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ASSESSING THE PLANT AVAILABILITY OF A NEW PHOSPHORUS FERTILIZER FORMULATION

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Declaration of originality

Hereby, I declare that this master thesis was written by me and that I did not use any other sources and means than specified. This master thesis has not been submitted at any other university for acquiring an academic degree.

Mai 2013, Tulln Paul Dickinger, Bakk.techn.

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Contents

Abbreviations:

ALT alternative fertilizer formulation
C_{CAL} CAL extractable P concentration
C_{DGT} time averaged concentration at the surface of the DGT device
C_{SOL} soil solution P concentration
CAL calcium-acetate-lactate
CDC critical deficiency concentration
DGT diffusive gradient in thin films
PUE phosphate-use efficiency
SSP single super-phosphate

1. Introduction

In every living organism there are numerous functions and processes, where Phosphorus (P) serves as a key element and cannot be replaced by any other element. Primarily being taken up by plant roots as orthophosphoric acid (H_3PO_4), orthophosphate (PO_4^{3-}) is an essential element in the structure of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and adenosine triphosphate (ATP). It is also involved in phospholipids that form all cell membranes. For DNA and RNA, it serves as a backbone-like component. It connects the pentose of one nucleotide with the pentose of the following nucleotide, leading to a phosphate-pentose-phosphate structure of the DNA and RNA, out of which the nucleobases stick out forming the genetic information. ATP, with its three phosphate molecules bound to an adenosine nucleoside, functions as an energy carrier and source of energy in cells (Schilling, 2000). Phosphorylation and dephosphorylation of histones play an important role in the accessibility of DNA during transcription processes and is subject to numerous studies about epigenetics where the outcomes of transcription are altered without changing the underlying DNA sequence (Jenuwein & Allis, 2001). Given these fundamental roles of P in plant metabolism, it goes without saying that a sufficient availability of P in soil is necessary to sustain a well-functioning plant production system, which in turn secures an adequate P supply for consumers.

Amongst the major plant mineral nutrients, P is considered to be the one with the smallest world resources. Thus, on a global scale, it is crucial that the agricultural use of it is managed more efficiently (Syers et al., 2008). Considering that P is the 11th most abundant element in the earth's crust and resources are expected to last between 104 and 470 years, one might think that there is no need to worry about the P supply for coming generations. However, the inefficient use of P does not only affect resources and reserves of P negatively, but may also lead to an over-proportional accumulation of P in agricultural soils and eutrophication of the surrounding environment by runoff into surface waters. Therefore, the emphasis on efficient P use in the last years is important for creating a state of awareness about the need of a sustainable P utilisation (Syers et al., 2008; Mason et al., 2010).

Jasinski (2010) stated, that over 77 % of the world reserves of phosphate bearing minerals (phosphate rock; PR), that can be exploited economically with current mining technologies, are found in only four countries (Morocco, China, Jordan and South Africa) and from the total global production of PR about 80 % goes to P fertilizer production, 5 % to animal feed purposes and the residual 15 % go to industrial uses. The mean P fertilizer application in Austria in the last five years (July 2007 – June 2012) amounted to nearly 12200 t P (Agrarmarkt-Austria, 2012). Ideally, every application of fertilizer (agricultural, horticultural etc.) has to be evaluated concerning its necessity, efficiency and adequacy for various reasons.

- Fertilizers are big annual expenses for farmers, which have to be employed economically according to the law of diminishing returns;
- Agricultural production systems have to ensure that crop production does not involve depletion of naturally accumulated P in the soils;
- PR is a finite global source and therefore has to be conserved to ensure the P supply of future generations because of its irreplaceable relevance for all living organisms;
- There is a need to keep the applied P in the production systems where it was applied and minimise run-off into water bodies;

For the most part, methods for evaluating P-use efficiency (PUE) of plants take the aspects listed above into consideration. Depending on the definitions adopted and the type of data included, a wide range of different issues in the plant-soil relationship can be addressed. Different concepts for evaluating PUE and altering fertilizer-use efficiency are described in chapter 2.2 and 2.4. For being able to interpret PUE calculations, one always has to bear in mind the presence of different so called P pools in soils. Within these pools soil P is in a constant state of shifting (Figure 1). Starting with a very limited P concentration in soil solution (normally ranging from 10^{-4} M (very high) to 10^{-6} M (deficient)) (Syers et al., 2008) which is immediately available for plant uptake by roots, a bigger amount of inorganic P is located in a second pool with a closer interaction with the surfaces of soil particles. These two pools are considered to rapidly adjust equilibrium and therefore the surface-adsorbed P is readily available for plant uptake within a short time span. Since lowering the P concentration in soil solution creates a concentration gradient within the soil solution, the release of surface-adsorbed P maintains equilibrium. P which makes up the third pool shows a stronger bond to soil components or may be adsorbed on inner surfaces of soil components. Therefore the accessibility of these P compounds for plant uptake is less. The forth pool includes P sources that are very strongly bonded to soil components, may be precipitated as only slightly soluble compounds or may show very low availability due to its location within soil component matrices, which lead to a very limited release (often over many years) of these P compounds into pools with higher plant availability (Syers et al., 2008).

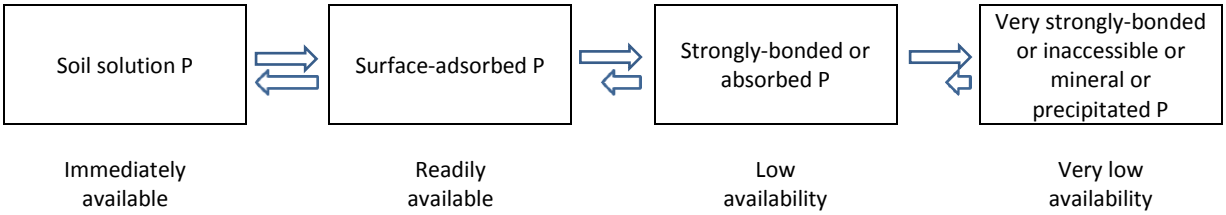

Figure 1 Diagram for forms of inorganic P in soils categorized in terms of plant availability; from Syers et al. (2008) p.24

The replenishment of one pool from another always depends strongly on the P-buffer capacity of the soil, i.e. to which extent the soil solution concentration resists changes, e.g. against the increase after a fertilizer application or against a decrease during P uptake by plants (Olsen & Khasawneh, 1980). Additionally, P solubility in soils is always in a close relationship with the soil pH. Whereas in alkaline soils calcium (Ca) is the major determinant of P solubility and therefore plant availability, in acidic and neutral soils we observe mostly high levels of P sorption to hydrous oxides of aluminium (Al) and iron (Fe) when they are present.

Testing soils to determine the amount of P in plant available pools is subject to continuous scientific investigation and discussion. Therefore multiple testing methods are applied worldwide to assess the state of P supply in soils. Whereas the majority of methods use extraction solution approaches with various extractants, soil to extractant ratios and deployment time, other methods try to mimic plant root behaviour more naturally by creating an infinite sink and induce diffusional transport of mineral nutrients to the measuring device.

A wide range of extractants is used worldwide but there is no general consensus which one produces the best predictions for plant available P. In Europe, mainly two methods are employed, the Olsen P method (using a 0.5 M sodium bicarbonate solution) and methods based on a calcium-acetate-lactate (CAL) extractant. The differences in extractants inevitably lead to different fractions of soil P being mobilized and designated as plant available P. Furthermore, many processes in the rhizosphere of plants affect the supply of readily plant available nutrients. Diffusional or massflux transfer of ions to the root, root growth associated with encountering fresh surfaces, secretion of root exudates and influences on root microenvironments are only a few to mention. Therefore, it is unlikely that one can combine all the above mentioned factors in one extractant solution, as long as none of the processes above is dominant. A different approach based on mimicking plant roots physio-chemical uptake of solutes and creating an infinite sink for nutrients, inducing a diffusional resupply from the solid phase, is subject to extensive investigations since the late 1990s leading to the establishment of the Diffusive Gradients in Thin-films (DGT) method (Davison & Zhang, 1994). Originally being developed for the investigation of trace metals concentrations in aqueous solutions, it since has become a powerful tool in soil science for the measurement of trace element and P bioavailability and recently also for chemical-imaging of rhizosphere processes (Zhang et al., 1998; Zhang et al., 2001; Santner et al., 2012).

Since research and the public put more and more emphasis on improved PUE of fertilizers, companies are trying to take advantage of this change in opinion and consequently offer fertilizers which are allegedly better in providing readily plant-available P to the crops. In the first part, this master thesis tries to give a solid background about the effect of applying fertilizers to agricultural

soils and what is the fate of applied P in soils. Following an introduction to PUE concepts, strategies of fertilizer producers as well as of plants for improved PUE are depicted and the novel DGT technique for assessing plant available P in soils is described. Afterwards, two experiments with a standard single super-phosphate (SSP) fertilizer and a fertilizer with an alternative formulation (ALT) for presumably better plant availability are presented.

The overall objective of this research work was to assess the differences in plant availability of two distinct P-fertilizer formulations acquired from Timac Agro Austria (www.at.timacagro.com) in terms of their effects on plant growth and development (phenology, biomass production and tissue nutrients concentrations). Additionally an incubation experiment of the fertilizers solely with the substrate used in the greenhouse experiment was conducted to evaluate the effects concerning plant-availability and extractability of soil P in a higher temporal resolution and under more constant conditions than would have been able to be established in greenhouse experiments.
Our scientific hypotheses were the following:

- The ALT fertilizer shows higher plant availability of P compared to the SSP fertilizer. Hence, we will see differences in plant growth, where plants which received ALT fertilizer show increased vigour in early phases because of the better supply with plant available P.
- With the induced early vigour and better plant availability of the applied P, plants fertilized with ALT will show higher biomass production and higher P uptake rates.
- Soil fertilized with ALT fertilizer will show higher amounts of plant available P with both the conventional CAL extraction method as well as with the DGT method, because of its higher resistance to sorption processes compared to the SSP fertilizer.
- Time series analysis of the incubation experiment will show lower declines in P plant availability for treatments that received the ALT fertilizer.

Finally we tried to draw conclusions from all the above and furthermore, future prospects are addressed.

2. State of the Art

2.1.Fate of applied P in soil

After an application of fertilizer containing soluble P species numerous reactions take place in soils within a short time span making the added P less plant available. These reactions are not only governed by the type of P added, but also by soil compounds, soil properties and non-P components of the fertilizer applied. Mostly, adsorption reactions on surfaces of soil particles, diffusion processes where P is moved deeper into the matrices of soil compounds, precipitation reactions which form new solid soil compounds, mineralisation of organic phosphorus and immobilisation of inorganic P are predominate (Hedley & McLaughlin, 2005; McLaughlin et al., 2011).

2.1.1. Sorption and precipitation of P

Where the P concentration does not exceed the P sorption maximum of the soil and soil solution concentrations of Fe, Al, Ca, magnesium (Mg), P and trace metals do not exceed their solubility product of mineral phases, sorption reactions are the key processes determining P solubility and therefore the efficiency of added P. Surface-based sorption reactions are commonly fast and reversible. However, diffusion of P below surfaces of Al and Fe oxides and subsequent sorption to inner surfaces is a slower process leading to higher strength of adsorption, thus resulting in lower plant availability of these P compounds. The amount of P present in each of these individual pools (Figure 1) depends mostly on the history of P fertilization on the individual site as well as the time span since the last fertilizer applications (McGechan & Lewis, 2002).

At the same time, precipitation reactions can become important in regions where P concentrations are high (e.g. around fertilizer granules or injection zones for fluid fertilizers). Whereas in acidic and neutral pH soils these processes are mostly governed by the abundance and type of Fe and Al minerals in the soil, calcium carbonate ($CaCO_3$) is the key determinant of P solubility in alkaline soils. This is a consequence of the solubility of Al-, Fe- and Ca-phosphates under varying pH values, where Al- and Fe-phosphates are little soluble in acidic and neutral soils and Ca-phosphates show the opposite behaviour being little soluble in alkaline soils (Schilling, 2000).

Additionally, after an application of mineral P fertilizers, zones with high rates of precipitation and zones with high rates of sorption develop around fertilizer granules. The dissolution of solid fertilizers needs soil pore water, resulting in a water movement that is directed towards the fertilizer granule and against the diffusion of P out of and away from the granule. This leads to a slowdown or restriction of the P ion's movement according to concentration gradients and causes high P concentrations and thus high rates of precipitation of P in these regions near the fertilizer granules. Further out, sorption processes are dominant due to the lower concentrations of P in the soil

solution. The inwards directed soil pore water movement is maintained as long as concentration differences are present and have not been balanced by dilution, sorption and precipitation where as a consequence P ends up in its least soluble chemical compound for each specific pH (Schilling, 2000; Hedley & McLaughlin, 2005).

