the P availability to plants over the entire growing season. However, Mundus et al. concluded that under field conditions the DGT method appeared to be superior to the other tested methods like Olsen when it came to the correlation of P tissue concentration during early vegetative growth.

It is worth mentioning that even though we observed strong correlations on many levels between the P extraction methods and shoot P respectively biomass, interpretations still have to be made with caution. Degryse et al. (2009) declared that as soil tests are generally developed after empirical methods, a strong correlation can be an indication for the factors and mechanisms that determine P uptake but it does not prove them.

To shed more light on the relationships between the many parameters influencing the results, a principal component analysis was performed.

3.6. Principal component analysis

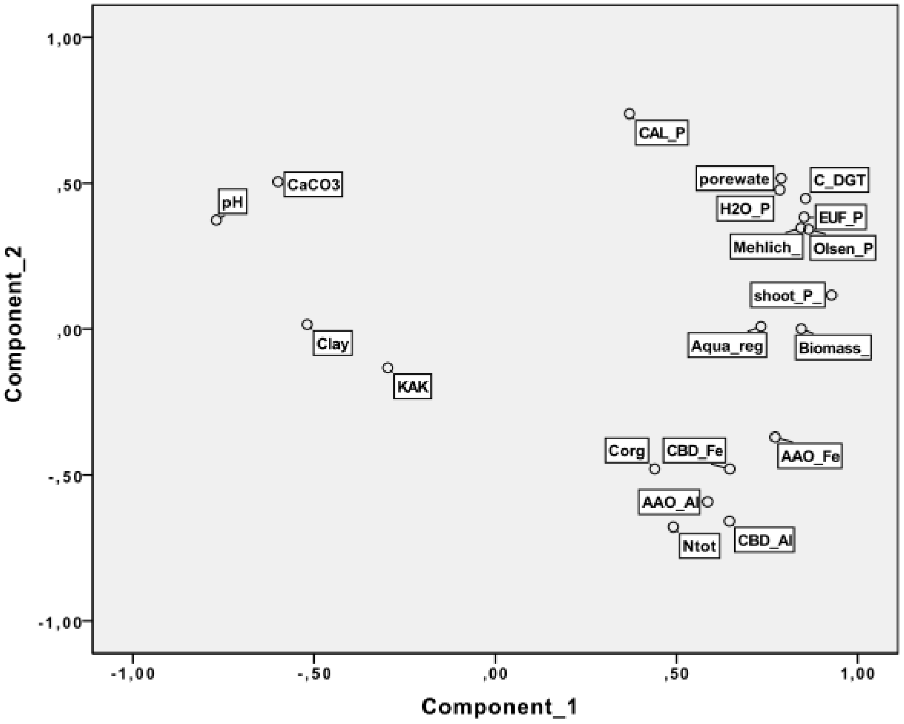


Figure 13: Principal Component Analysis (PCA) for soil parameters, extraction methods and plant response

In order to review our findings, we performed a Principal Component Analysis (PCA) in which we extracted 4 components. Component 1 explained 49.6 %, while component 2 accounted for 19.4 % (together 69 %) of the total variance. The PCA confirmed our previous assumptions. The investigated P fractions extracted with different methods were closely clustered. This indicates that even though the P amounts extracted from a soil varied depending on the method chosen, when viewed over a wide range of soils, it was proportionally always rather constant relating to total P. As expected due to the previous investigations, the CAL method was aloof from the rest of the extractions.

Another remarkable point concerns the position of plant response, i.e. biomass and P concentration in the shoot tissue. These two parameters were closely clustered with P extraction methods, particularly to the *aqua regia* P. This indicates that it was primarily the total amounts of P in the various soils which was responsible for the plant response. Another sign for this is the position of the AAO and CBD extractions. These two methods that extract the amorphous and crystalline Fe- and Al-oxides were situated aloof the cluster of the P extraction methods which indicates that they did not influence them to a great extent.

4. Conclusion

The amount of extracted P by 9 different methods was compared and it was established that a comparison based on extracted amounts of P was not productive. In the case of DGT, a direct comparison with the other methods was not possible. This was not because of the manifold factors that in general make the comparison of different techniques problematic but due to the differing unit of measurement which for DGT (and soil porewater) was µg L^{-1} while for all the other methods it was mg kg^{-1}. Even though the direct comparability was not possible, a relative comparison based on the application of correlations was feasible. The correlation of extracted P by the various methods showed the strong relatedness of DGT not only with the other weak extraction methods (I-methods) but also with two of the stronger (Q-methods) ones; namely Mehlich 3 and Olsen. This result indicated the strong effect of certain soil parameters on the P extractability.

The soil parameters that exerted the strongest effect on P extractability over the whole spectrum of methods appeared to be pH, texture and the amount of Total P in the soil. The latter correlated with all methods except CAL. This suggested that in our study the amount of extractable P was to a high degree determined by the Total P status of a soil and suggested that this parameter also asserted a big influence on the easily available fractions in the soils. The higher the Total P, the more P was apparently stored in proportional labile fractions. Hence, P diffusion will likely not be the limiting factor for plant availability as it is typically the case in highly weathered tropical soils.

In contrast to the findings of many other studies, in our experiment DGT did not perform markedly better than other methods in predicting the plant biomass and plant P uptake. The P extracted by DGT did show the highest correlation in comparison to all the other methods with regard to plant P uptake of *Secale cereale* after 6 weeks (measured concentration of P in the shoot) but became second after the Olsen method regarding the early dry matter. Many authors had reported a superiority of DGT over other extraction methods when the tests were conducted on highly weathered tropical soils. Nawara et al. (2017) reported that on European soils, were the P supply was not determined by diffusion, these results could not be reproduced. Our study confirmes their findings and stresses the central role of the soil characteristics with regard to the performance of a specific soil test. DGT could show its advantage when the P availability of soils was influenced predominantly by diffusion which is the case in highly weathered tropical soils. In the Austrian soils that were examined in this study, the quantity of P seemed to be the decisive factor and hence there was no clearly superior extraction technique with regard to the predictive power of P availability to plants.

The P that becomes available to the plant during the next growing season and beyond is dependent on many factors. Likewise, the predictive power of an extraction method depends on many factors, e.g. on the crop grown and its respective P uptake mechanisms, the point in time the plant sampling takes place, the climatic situation, the soil characteristics to name but a few. DGT did not fulfill the promise of being the long sought-after universal P test that performs superior than all other methods over all kinds of soils. Instead, the prevailing conditions have to be taken into account before choosing an appropriate soil P test. Nevertheless, in our experiment DGT performed very well in comparison to the other methods and further research should include a diversity of different soil - climate - crop situations (field experiments) in order to determine under which circumstances DGT can provide a valuable alternative to other P extraction methods.

5. Literature

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