2.1.2. Mineralisation and immobilisation of P

Mineralisation, i.e. the release of inorganic P from decomposition of organic P compounds, and immobilisation, i.e. the incorporation of inorganic P from soil into organic P compounds, are important sources and sinks for plant available P. Whereas for N these two processes can be quantified to some extent via critical carbon over nitrogen (C:N) ratios for microbial activity, attempts to establish critical C:P values and subsequently estimate release rates due to mineralisation have not been successful. This is because of the fact that firstly most of the P in green or senescent plants is already inorganic P. Hence, the release of inorganic P out of organic matter by microbial decomposition is no real mineralisation and therefore does not need critical C:P ratios. Secondly, microorganisms are able to store P in their cells as condensed phosphates during high P concentration periods, which leads to release or uptake of P independent of C:P ratios in soils. (McLaughlin et al., 2011).

2.2. Concepts for calculating PUE

For the assessment of nutrient-use efficiency of plants numerous indices, methods and concepts can be applied, which mostly differ in terms of applied definitions and whether yield, nutrient uptake, fertilizer amount, fertilizer recovery or soil analysis is taken into account for calculations. According to Cassman et al. (1998) five major concepts are being used for evaluating nutrient use efficiency and consequential PUE of plants:

- Direct method: Fertilizer uptake by plants is measured directly by radioactive labelling of fertilizer (e.g. ^{32}P labelled) and afterwards comparing it to the applied fertilizer amount. Due to the short half-life of ^{32}P and the highly sophisticated procedures, studies are inevitable short and costly.
- Difference method: Differences in yields or fertilizer uptakes are calculated between non-fertilized and fertilized plots and divided by the applied fertilizer amount. One disadvantage of this method is that the outcome depends largely on the yield or fertilizer uptake of the non-fertilized plot, and this, in turn, depends largely on the amount of plant available P in the control soil and not entirely on the efficiency of the applied fertilizer itself. Furthermore differences in environmental conditions between years make comparisons of calculated PUE more difficult.

- Balance method: The total amount of P taken up by plants is divided by the applied fertilizer amount. It does not make any differentiation between yield of non-fertilized and fertilized plots and in one sense, uses a system approach where changes in all nutrient pools are taken into account irrespective of the origin of the nutrient (i.e. fertilizer P or soil P).
- Partial factor productivity index: Yield of plants is put in direct relation to the applied fertilizer amount, giving a value that depicts a product produced per kilogram of nutrient applied relationship.
- Physiological efficiency index: The differences in yield of non-fertilized and fertilized plots are put in relation with the increase of nutrient uptake by the plants.

Additionally, PUE can be evaluated by measuring the increase of P fraction in readily plant available P pools. Total P content of soils (consisting of plant available as well as not plant available P) will not change significantly after an application of P fertilizer since the addition of P at amounts usually applied in cropping systems only influences the total P content of soils to a relatively small extent. However, extraction methods and other soil analysis methods (chapter 2.8 and 3.2) may be able to display changes in the labile P pools following an application of P more clearly, although these methods are accompanied with some disadvantages that have been addressed in the introduction (Syers et al., 2008).

As previously described in chapter 2.1, applied P is subject to a number of soil reactions that lead to a more or less temporary fixation and removal of P from plant available P pools. However, this does not mean that it becomes completely unavailable to plants. Measurements with the direct methods only attribute 25 % of P taken up by plants to the fertilizer which was applied to the crop in the first year and the rest must come from soil reserves that also have to be replenished to sustain the level of plant available P in the soil. Thus, the balance method, which measures PUE as total P uptake as a percentage of the P applied is a good choice for PUE calculations and uses a system approach where changes in all P pools are taken into account (Syers et al., 2008). For this reason, the balance method and also methods including soil analysis have mainly been used in this thesis to assess PUE of the two applied P fertilizer formulations.

2.3. P transport to plant roots and P uptake by plants

Besides interception, where plant roots interact directly with nutrients in soils and grow towards them, two main nutrient supply pathways to plant roots exist in soils: mass flux and diffusion. Mass flux means that solutes are supplied with the flow of their solvent (i.e. water) towards the root surface, where they are taken up. This process is induced by the transpiration of plant's above ground biomass resulting in a flow of water towards plant roots and up to the shoots. Multiplying nutrient concentrations in soil water and the amount of water transpired by the specific plant, one

can evaluate if nutrient accumulation in plant material is explained by this specific process alone. For N, Mg and Ca this is the standard case. However, for some nutrients (e.g. P), concentrations in soil solutions is sometimes as low as 10^{-6} M and therefore the accumulated amount of P in plant material cannot be explained by mere mass flow but diffusional transport must have a higher importance here. It means, that in contrary to mass flow, not the movement of the solvent itself is important, but the movement of ions in the solvent from zones with high concentrations to zones of low concentrations. This continues until concentration equilibrium is reached. Nutrient uptake by plants creates such concentration gradients in the rhizosphere by actively lowering the concentration, which initiates a diffusional resupply of ions according to the Brownian motion. Continuous nutrient uptake leads to the formation of a sink, where more ions diffuse to. Besides P this is the main supply mechanism for manganese (Mn) and copper (Cu) as well. (Schilling, 2000)

2.4.Altering the PUE of P fertilizers

Improving the efficiency of P fertilizers has been at the forefront of plant nutrition research for decades and using the right P source at the right rate, right time, and right place ("The 4 R's") is crucial for its efficiency (IPNI, 2012). To begin with, PUE of a P fertilizer can be influenced by altering its placement in the soil. As P supply is primarily ensured by diffusional transportation and diffusion coefficients in soils are commonly small ranging from 10^{-12} to 10^{-15} cm^2 s^{-1} (Marschner & Rengel, 2012), plants need readily available P nearby their root zone for an optimal plant growth. This can be achieved by banding of P fertilizers near the seedlings. Furthermore, a precise placement may optimize the usage of available soil pore water in lower soil horizons resulting in a better dissolution of fertilizer granules and thereby improve the diffusion of P to the rhizosphere.

A different approach would be altering fertilizer formulations to improve PUE. Since poor PUE may come from various soil or fertilizer properties, a new formulation has to address the main reason for low PUE in a specific soil. In this context, most scientific investigations were done on either altering the pH around fertilizer granules, slowing down the release of P from fertilizer granules or increasing P solubility.

As mentioned in the introduction, soil pH has a major influence on the solubility of P in soils and the removal of P from the soil solution. The co-granulation of P with elemental sulphur (S^0) or ammonium salts has already been put into practice for a long time for the use on neutral and alkaline soils. However, the effect of S^0 was more investigated concerning its plant nutritional benefits than its effect on P solubility. Yet, there is evidence that addition of S^0 may assist PUE of insoluble sources of P (e.g. ground phosphate rock) (McLaughlin et al., 2011). Usage of slow release P fertilizers may be applicable for slowly growing perennial species and may be stretched to their limits when it comes to fast growing annual crops with a high demand of readily available P during their major growth period

(Rahmatullah et al., 2006). An example would be the coating of fertilizer granules with synthetic polymers to reduce the release of P and stretch it over a longer period of time. Increasing the solubility of the applied P, preventing it from being adsorbed to or precipitated by various soil compounds or even mobilizing accumulated P reserves in soils is an important field of investigation both by commercial as well as scientific research. At present, only few of these new technologies went through extensive scientific research and reports about effectiveness are mixed with positive and negative findings. Commercial attempts for reducing P retention by precipitation include the addition of polymers or polymeric organic acids or silicon-based compounds to complex possible precipitation partners in the soil to anticipate subsequent reactions with Al, Ca, Fe and Mg. Mobilizing P out of strongly bound P pools with the addition of special agents is very little addressed by scientific research, thus we will not provide any findings about such, as detailed review of the claimed mechanisms is difficult and goes beyond the scope of this thesis (McLaughlin et al., 2011).

2.5. Excursus: Plant strategies for improving their PUE

Despite the fact that we struggle with quantifying plant available P pools in soil and putting effort in trying to make the use of P fertilizers more efficient, plants themselves have developed diverse strategies to improve their efficiency in P uptake which can be used in breeding programs to help improving the PUE of agricultural systems.

Richardson et al. (2011) concluded that three main strategies can be distinguished concerning improving P uptake by plants. They can improve their acquisition of soil P through “root foraging strategies”, where plants alter their root morphology and architecture to be able to exploit soil volume more efficiently, by formation of denser and longer root hairs or increasing the amount of root classes that are less metabolically demanding per unit P taken up. The latter may be achieved by increased formation of root cortical aerenchyma, where living cortical tissue is converted to air space, consequently reducing nutrient and carbon costs of soil exploration, while at the same time maintaining surface area for nutrient uptake. Additionally, plants can actively increase the total number of P transporters in cells that are at the forefront of P uptake, i.e., cells of root cap, root hairs and epidermis to increase the amount of P taken up per root area (Smith et al., 2000). Since the concentration of plant available P is commonly highest in the topsoil, changes in root architecture for enhanced topsoil foraging may include shallower growth angels of axial roots, greater dispersion of lateral roots and enhanced adventitious rooting. A different approach would be to enhance desorption and solubilisation of soil P by more efficient “mining strategies”, thereby promoting the P release from sparingly-available P pools. These alterations of soil chemistry by releasing root exudates (organic anions; enzymes; phenolic acids and protons) are important acquisition strategies under P deficient conditions (Hinsinger, 2001; Oburger et al., 2011). Finally plants can, to some extent, modify their “internal P-utilisation efficiency” by reducing P content in harvested material

and therefore leading to a reduction in the export of P from fields at harvest or actively reducing internal critical P concentrations which enables them to keep up their metabolic activity even under low P supply. However, the genetic basis of translocation of P within plants and remobilisation to grain requires more detailed investigation.

All these strategies are under constant investigation and important for breeding towards higher efficiency of P use by crops. Hence, the amount of P fertilizer for sustained or maximized production could be reduced and production in low P conditions could be increased.

2.6. Critical deficiency concentration (CDC)

Elemental analysis of plant tissues for assessment of nutritional status and furthermore assessment of plant availability of nutrients in soils requires sophisticated knowledge about reactions of plant species to applied fertilizers and their behaviour under deficiency conditions. For giving appropriate recommendations about critical nutrient concentrations in plant tissue, these calibrations presume intensive investigations of plant growth and yield response curves related to the measured concentrations of nutrients in plant tissue and soil. Additionally, specific and unspecific interactions of particular nutritional elements have to be considered not only in the plant itself but also in the rhizosphere. Critical deficiency concentrations (CDC) of plants condense this obtained knowledge in terms of what concentration of a particular element has to be present in plant material to achieve 90 % of dry weight or yield. CDC values vary between plant species, varieties and also change between developmental stages of plants (Römheld, 2012).

Interaction between N and P represent an example for non-specific interactions, which are of most importance when the concentrations of both nutrients are near or at critical deficiency concentrations. An increase of one element alone does not influence the CDC of the other but might induce, through better growth conditions, a deficiency in the other nutrient by dilution. However, evaluating ratios alone is not enough, as these can be obtained as well when both nutrients are present in deficiency concentrations (Jarrell & Beverly, 1981). Competition between potassium (K) and Mg ions for uptake by roots may serve as an example for a specific interaction which also affects CDC of the corresponding nutrients and high concentrations of K in the rhizosphere may induce Mg deficiency in plants (Hawkesford et al., 2012).

2.7. Soil analysis with extraction methods

Looking at how long implementation of the nowadays still valid official P testing methods date back (Olsen P method 1954; CAL method 1969), it is obvious that recommendations concerning P input for agricultural production strongly rely on them for decades. However, as briefly mentioned in the introduction, potential drawbacks or errors of these methods have to be considered. Firstly, nearly every European state has its own legislation concerning soil tests recommended for determining

plant available P in agricultural soils resulting in a limited comparability of P supply status of soils across Europe. Secondly, it is known that different extractants inevitably lead to differing conclusions about plant available P in soils because of their differences in extraction strength of given P fractions in soils and their differences in aggressiveness concerning dissolving P from soil compounds. Whereas mild extracting agents (e.g. H_2O) extract the smallest P amounts and can depict the most readily available P pools and therefore be ideally used for immediate fertilization recommendations (Neyroud & Lischer, 2003), stronger extracting agents (e.g. sodium bicarbonate (Olsen P method) or calcium-acetate-lactate solutions (CAL method)) include P fractions that are more strongly bound to soil compounds and thus should be included in long-term fertilization strategies. Thirdly, due to the fact that mechanisms of P supply from more stable pools in different soil types across Europe vary drastically, it is not expected that international conventions are set up for unifying soil analysis in Europe. Recently some studies suggest unsatisfying performance and low reproducibility of some soil tests concerning plant available P based on extraction methods (Kleinman et al., 2001; Neyroud & Lischer, 2003; Tandy et al., 2011). However, the majority of standard methods used in Europe (and as a consequence used for recommendations concerning P input for agriculture) rely on them. One of the main reasons for this might be the high sample throughput of most of the extraction methods and their easy-to-handle work procedure (Schilling, 2000).

After conducting a soil analysis for extractable P pools, soil samples can be categorized according to the amount of P extracted with the specific method. The Austrian Ministry of Agriculture periodically publishes guidelines for the interpretation of soil analysis results and gives recommendations concerning fertilizer applications (BMLFUW, 2006). Classification is made in five categories (Table 1) according to the content of CAL-extractable P per kilogram of dry soil. Depending on the classification of the soil sample, additions or deductions of the recommended amounts of applied P fertilizer are made. These adjustments are based on average removal by harvest (= 100 %) and range between +50 % amount of fertilizer for sites very low in plant available P, to recommendations of no application of P fertilizer on sites with very high supply of plant available P. For sites already having a high supply of P (Table 1) clay content and planted crop species are also taken into consideration.

Table 1 Categorization of soil analysis results concerning CAL extractable P (BMLFUW, 2006)

Class	Nutrient supply	CAL extractable P mg kg^{-1}
A	very low	less than 26
B	low	26 – 46
C	adequate	47 – 111
D	high	112 – 174
E	very high	above 174

2.8. DGT technique and calculation of C_{DGT}

As briefly mentioned in the introduction, Davison and Zhang (1994) established the DGT technique initially using it for quantifying trace metal concentrations in natural waters and sediments. DGT measures compounds, which are moving across a diffusive gradient. First passing a protective filter layer and a hydrogel of known thickness, the solutes are finally bound to a second hydrogel containing a binding agent, which acts as a zero sink for the target element (Figure 2). For measurement of P, commonly a binding layer containing ferrihydrite is used (Zhang et al., 1998; Santner et al., 2010).

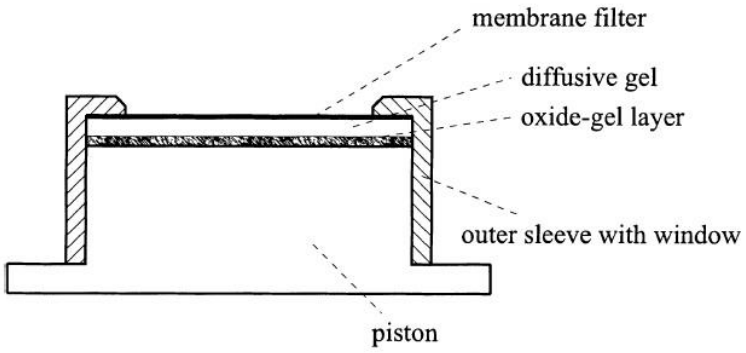

Figure 2 Cross-section through a DGT device (Zhang et al., 1998)

In the case of soil samples, the diffusion of P through the diffusive gel with subsequent binding to the ferrihydrite layer causes a depletion of soluble P and consequently a non-equilibrium between bound and dissolved P fractions of the soil sample (Figure 1). This initiates a constant resupply of P by desorption processes from soil compounds. As the binding layer is not reaching its maximum capacity during standard deployment times, depletion and desorption processes are sustained. The process of DGT-induced P depletion is comparable to P uptake by a plant root. Knowing the exact geometrical properties of the devices and the deployment time, we are able to calculate time-averaged fluxes F (1) into the sampler and the concentration C_{DGT} (2) at the sampler surface.

$$F = \frac{M}{At} \qquad (1)$$

$$C_{DGT} = \frac{M\Delta g}{DAt} \qquad (2)$$

Calculation of F and C_{DGT} requires knowledge about the exposed gel surface, A [cm^2], the deployment time, t [s], the diffusion coefficient for the specific compound in the gel at a given temperature, D [$cm^2\ s^{-1}$], the thickness of the combined layers, Δg [cm], and the mass of the compound bound in the

gel, M [µg]. For P measurements, M is usually determined colorimetrically after dissolving the ferrihydrite gel in sulphuric acid. The value for D at 20 °C incubation temperature is 5.27×10^{-6} cm^2 s^{-1} and was provided by DGT Research Ltd, Lancaster (www.dgtresearch.com).

In contrast to bulk soil concentrations (e.g. by measuring concentrations of centrifuged soil pore water; C_{SOL}), C_{DGT} values are determined by the resupply rate from the soil solution as well as from the solid phase and thus can be used as an indicator for the bioavailability of labile P pools in soils. This is further supported by investigations by Tandy et al. (2011) that show high correlation of plant tissue P concentrations with C_{DGT} measurements. However, Degryse et al. (2009) stated that DGT measurements only predict the bioavailability of elements to plants correctly, when uptake of a specific element by plants is limited by diffusional supply, which is the case for P in soil. Furthermore, as described in detail in chapter 2.5, plants have the ability of increasing nutrient supply to roots by various strategies. The DGT technique alone does not take this ability of plants into consideration and for a correct prediction of nutrient availabilities for specific plant species, one has to be particularly careful when investigating species or varieties that are well adapted to low P environments and mobilize high amounts of P by secretion of root exudates or altered root morphology. As described by Fitz et al. (2003) these root-induced chemical changes of bioavailability in the rhizosphere (in their particular article for arsenic) may be investigated with rhizobox experiments, where bulk and rhizosphere soil can be collected and analysed separately.

3. Materials and Methods[1]

Two experiments were conducted to assess the plant availability of two different P fertilizers over time under controlled conditions in Tulln (Lower Austria) from April 5th to June 26th (greenhouse experiment) and from September 24th to November 7th 2013 (incubation experiment). Whereas the first investigation focused on plant growth following the application of P fertilizers, the second experiment focused solely on the behaviour of the fertilizers in soil.

The soil used in both experiments was collected on March, 14th 2012 from a site near Mattersburg (Burgenland) and spread out to dry for the following two weeks with frequent thoroughly mixing. Prior to any further usage, the dried soil was sieved (<5 mm), homogenised again and subsequently mixed with sand in a volumetric ratio of 1:2 (soil : sand) to receive a soil that has low to very low plant available P supply (Table 1) and is easy to handle in the pot experiment. A sample of the resulting mixture was analysed for selected physio-chemical characteristics (Table 2).

Table 2 Selected physio-chemical characteristics of the soil-sand-mixture

pH $CaCl_2$	pH H_2O	Field-capacity	P (CAL) mg kg^{-1}	P (C_{DGT}) µg cm^{-3}
7.66	8.45	16.1 %	22.05	0.111

3.1. Assessment of P availability by plant growth and dry matter analysis

Mitscherlich pots (20 cm diameter and 15 cm height) were used for the experiment, for which the preparation of the soil-sand-mixture mentioned above was done individually for every pot. Soil and sand, 1880 g and 5020 g respectively, were mixed thoroughly by hand and afterwards 750 ml of unfertilized mixture was put to the bottom of the pot to prevent accumulation of fertilizer in the subsoil. Both N and P fertilizers were ground with a mortar and the corresponding amounts were mixed thoroughly with the sand soil mixture. N was supplied equally to each pot (0.265 g N per pot, equivalent to 100 kg ha^{-1} N) as ammonium sulphate ($(NH_4)_2SO_4$). P supply was established with two different fertilizer formulations with the same percentage of water soluble P (Single super-phosphate (SSP) and an alternative formulation (ALT)) at two different levels (0.141 g P_2O_5 per pot, equivalent to 53.13 kg ha^{-1} P_2O_5 (ALT1 and SSP1) and 0.282 g P_2O_5 per pot, equivalent to 106.25 kg ha^{-1} P_2O_5 (ALT2 and SSP2)). Both fertilizer formulations were acquired from Timac Agro Austria (www.at.timacagro.com) and have the same neutral ammonium citrate soluble P_2O_5 content (18 %) and the same water soluble P_2O_5 content (16.75 %). According to the company the usage of the ALT fertilizer reduces the amount of added P being adsorbed to or precipitated by soil components.

[1] Pictures of methods are provided in Appendix 7 and Appendix 8.

Therefore, the recommended use of this formulation focuses on soils, where the efficiency of conventional P sources is limited by these processes, i.e. in acidic and alkaline soils.

The applied fertilizer amounts were calculated according to the guidelines issued by the Austrian Ministry of Agriculture (BMLFUW, 2006). The underlying assumptions for the fertilizer calculations were a middle rate yield expectation, a middle deepness of the soil, a moderate mineralisation rate and based on the P supply classification of the soil, a 25 % addition of P amount was taken into account. The control treatment only received N supply and no additional P fertilization. For each of the five treatments eight replicates were arranged in a completely randomised design.

Prior to planting, the soil was saturated and then allowed to drain. The soil water content was kept at around 80 % of field capacity throughout the experiment. Maize (*Zea maize*) was grown in the pots starting on April 5th 2013 with five seeds sown in a star pattern in a greenhouse under the following conditions: natural light regime supplemented with artificial lightning with several 400 W SON-T lamps to ensure a minimum photon flux of 220 µmol s^{-1} m^{-2} and 25 °C / 15 °C day / night temperatures. Plants were thinned out 11 days after sowing (DAS) keeping the two plants which were the strongest and most far away from each other. During the experiment, the phenological stages of maize were determined 10 times using the BBCH-scale (Lancashire et al., 1991).

Eight weeks after sowing (1st harvest; H1), half of the pot trial (four replicates of each treatment) was harvested. Leaves, stems and roots (washed out in a root washbasin on sieves to prevent loss of small roots) were separated and oven-dried at 60 °C for at least 24 h. 12 weeks after sowing (2nd harvest; H2) the rest of the trial (four replicates of each treatment) was harvested and green leaves, senescent leaves, stems and roots (same procedure as at H1) were collected separately and oven-dried at 60 °C for at least 24 h. After milling (Retsch knife mill GRINDOMIX GM 200), subsamples were prepared for dry matter analysis as described below.

Furthermore, soil samples were collected (six samples per pot in a star shaped pattern with a boring rod to obtain a representative sample of the whole pot profile) from each pot individually to investigate soil P content at both time points (eight and 12 weeks after sowing). Prior to any further tests, the soil samples were spread out and air-dried at approximately 25°C overnight and then sieved (<2 mm) as stated in chapter 3.2.

3.1.1. Combustion analysis

Analysis for N content of the harvested plant material was conducted with an Elementar vario MACRO cube combustion analyser. Sample preparation consisted of oven-drying the milled samples at 103 °C for 4 h, and afterwards 40 mg of the sample were weighed and wrapped into tinfoil in two replications and supplied to the machine.

3.1.2. Atomicabsorptionspectroscopy (AAS) and Spectrophotometry

For analysis of P and K content of the maize material, the samples were oven-dried at 103 °C for 4 h and 500 mg of the sample were weighed into 50 ml volumetric flasks in two replications. Open acid digestion of the material was done with a mixture of nitric acid (HNO_3), sulphuric acid (H_2SO_4) and perchloric acid ($HClO_4$) (10 parts; 1 part; $^1/_2$ part respectively) over a time span of 8.25 hours on a programmable heating plate. Finally the flasks were filled up with deionised water.

Analysis for K content of the harvested plant material was conducted with a Varian SpectrAA 300 Atomicabsorptionspectrometer (AAS), where K concentration was measured in a 1:10 dilution with 11 mM caesium chloride solution.

P concentrations were determined colorimetrically using the vandate-molybdate method (yellow method), where absorbance of the phosphovandate/phosphomolybdate complex was measured with a Varian DMS 200 Spectrophotometer at 436 nm. Calibration standards were prepared using 0.3 M HNO_3 in a range between 2.5 and 30 mg l^{-1} P.

3.1.3. Nutrient use efficiency calculation

Following the presented concepts for calculating nutrient use efficiencies, these were done for N (N use efficiency; NUE) and P (P use efficiency; PUE) eight and 12 weeks after sowing based on the balance method (chapter 2.2). For this purpose, the amount of accumulated nutrient in plant tissue was calculated via nutrient concentrations in plant organs and their corresponding dry weight. The following calculation,

$$PUE = \frac{plant\ P\ uptake\ [mg]}{applied\ fertilizer\ P\ [mg]} \qquad (3)$$

gives us estimations about the recovery of the applied P fertilizer by the plants.

3.2. Assessment of P availability by CAL, DGT and soil solution extraction

In addition to the initial plant growth experiment described in detail above, a second experiment was set up to investigate the fate of the applied fertilizer in soil. Sand and sieved soil (<2 mm) was mixed again in a 2:1 volumetric ratio to gain 5.5 kg of homogenous substrate. This starting material was divided equally into five plastic shaking-bottles (size: 2000 ml), where the respective amount of fertilizer (see chapter 3.1) was added and then thoroughly mixed with the substrate. While P was supplied with two different P fertilizers (ALT & SSP) at two different levels (22.5 mg P_2O_5 and 45 mg P_2O_5 per bottle, equivalent to 53.13 kg ha^{-1} P_2O_5 and 106.25 kg ha^{-1} P_2O_5), N was supplied equally to all bottles with 42.3 mg N per bottle, equivalent to 100 kg ha^{-1} N as $(NH_4)_2SO_4$. Water was added in small portions until 70 % of field capacity was reached, which in turn means 11 % total water content for this particular substrate (gravimetrical determination). The five bottles containing the substrate-fertilizer mixture were sealed with parafilm to prevent desiccation and incubated at 15 °C for 44

days. During incubation, samples were taken for analysis 0, 3, 7, 10, 15, 29 and 44 days after mixing. At every sampling, the weight of the bottles containing the soil was monitored before and after soil was taken out, to be able to compare the weight at the beginning of a new sampling with the weight at the end of the previous sampling. Thereby the current water content could be calculated and a constant water content of 11 % of the substrate could be ensured throughout the 44 days.

3.2.1. CAL method

The CAL extraction method (Schüller, 1969; Austrian-Standards-Institute, 1993) uses an extraction solution containing 70.6 mM calciumlactate, 50 mM calciumacetate and 0.3 M acetic acid (pH 4.1). After 100 ml extractant and 5 g of air-dried sieved soil were shaken for 2 h in an overhead shaker, the suspension was filtrated through pleated filters. The extraction was done with one sample for every pot in the first experiment (four replicates per treatment per harvest) and repeated three times per treatment in the second experiment (three replicates per treatment per sampling). The filtered solutions were kept in a fridge at 4 °C and were further analysed within the next 24 h. P concentrations in the filtrate were determined colorimetrically using the molybdate 1 method (blue method 1) where absorbance of the phosphomolybdate complex was measured with a Hitachi U-2000 Spectrophotometer at 660 nm. Calibration standards were prepared using CAL extraction solution in a range between 0.5 and 25 mg l^{-1} P.

3.2.2. DGT method

For the DGT test a binding layer containing ferrihydrite was used. For both, the binding layer and the diffusive layer, gel solution was used that consisted of 15 vol.% acrylamide and 0.3 vol.% cross linker obtained from DGT Research Ltd, Lancaster (www.dgtresearch.com). The casting of the gels was done according to published procedures (Zhang & Davison, 1995). The used plastic DGT devices consisted of a base and a ring with an exposure window (A = 3.14 cm^2). On top of the 0.4 mm thick ferrihydrite binding layer (A = 4.52 cm^2) a track-etched membrane (Whatman Nucleopore; 25 mm diameter; 0.2 µm pore size) was placed to function as a separating layer to the 0.8 mm thick diffusive gel (A = 4.52 cm^2) for easier disassembly of the device after exposure to the soil. As protection and separation for the diffusive gel from the soil sample, a membrane filter (Pall Supor® 450; 25 mm diameter; 0.45 µm pore size) of 0.14 mm thickness was applied on top which acts as an extended diffusive layer (Figure 2) (Zhang & Davison, 1995).

Immediately after each sampling, the soil sample was adjusted to maximum water holding capacity (WHC) as assessed by visual inspection (glistening of water on the soil surface without having any free water on the side of the beaker and easy gliding off of soil paste from a spatula) with the addition of deionised water. If needed, the water content was further adjusted over the next 2 h according to visual inspection. Prior to any further handling, this soil-water paste was sealed with

parafilm and incubated 24 h at 20 °C for establishing new equilibrium at maximum WHC. The next day, soil-paste was put on the DGT devices (one replicate per pot for the pot trial and three replicates per treatment for the incubation experiment) taking special care that the paste was spread equally over the exposure window and no air bubbles were visible. Concurrently two blank DGT devices without soil were prepared, which were treated identically to the DGT devices being loaded with soil. The loaded devices and blanks were placed in an incubator in a sealed box to prevent desiccation. After 24 h deployment at 20 °C, the DGT devices were rinsed with deionised water to remove any adhering soil particles and exact deployment time was noted down for subsequent calculations. Before the ferrihydrite binding layers were eluted in 10 ml 0.25 M H_2SO_4 they were rinsed again with deionised water to minimize the possibility of contamination of the elution solution with soil particles.

The P concentration in the elutant was determined colorimetrically using the molybdate method (blue method) where absorbance of the phosphomolybdate complex was measured with a Hitachi U-2000 Spectrophotometer at 881 nm. Calibration standards were prepared using 0.25 M H_2SO_4 in a range between 25 and 1000 µg l^{-1} P. The difference (to the CAL method) at which wavelength the phosphomolybdate complex was measured arises from differences in published standardized procedures (Austrian-Standards-Institute, 1993).

3.2.3. Soil pore water extraction

Since conventional centrifugation of the soil sample and subsequent collecting of soil pore water with a syringe with a filter tip was not possible due to the little amount of obtained centrifugate, a different procedure was applied for obtaining enough solution for subsequent colorimetrical analysis of P concentrations in the pore water of the soil sand mixture (adjusted to maximum WHC (chapter 3.2.2)). 0.5 g glass wool was put to the bottom of 20 ml syringes, to prevent the syringe tip from being clogged by soil particles. After being filled with approximately 50 g of soil-paste, the syringes were put into 50 ml centrifuge vials and centrifuged at 3000 g for 5 min. To settle fine particles in the obtained solution, the vials were again centrifuged without the syringes at 10000 g for 10 min. Finally, the maximum amount of solution (about 2 ml) was pipetted out of the centrifuge vials and acidified with H_2SO_4 for conservation and subsequent colorimetrical analysis.

The procedure of measuring the P concentration in the soil solutions was done according to chapter 3.2.2.

3.2.4. Calculation of C_{DGT} and R

The calculation of C_{DGT} values from the measured P concentrations in the binding layer elution solution allows evaluation of the time averaged soil solution concentration at the surface of the DGT device. This has been discussed previously by Zhang et al. (2001) and is described in detail in chapter

2.8. Including the obtained P concentrations in the soil pore water centrifugates (chapter 3.2.3) by calculating a ratio R, which represents the ratio of DGT concentration to soil pore water concentration during the DGT deployment (C_{DGT}/C_{SOL}), we can also make estimations about the possible P resupply capacity from labile pools of the solid phase in the particular substrate (Harper et al., 1998; Degryse et al., 2009).

3.3. Statistics

Descriptive statistics, t-tests, univariate and multi-factorial analysis of variances (ANOVA) were carried out using the statistical software PASW Statistics 18 (SPSS Inc. 2009). A general linear model with repeated measurements and a post-hoc analysis with Tukey-Kramer method were carried out using the statistical software SAS 9.2 (SAS Institute Inc. 2008).

4. Results[2]

4.1. Plant growth experiment

Environmental conditions during the plant growth experiment

Due to the lack of a cooling system in the glasshouse the temperature regime of 25 °C / 15 °C day / night could not be maintained throughout the maize growth period (chapter 3.1). Thus the temperatures during hot days were higher than targeted (Figure 3; increases of up to 24 °C during 24 h). Consequently, despite frequent watering, plants suffered water stress. Furthermore, it has to be remarked, that the artificial lightning was not equipped with the proposed SON-T lamps, leading to a reduced photon flux and therefore increased internode lengths.

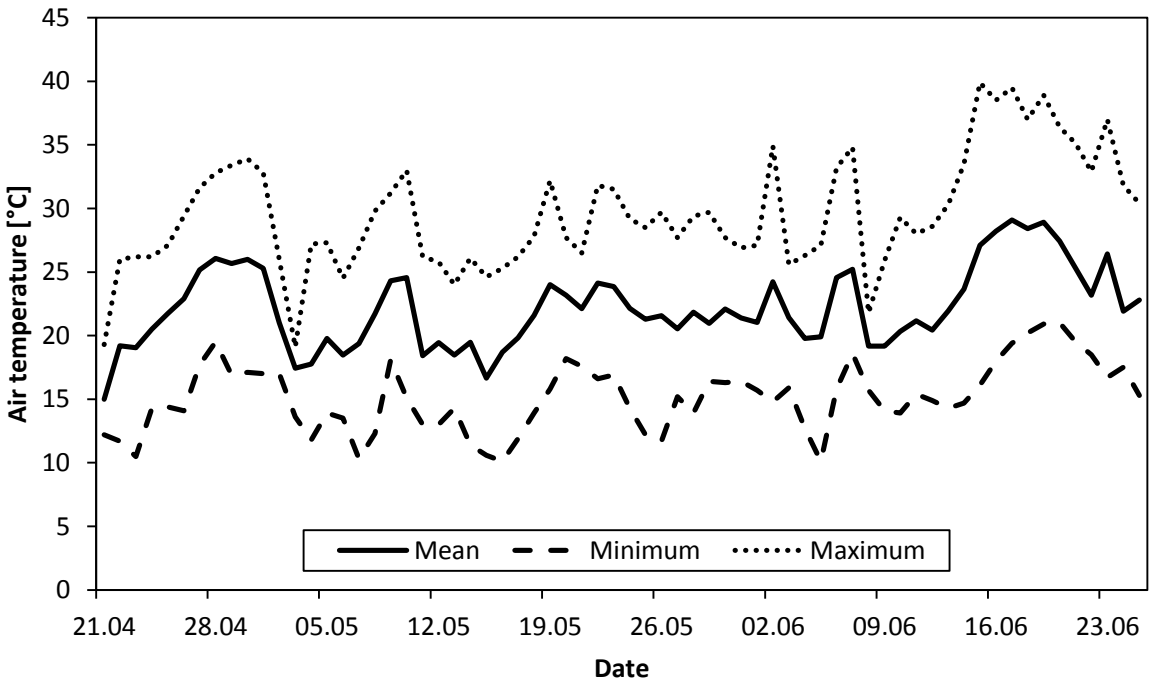

Figure 3 Daily minimum, maximum and mean air temperature in the greenhouse compartment; data for the first 15 days not included due to lack of long-term storage of temperature data

[2] Tables of mean values of the presented results are provided in the Appendix

Biomass production and plant development

Figure 4 depicts the biomass production per pot of maize plants in the growth experiment after eight (H1) and 12 weeks (H2). In the graph biomass for each treatment was split according to separately harvested plant organs. For the second harvest, the separately harvested leaf material (green leaves and senescent leaves) was summed up, as no significant differences in weight could be determined. No statistical significant differences in biomass production between the five different treatments, neither at the first nor at the second harvest, could be verified. However, an average 2.6 fold total biomass increase could be observed in the time span between H1 and H2. Concerning maize phenology according to the BBCH scale, no differences could be determined (data not shown).

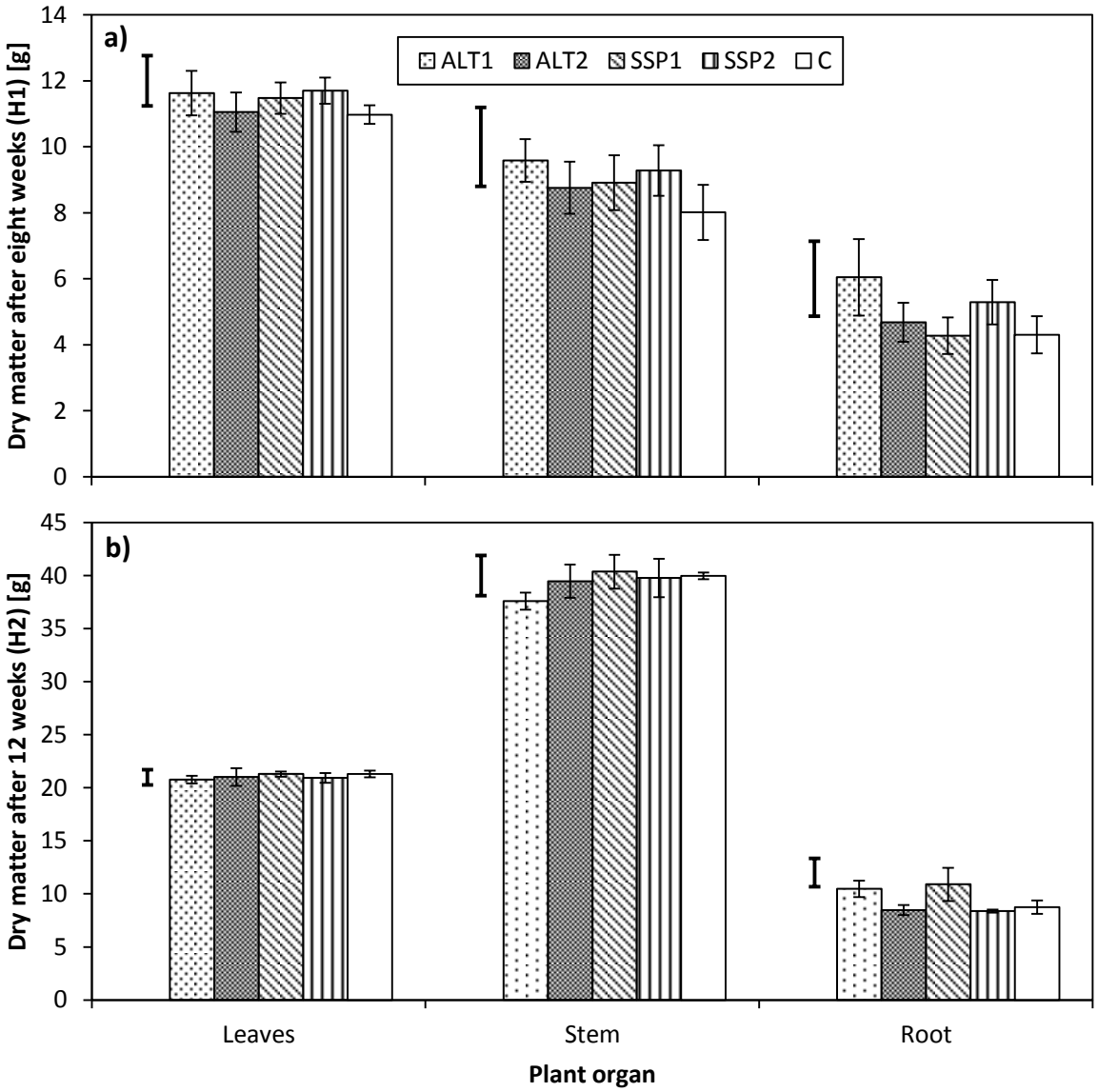

Figure 4 Dry matter of leaf, root and stem of maize eight (a; H1) and 12 (b; H2) weeks after sowing. Mean values of four replicates. Error bars are standard errors (SE); bold vertical bars: least significant differences (LSD) for $p < 0.05$; ALT1 & ALT2: 53.13 kg ha^{-1} P_2O_5 and 106.25 kg ha^{-1} P_2O_5 respectively; SSP1 & SSP2: 53.13 kg ha^{-1} P_2O_5 and 106.25 kg ha^{-1} P_2O_5 respectively; C: no additional P fertilization

N uptake and concentration in plant tissue

Figure 5a shows the mean N concentrations in maize plants averaged for each treatment. The most obvious result that we can draw from this comparison of the first and second harvest is the enormous decrease in N concentration in the tissue of plants during this four-week period. Starting from mean values between 1.9 % and 2.2 % (no significant differences between the treatments), these concentrations dropped to values of slightly over 0.5 %, where treatments which where fertilized with the ALT formulation achieved significantly higher mean N concentrations in maize. Taking into consideration the total N that was taken up by the plants shown in Figure 5b, we can state, that little to no N uptake by the plants took place between the first and the second harvest, resulting in N dilution in plant biomass. The increase in biomass (Figure 4) and the decrease in N concentration in plants show similar magnitudes, underlining again that N uptake stopped after the first harvest. Furthermore, Figure 5b shows that concerning total N uptake at the second harvest, a significant difference between ALT and SSP treatments could be measured ($p < 0.001$). This significant difference has to be attributed to a significantly higher N concentration in the leaves of plants fertilized with the alternative formulation fertilizer ($p < 0.001$; Figure 6). N use efficiency calculations (chapter 3.1.3) revealed that plants extracted on average the 1.8 fold amount of N that was supplied to the pots by fertilizer.

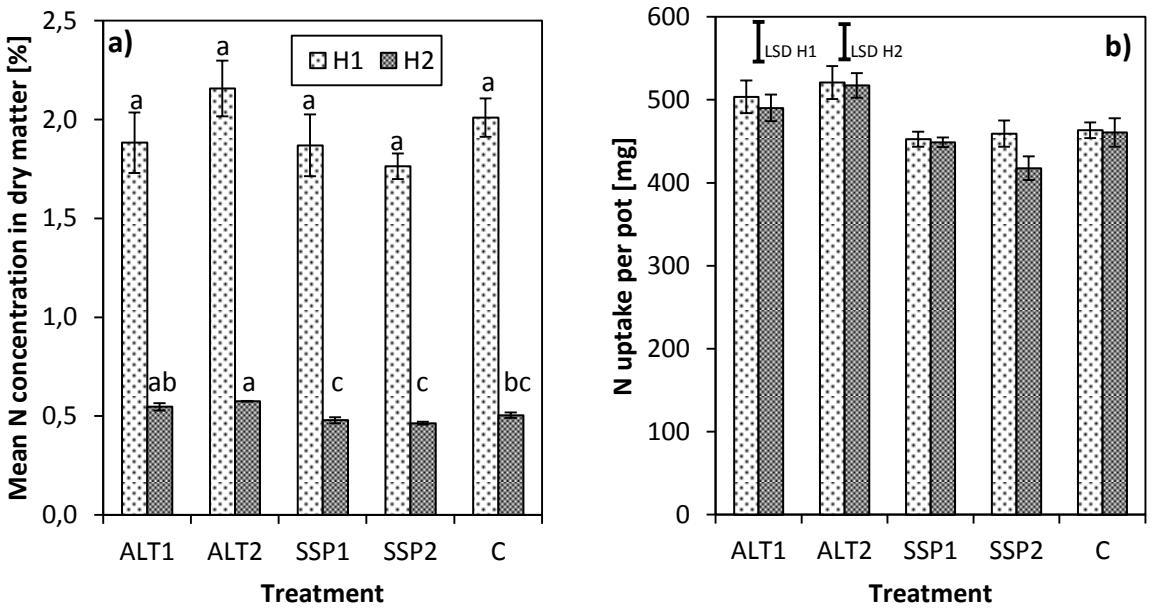

Figure 5 a) Mean N concentrations in plant material eight (H1) and 12 (H2) weeks after sowing. Means of H1 and H2 with different overhead letters are significantly different at $p < 0.05$; b) N uptake of plants per pot eight (H1) and 12 (H2) weeks after sowing. Mean values of four replicates. Error bars are SE. bold vertical bars: LSD for $p < 0.05$ Labelling according to Figure 4

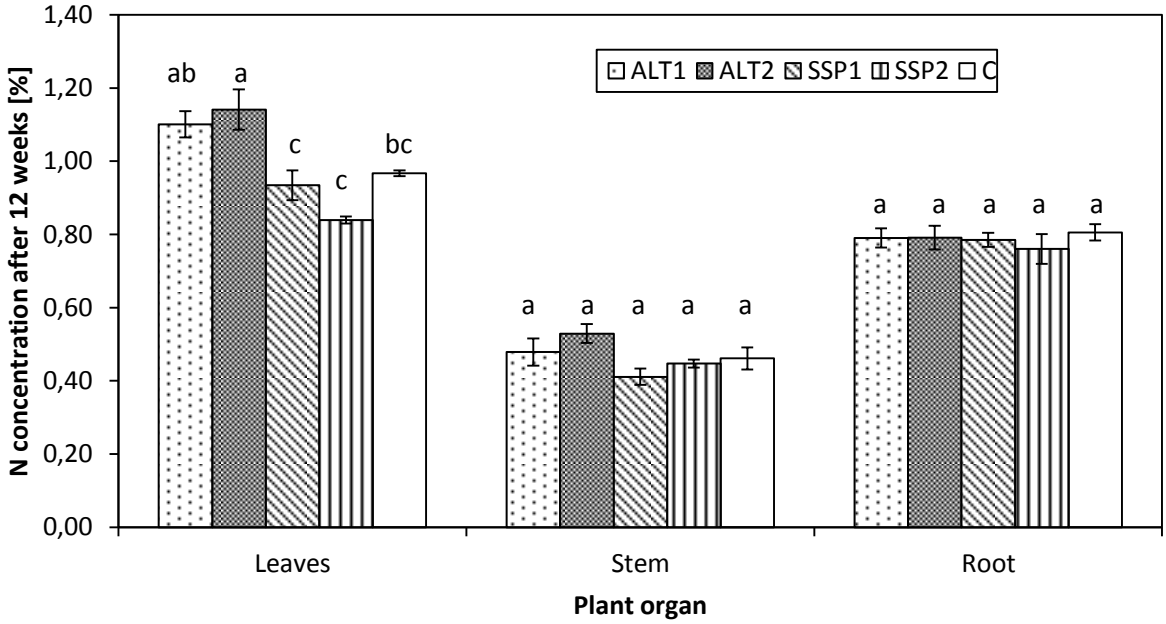

Figure 6 N concentrations in plant organs 12 weeks after sowing. Mean values of four replicates. Error bars are SE. Means within an organ category with different overhead letters are significantly different at $p < 0.05$; labelling according to Figure 4

P concentration in plant tissue and PUE based on balance method

Similar to the average N concentrations in plants (Figure 5a), the mean P concentrations in plant material dropped significantly from the first to the second harvest (Figure 7a). However, the P dilution does not show the same high magnitude as the drop in N concentration since there was a significant increase in accumulated P in plant material at the second harvest compared to the first harvest. This shows that P uptake still took place between the two harvests while N uptake stopped after the first. Analysis of the P amount accumulated in different plant organs for the first harvest showed lower concentrations for leaves of plants that grew under fertilization with ALT (Figure 7b) and the highest concentration values were obtained from plants that received no P fertilization. For the second harvest these differences were not significant anymore, resulting in homogenous overall P concentrations and P uptake in all five treatments.

Figure 8 depicts the calculated PUEs according to the balance method (chapter 3.1.3) as the amount of P taken up by the plant in relation to the amount of P supplied to the plants. There is a distinct separation of treatments which received the full amount of fertilizer from the ones that received only half the amount. However, between the two fertilizer formulations no significant differences could be determined and therefore for the following comparisons these were averaged for the two fertilizer levels. For the first harvest, PUE was about 15 % for treatments with the full amount of fertilizer and about 30 % for the ones supplied only with half of it. These values increased to about 24 % and 46 %, respectively, at the second harvest.

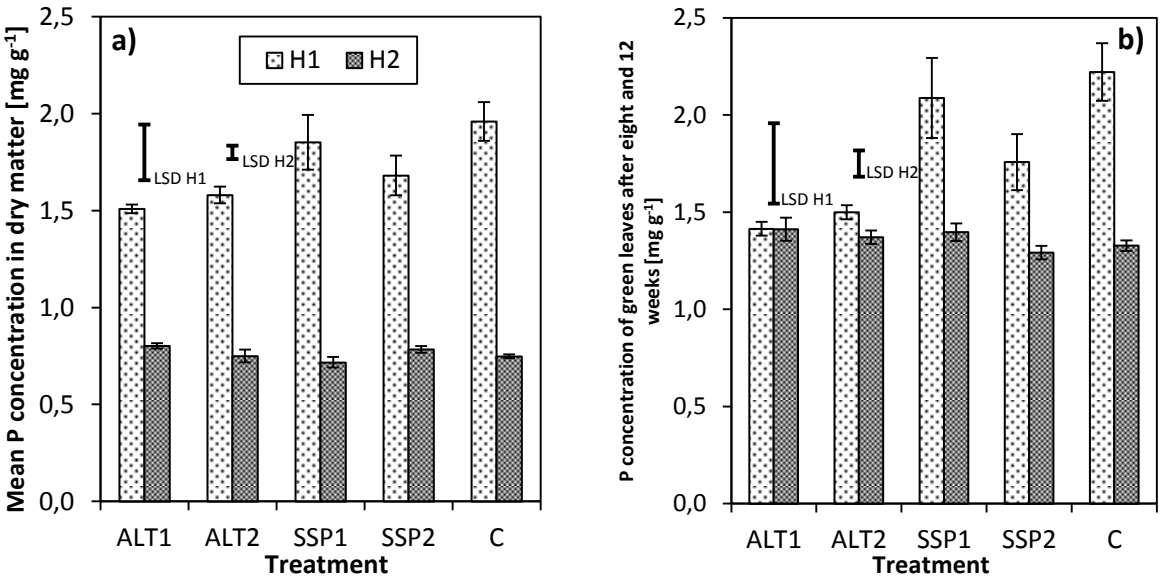

Figure 7 a) Mean P concentrations of plant material eight and 12 weeks after sowing; b) P concentration in maize leaves after eight and 12 weeks. Means of four replicates. Error bars are SE. bold vertical bars: LSD for $p < 0.05$; Labelling according to Figure 4

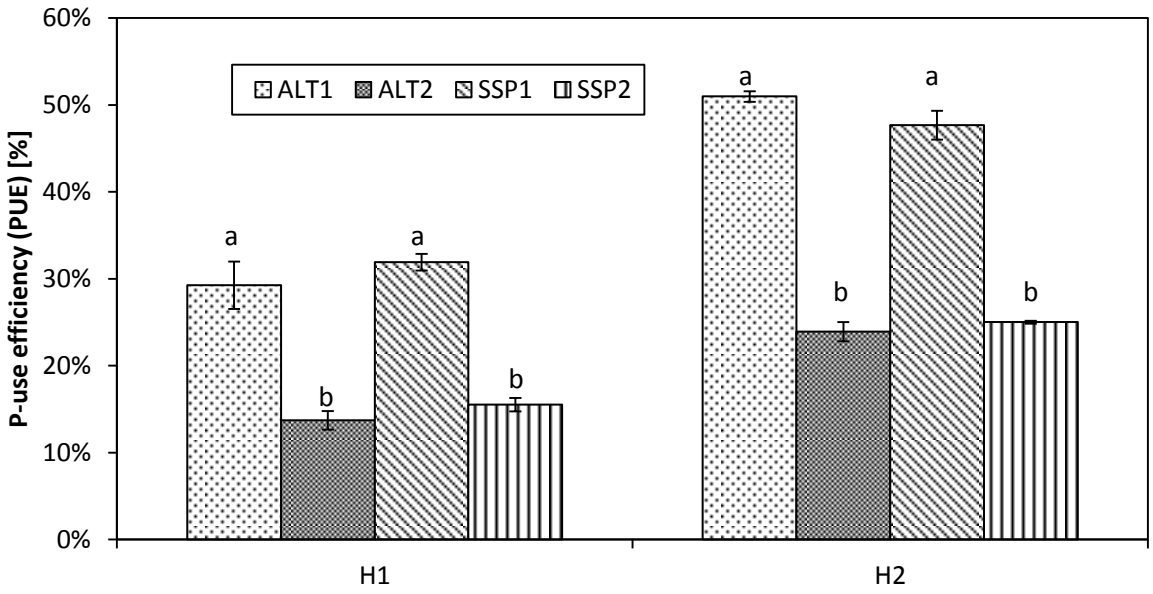

Figure 8 PUE of maize calculated according to the balance method eight (H1) and 12 (H2) weeks after sowing. Means of four replicates. Error bars are SE. Means of treatments with different overhead letters are significantly different at $p < 0.05$; Labelling according to Figure 4

Analysis for K concentration in plant tissue neither revealed significant differences for different plant organs nor differences between the two harvests or any effects of P fertilizer treatments.

Soil P content based on CAL and DGT method

Figure 9 depicts soil P content based on the CAL extractions in the plant growth experiment. Statistical analysis of each harvest showed that significant differences between treatments were present in both harvests ($p < 0.001$). At the first harvest ALT2, showed significantly higher P concentration than ALT1 and SSP1 but was not statistically different from SSP2 ($p = 0.070$), which in turn was not statistically distinguishable from ALT1 and SSP1. Whereas at the first harvest all treatments that received P fertilizer achieved higher CAL extractable P concentrations than the control treatment, the second harvest showed a different picture. Here, the half fertilized treatments cannot be separated anymore from the control on a 0.05 significance level ($p = 0.058$), whereas ALT2 and SSP2 showed again the highest concentrations. Worth noting is that for four out of five treatments the mean CAL extractable P concentrations increased. Yet this is only a tendency and not statistically significant and might be attributed to common technical variation in the CAL extraction and the following colorimetrical measurement.

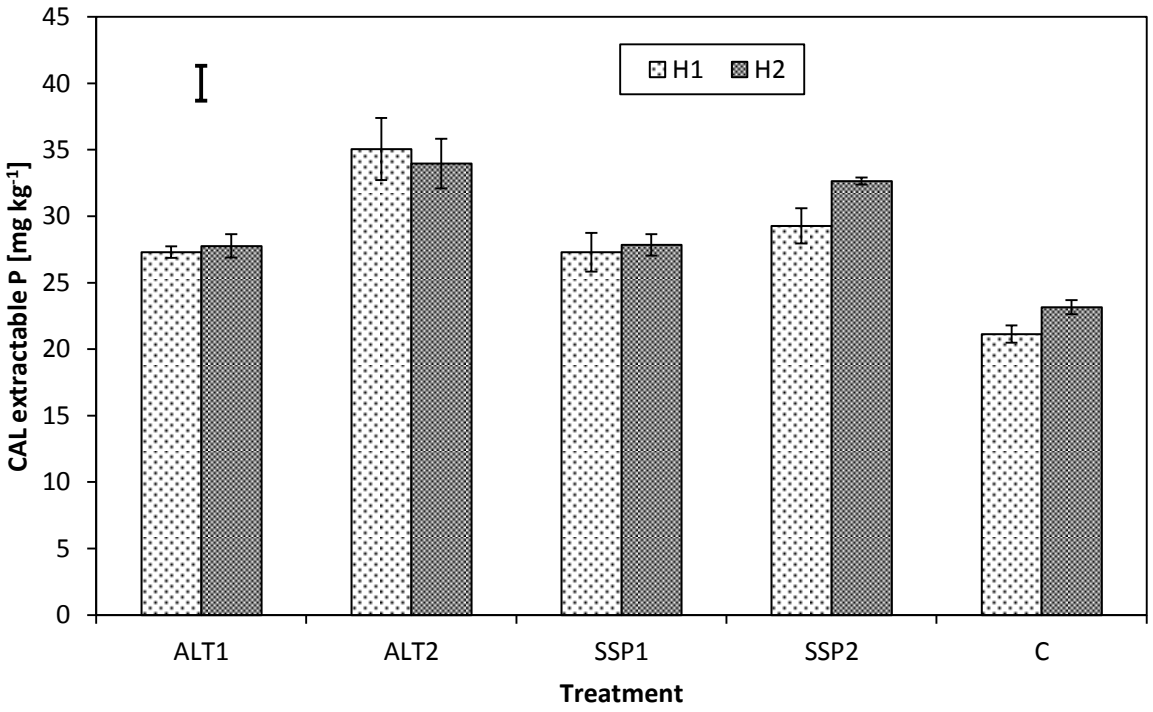

Figure 9 CAL extracted P concentrations in soil samples eight (H1) and 12 (H2) weeks after sowing. Means of four replicates. Error bars are SE. bold vertical bars: LSD for $p < 0.05$; Labelling according to Figure 4

The obtained C_{DGT} values from the DGT applications showed a picture similar to the CAL measurements but made a better distinction between the two fertilizer levels applied (Figure 10). For both, the first and the second harvest, ALT2 and SSP2 were significantly different from the other treatments and showed the highest C_{DGT} values. Whereas a clear distinction between the treatments with half fertilizer amount and the control treatment could be made at the first harvest, the same did not occur for the second harvest, however, the difference was still close to significance ($p = 0.059$). In general, we saw no significant differences between the C_{DGT} values of the first and the second harvest.

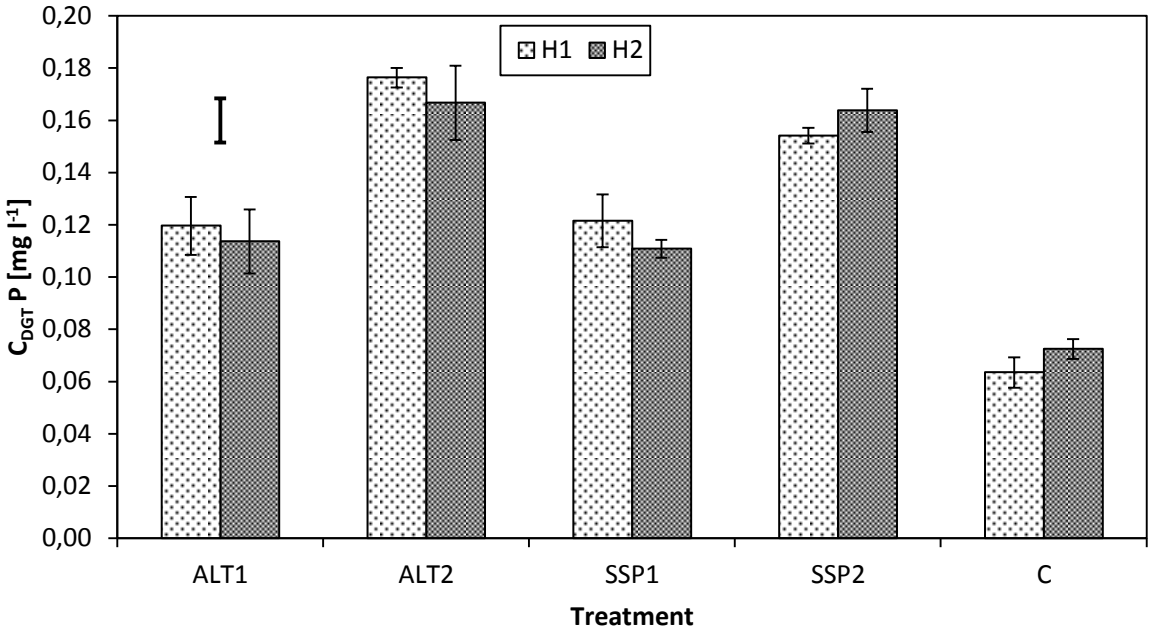

Figure 10 Measured C_{DGT} P concentrations in soil samples eight (H1) and 12 (H2) weeks after sowing. Means of four replicates. Error bars are SE. bold vertical bar: LSD for $p < 0.05$; Labelling according to Figure 4

4.2.Incubation experiment

As described in chapter 3.2, initially seven samplings were scheduled over 44 days of the incubation experiment. However, due to technical difficulties, measurements obtained from the fifth sampling that took place at day 15 could not be used for further analysis, as the data showed very unusual results probably due to errors made during sampling, preparation of samples, or analysis of the soil material. Hence, these data were taken out of statistical analysis and only data from six samplings (0; 3; 7; 10; 29; 44 days after incubation) are represented in the following figures.

Soil P content based on the CAL method

As presented in Figure 11 the measured CAL-extractable P concentrations of the soil samples were grouped into three statistically significant groups ($p < 0.001$). Samples from treatments that received the full amount of P fertilizer showed significantly higher concentrations throughout the whole experiment compared to samples from treatments with half the amount. Furthermore, samples from the control treatment showed the lowest concentrations. At the beginning of the experiment, the data indicated an increase of available P in the soil within the first five days with a subsequent decrease over the next weeks. The peak in the measurements at day 29 might be considered to be a systematic error, since there is no reason that the control treatment, which received no additional P, should show an increase in plant available P after 14 days of incubation. Therefore the values are assumed to have stayed approximately constant after day 10 of the experiment.

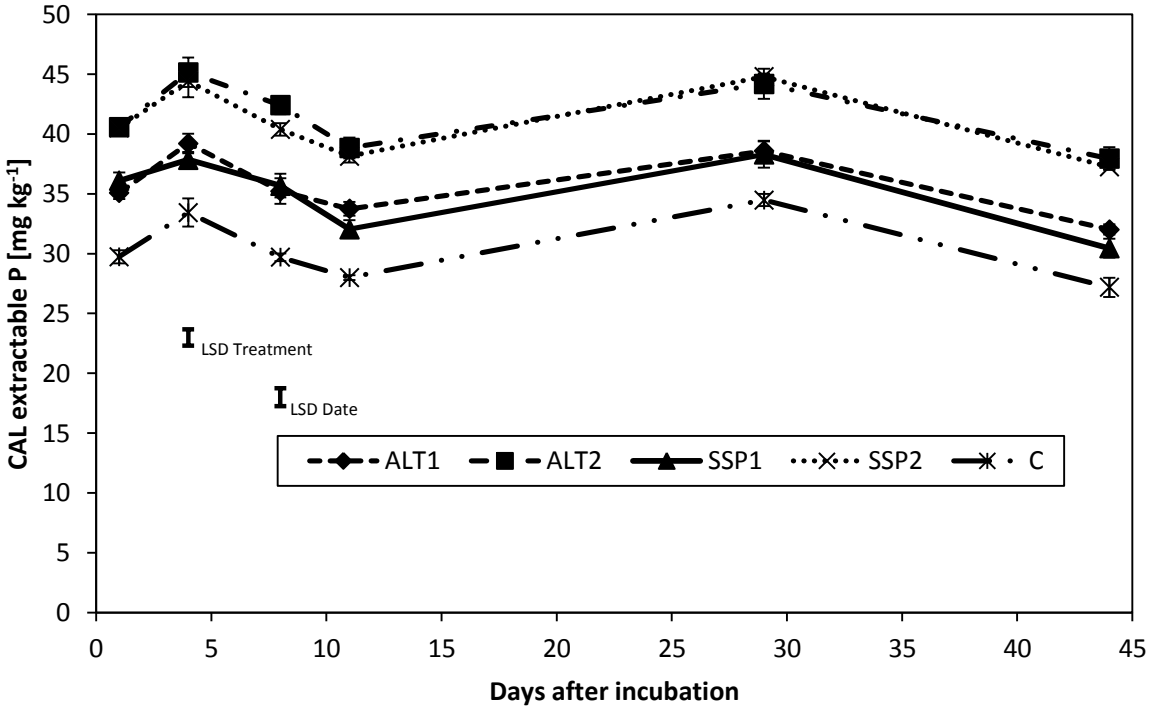

Figure 11 Time series of CAL concentrations over a time span of 44 days. Means of three replicates. Error bars are SE. bold vertical bars: LSD for $p < 0.05$; Labelling according to Figure 4

Soil P content based on the DGT method

Looking at the C_{DGT} values measured over time in Figure 12, some obvious differences but also similarities to the measured CAL extractable P concentrations in Figure 11 can be observed. Again, there was a clear separation of the treatments that received the full amount of fertilizers from the ones with half the amount and no P fertilization ($p < 0.001$). However, the separation and tendency was clearer compared to the CAL measurements. Within the two groups that received P fertilizer, no distinction could be made between the two P fertilizer formulations throughout the whole experiment on a basis of a 0.05 significance level. Although the new formulation showed a significantly higher C_{DGT} value at the last sampling, it is rather insecure if this really was due to the alternative formulation or has to be considered as an artefact. In the control treatment mean values would have indicated a slight decrease in P availability due to the incubation within the first seven days. However post-hoc analysis showed that there was no statistically significant decrease and the obtained C_{DGT} values are assumed constant during the whole time span. For the fertilized treatments the picture was different. For the first four measurements of ALT2 and SSP2 there was a significant decrease in the measured C_{DGT} values which was followed by a period of constant values until the end of the experiment. For ALT1 and SSP1 the C_{DGT} values decreased until the third sampling and stayed constant thereafter. This can be observed in all fertilized treatments. However, the reduction rates appeared to vary between fertilizer levels.

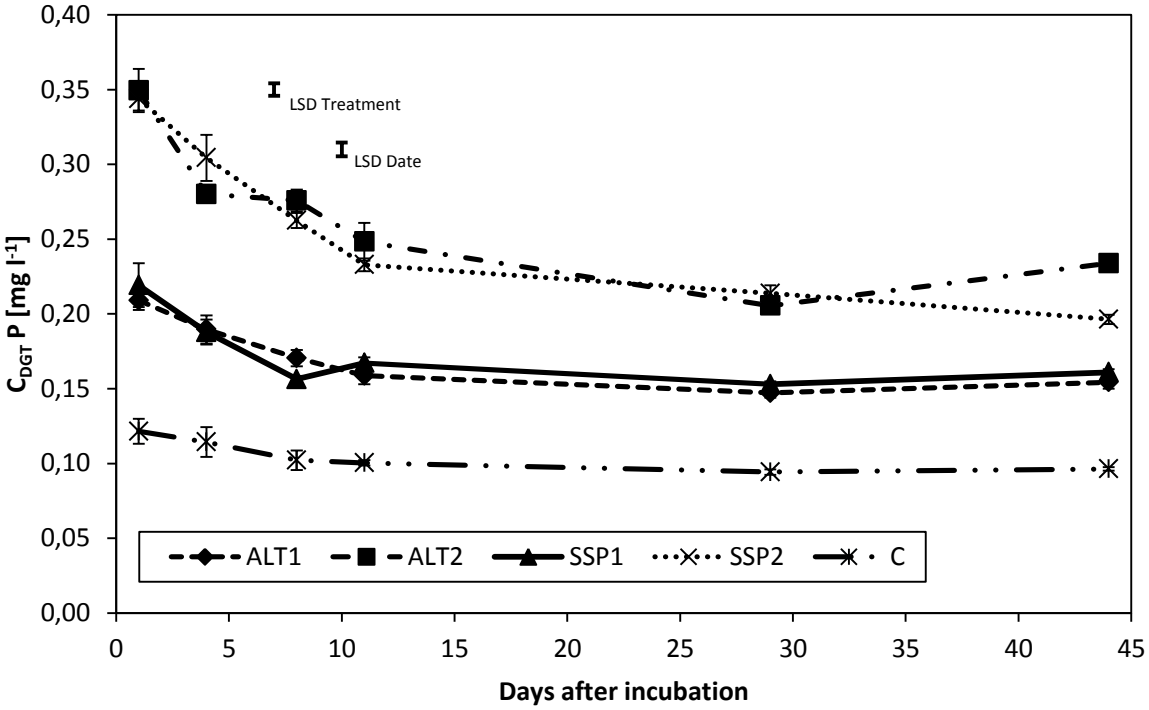

Figure 12 Time series of C_{DGT} values over a time span of 44 days. Means of three replicates. Error bars are SE. bold vertical bars: LSD for $p < 0.05$; Labelling according to Figure 4

In the treatments with the full amount of fertilizer C_{DGT} values dropped from 0.349 (ALT2) and 0.344 mg l^{-1} (SSP2) at the first sampling to 0.248 and 0.233 mg l^{-1} at the fourth sampling, representing a

decrease of 28.9 and 32.3 %, whereas for ALT1 and SSP1 the decrease during the same time was 24.1 and 23.8 %. Subsequently, the further slight decrease was negligible and the values can be considered constant from day 10 onwards. Furthermore it is worth to mention to what extent the measured C_{DGT} values were increased due to the fertilization, compared to the increases shown in the CAL extractions. Whereas the CAL measurements showed an average increase of about 36 % for the fully-fertilized and 20 % for the half-fertilized treatments compared to the control treatment at the beginning, the C_{DGT} values increased 185 % and 76 % respectively. This effect of P fertilizer addition on C_{DGT} values was considerably smaller at the end of the experiment, where the average increase of C_{DGT} values compared to the control are 122 % and 59 % for the fully and half fertilized treatments, respectively. Hence, the fully fertilized treatments showed a significantly stronger reduction in potential P plant availability than treatments with half of fertilizer amount applied.

Soil solution centrifugates

As described in chapter 3.2.3 soil pore water was extracted by centrifuging soil samples and measuring P concentration (C_{SOL}) colorimetrically (Figure 13). Since only one replicate per treatment was carried out, no error bars are represented. Without having any means to determine statistical variation, we can only state that the shape of the curves showed high similarities to the ones from the C_{DGT} measurements. Again, three groups (fully fertilized, half fertilized and control treatments) could be distinguished and a decline in P concentration occurred approximately until day 10 of the experiment. After that, C_{SOL} seems to be more or less constant. Whether the reduced values at sampling three were actually a real decline followed by a significant increase or an analytical error cannot be stated with sufficient certainty.

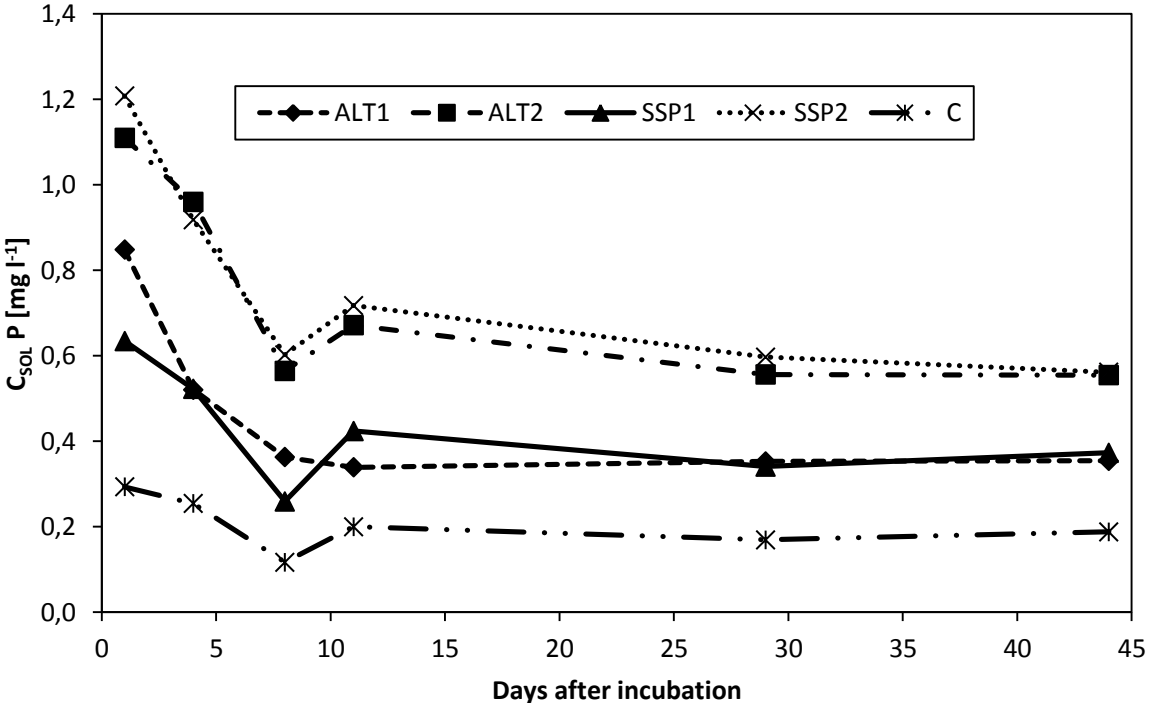

Figure 13 Time series of soil solution P concentration; one replicate per data point; Labelling according to Figure 4

R calculation

The obtained C_{DGT} and C_{SOL} values of the different treatments can be set into relation to each other by dividing C_{DGT} by C_{SOL}. Interpreting this ratio gives us the possibility to make estimations about the potential P resupply from labile pools of the soil solid phase and furthermore we can describe the behaviour of different fertilizer formulations when they interact with soil particles. Since the control treatment did not receive any P fertilizer and hence the measured C_{DGT} and C_{SOL} are solely determined by the substrate itself, Figure 14 only includes treatments where P fertilizer was added. For every sampling, the R values of the control treatment would be larger than the other treatments. In contrast to CAL-extractable and DGT-available P, the order of the treatments for the R value is opposite. Treatments that received the full amount of P fertilizer showed in average significantly lower R values than the ones that received half of the amount. The peak at sampling three is a consequence of the potential analytical error in the measured C_{SOL} concentrations at the third sampling. Distinguishing different groups cannot be done with the same certainty like in the previous figures. Assuming that the measured C_{DGT} value for the alternative formulation fertilizer at the last sampling was an artefact (see above), the curves for ALT2 and SSP2 show higher similarities and thus a better grouping for the last sampling could be achieved.

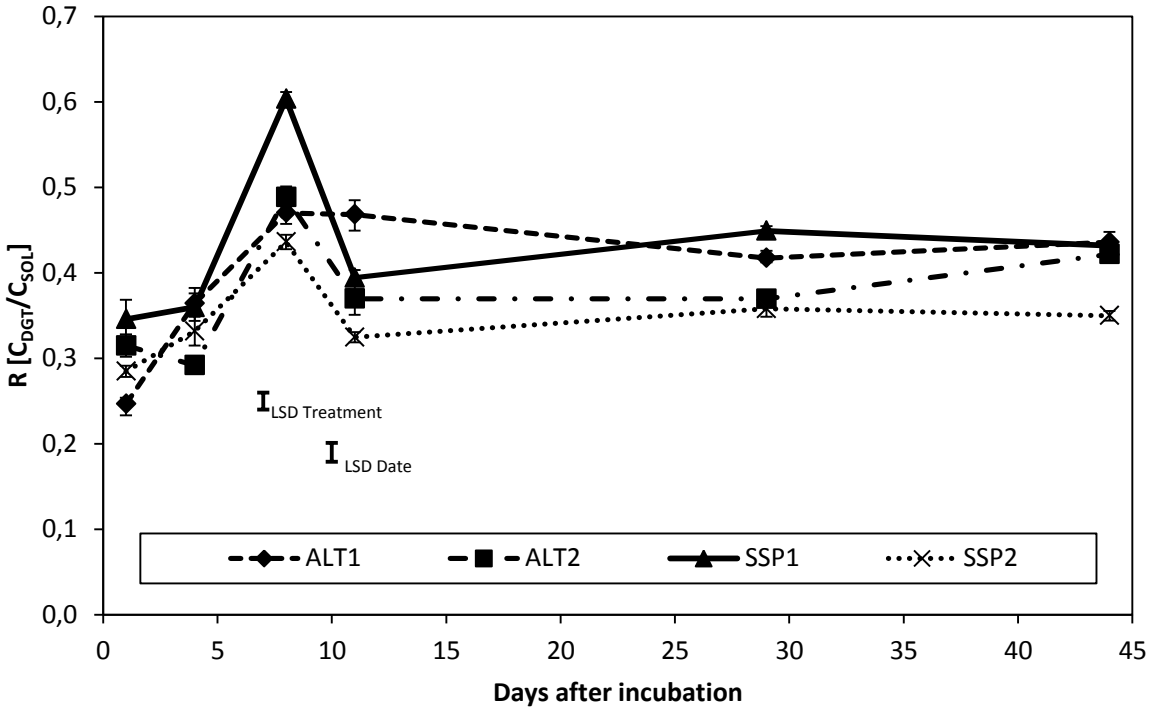

Figure 14 Time series of calculated R values. Means of three replicates. Error bars are SE. bold vertical bars: LSD for p < 0.05; Labelling according to Figure 4

5. Discussion

Assessing differences of fertilizer formulations in their capability of supplying plant available nutrients to plants is crucially important to evaluate their efficiency as fertilizers. Not only their elemental composition but rather their direct impact on plant growth and their behaviour when added to soil have to be investigated. By conducting a plant growth and an incubation experiment, this study aims to evaluate two distinct fertilizer formulations according to their supply of plant available P to soil and furthermore to the plant itself.

As described in chapter 4.1 and depicted in Figure 3 the environmental conditions during the plant growth experiment were not favourable for optimum plant growth. Plants experienced high fluctuation in temperature during their major growth period in the greenhouse compartment. Although the plants showed symptoms of drought stress during the hottest days, they achieved a 2.6 fold biomass increase in the four weeks between the two harvests (Figure 4). Chemical analysis of the plant material revealed that most of the N that was present in the analysis of the second harvest was already taken up at the time of the first harvest and the increase in biomass led to substantial dilution of N in plant material. The initial average N concentration in plant material at the first harvest was around 2 % in the dry matter, which is still within the range of 1.5 and 2.5 %, that Schilling (2000) states as common concentrations in green maize. Afterwards, mean concentrations dropped to around 0.5 %, which indicate a state of critical deficiency.

The amount of added N fertilizer to the pot experiment was calculated according to recommendations for Austrian agriculture that mostly rely on field trials. Within the first eight weeks of the experiment maize accumulated 1.8 times the applied fertilizer amount in its biomass and in the remaining four weeks of the pot experiment no N uptake took place. Comparison of the amount of N taken up by plants and the initial N fertilization amount shows clearly that the N demand of maize was vastly underestimated in this experiment. Assuming plant availability of 100 % of the added N fertilizer, the input amount of 265 mg N per pot only accounts for slightly over 50 % of N accumulated in plants eight and 12 weeks after sowing. N mineralisation in the substrate must have supplemented about 235 mg N, representing a rate of weekly mineralisation of about 16 mg N per kilogram of soil in the sand-soil mixture until the first harvesting. Reasons for the underestimation of N demand may be a too high plant density in the pot experiment compared to agricultural practice. Whereas on fields densities of nine to ten plants per m^2 are recommended, the two plants grown in the Mitscherlich pots in the experiment represent an about six times higher plant density resulting in less soil volume per plant available for nutrient acquisition. Therefore the N foraging capability and NUE of the maize plants cannot be compared to results coming from field trials, since the prerequisites are completely different. Apparently, the N fertilizer application and N mineralisation of the substrate supplied adequate amounts of plant available N to the maize until the first harvest

resulting in N concentrations in maize tissue within the range stated as common concentrations. Since no additional N fertilizer was applied after the first harvest, resupply of N was solely based on mineralisation until the second harvest. As previously mentioned, plants experienced high air temperatures during this time (Figure 3) which further deteriorated N supply for plants since the ratio between N mineralisation and immobilisation decreases with increasing temperature, i.e. the immobilisation increases at a higher rate than the mineralisation of N (Andersen & Jensen, 2001). This effect might have been enhanced since we used a substrate mixture consisting of a high amount of sand thus decreasing the mineralisation rate even further (Hassink, 1992).

Looking at the overall P concentrations of plant dry matter in Figure 7, there was to some extent the same tendency of P dilution in plant material visible. However, the magnitude was by far not as large as we saw for N. P was still taken up by plants when N uptake already stopped. According to Schilling (2000) 2 - 3 mg g^{-1} P are considered common average P concentrations found in healthy maize plants. Since at the first harvest P concentrations in dry matter of plants in the pot experiment were in a range between 1.5 and 2 mg g^{-1} we assume that these plants did not suffer severe P deficiency at this moment. Nevertheless, we see a decrease of concentration from the first to the second harvest. On average the values dropped to mean concentrations of 0.8 mg g^{-1} P in dry matter showing the dilution effect mentioned above and indicating a state of P deficiency at this moment. However, among others, Römheld (2012) suggests taking highly metabolic active plant organs as ideal sampling material, therefore, when only looking at P concentrations in green leaves, the concentrations dropped to on average 1.36 mg g^{-1}, which still indicates a state of deficiency although not as bad as calculated by combining all plant organs. The concentrations are slightly below the critical plant tissue concentration of 1.4 mg g^{-1} for maize stated by Zia et al. (1988). The concept of critical deficiency concentration (chapter 2.6; CDC) (Bouma, 1983) can also be used for assessing interactions between nutrients in plants. In literature ratios between element concentrations, instead of concentration of a single element, are used for better assessing the nutritional status and to take specific or unspecific interactions between nutritional elements into consideration. As it was stated before, the comparability of the pot experiment results with data from the literature is limited, but it may serve as a possibility to estimate tendencies. When comparing published CDC values for N/P ratios of maize ear leaf tissue from Walworth and Sumner (1988) with the ratios calculated for green leaves in our experiment, we can conclude that at the first harvest plants were already below these critical concentration ratios (9.0 in our experiment; 10.1 published ratio for maize). When looking at N/P ratios at the second harvest, the same picture is seen. The ratio did not change and we have to state once again, that N supply was most probably the biggest stress factor for plant growth in our pot experiment.

The calculated PUE figures depicted in Figure 8 are well in line with PUE developments over time described in literature. We see a distinct separation of treatments which were fully fertilized from the ones which received only half of the fertilizer amount. Whereas fully fertilized ones reached about 15 % recovery, the half fertilized ones reached about 30 % at the first harvest. During the four weeks between the first and the second harvest, the percentage points of P recovery of the applied fertilizer rose about 10 % for the fully fertilized treatments and nearly 20 % for the treatments that received half the fertilizer amount (Figure 8). This results go in line with Syers et al. (2008), who concluded that over time lower amounts of added P lead to higher recovery rates and larger increases in recovery rates. However, in some cases the withdrawals through harvested products can deplete the P reserves of the soil which has to be kept in mind and avoided. One of the reasons for this development is that with higher amounts of P applied within a short period of time, large amounts of readily soluble P species are added to the soil solution and therefore the equilibrium with the other P pools is not in balance anymore, leading to a shifting of readily available P into less available P pools (McLaughlin et al., 2011).

Since there was no significant difference in the amount of P taken up by the plants grown in the five different treatments at the first and at the second harvest, though we applied different amounts of fertilizer, it is obvious that we did not create any state of primary P deficiency for the plants and the low P concentrations at the second harvest have to be attributed to a severe N deficiency. Therefore the acclaimed better plant availability of the alternative P fertilizer formulation ALT cannot be validated with the biomass analysis of the plant growth experiment.

The soil P analysis showed significant differences between the treatments ($p < 0.050$) at both harvests (Figure 9), thus application of P fertilizers increased the CAL extractable P concentration significantly. The higher concentrations in the fertilized treatments can be attributed to an addition of readily available P to the soil through the added P fertilizer. At the second harvest, the increase in plant available P for the half fertilized pots compared to the control treatment was about half of the increase seen for the fully fertilized pots, which leads us to the conclusion, that the amount of P that was added to the pots underwent the same degree of immobilisation, sorption or precipitation. At the first harvest the amount of extracted P by CAL increased on average 14 mg kg^{-1} for ALT2, that means an increase of 96 mg plant available P per pot due to the fertilizer application. The overall P content of the harvested biomass was on average 39 mg P per pot. An addition of these two figures gives us nearly exactly half the amount of P that was added to these particular pots at the beginning of the experiment (282 mg P per pot). Hence, we can state that according to the CAL measurements for the first harvest, half of the added fertilizer P was still plant available at this moment, although the P taken up by the plant naturally comes from multiple sources and not only from fertilizer P. Since we saw no significant decrease in plant available P measured by CAL extraction in the second

harvest, but a significant increase in P accumulated in plant biomass of an average 28.8 mg for ALT2, the maintaining of constant CAL values depended on resupply of plant available P from the substrate. It is unclear and would need measurements with labelled P species if this P came from the P already available in the soil prior to the experiment or from the fertilizer that was added.

The clearer distinction of treatments by the DGT measurements (Figure 10) has to be attributed to the fact that the DGT technique only affects the very labile pool of P in soil via desorption processes, whereas the CAL extraction uses a chemical extractant and might also include some less labile P sources into plant available pools. In the plant growth experiment the outcomes of these two methods did not differentiate in their main message. The addition of the two fertilizer formulations to the substrate increased the amount of plant available P significantly but at both harvests we could not make any distinction between the two fertilizer formulations that were used. Due to the fact that C_{DGT} values cannot be seen as content of P per kilogram air-dried soil, the direct comparison with absolute values of the CAL measurements is limited. Nevertheless, the impact of the fertilizer application in relation to the control treatment can be investigated. While the degree to which the CAL concentrations increased, compared to the control treatment, reflected nearly exactly the ratio of applied fertilizer between fully and half fertilized pots, the C_{DGT} figures showed a different trend (Figure 10). At the second harvest the fully fertilized treatments ALT2 and SSP2 showed increases of 130 and 126 % while ALT1 and SSP1 only reached increases of about 57 and 53 % respectively which did not reflect the 2:1 ratio of applied P at the start. This might be a result of the fact that DGT measurements, as stated above, only take into consideration very labile forms of P, which are present in soil solution and are desorbed from the soil solid phase. While CAL measurements do not differ between different pools of very labile P in soils, the DGT technique, suggested by the results presented above, even illustrates differences in these pools.

Since we see a clear distinction in plant available P in the soil analysis results between fertilized and the control treatment in both CAL and DGT, but not in the plant growth results, we have to conclude once more that other stress factors than P deficiency have to be attributed for differences that came up in the plant experiment, which already have been addressed above.

With the second experiment, we did not address the impact of the added fertilizer on plant growth but the dynamic of soil P over time. Fertilizers may have different efficiencies, which can be altered by e.g. special positioning in the soil, altering of soil pH or increasing the solubility of the applied P. Although Figure 11 and Figure 12 do not support the allegedly better plant availability of the P fertilizer with the alternative formulation, we can interpret the development of the CAL and DGT measurements concerning the illustration of P retention by sorption and precipitation processes of P after the application of P. For CAL we saw some fluctuation that might indicate an increase of P plant availability during the first week of the incubation, this increase was not statistically significant

though, and statistical analysis suggests that from the start on there was neither an increase nor a decrease of potential plant availability. Again, the reason for this phenomenon might be due to the characteristics of the CAL extractant. Many studies came to the result that extraction methods including CAL do not give an accurate view on the plant availability of nutrients in soil samples (Mason et al., 2010; Tandy et al., 2011). The major restraint of using chemical surrogates to mimic plant nutrient uptake is that numerous factors affect the underlying mechanisms. Which extractant is used influences enormously the amount of nutrient extracted. Furthermore, the defined operational pH value, extraction time and soil to extractant ratio of a given extraction methods has a major impact on the results of the measurements and therefore really plant available as well as more stable compounds can be subjected to extraction. The amount of plant available P in soils is subsequently overestimated and recommendations concerning fertilizer applications do not build on P supply statuses of soils in the field. (Neyroud & Lischer, 2003). From the CAL measurements during the 44 days alone, a conclusion that there was no decrease in plant availability of the added P by sorption and precipitation process might be obvious. However, literature suggests just the opposite and it appears that in the present study the CAL extractant is not well suited for estimating P plant availability after a P fertilizer application. P added to the soil undergoes various processes as described in chapter 2.1 with the result that the majority of P becomes distributed between readily-available and less readily-available P pools in the soil by adsorption and absorption reactions, thus leading to lower plant availability over time (Zhang, 1996; Syers et al., 2008).

The expected decline of available P was well shown with the six DGT measurements conducted within 44 days (Figure 12). The initial effect of the fertilizer application decreased significantly within the first two weeks of the experiment with subsequent stabilisation at lower C_{DGT} values. The decrease in P availability for the control treatment was not statistically significant and might be attributed to the effect of moistening of the soil-sand mixture prior to the incubation. Therefore the C_{DGT} values of the control treatment are considered constant over the 44 days. The extent to which the C_{DGT} values initially increased due to the added P showed similar magnitudes as were shown in the DGT measurements of the plant growth experiment, where the half fertilized treatments showed a significantly lower increase than would have been expected according to the proportion of amounts which were applied to the fully fertilized treatments. At the beginning of the incubation experiment ALT2 and SSP2 showed increases of 188 and 183 % respectively, whereas the half fertilized treatments ALT1 and SSP1 only showed increases of 72 and 81 %. Over the following two weeks this gain of plant available P decreased with different declines and at day 29 of the experiment ALT2 and SSP2 showed gains of 118 and 127 %, while ALT1 and SSP1 achieved 56 and 62 %, respectively, compared to the control treatment. This reflects exactly the ratio 2:1, in which the amount of fertilizer was applied at the beginning of the experiment. This, at the first sampling

unexpected differences to the underlying proportional application amounts, which were not seen in the CAL results, and the subsequent balancing of them to the expected 2:1 ratio, have to be attributed to P sorption kinetics in soils. Since the used substrate was low in plant available P, firstly short-term sorption processes were more pronounced in the half fertilized treatments compared to the fully fertilized treatments, resulting in C_{DGT} values lower than expected at the first sampling (McGechan & Lewis, 2002). These processes reached equilibrium for the half fertilized treatments after one week due to the lower amount of added plant available P but they continued to remove P from readily available pools in the fully fertilized treatments for one more week (see also Figure 12). This led to the final 2:1 ratio of plant available P gained from day 29 onwards. Since a full speciation of the individual fractions of soil P would have gone beyond the scope of this thesis, we can state, that when considering the pH_{CaCl2} of the used substrate (7.66) the decline in plant availability has to be attributed most probably to sorption and precipitation reactions with Ca cations, which are the key determinant of P solubility in alkaline soils. Adsorption and precipitation lead to stronger retention of P and hence a decreased availability of P (Hinsinger, 2001; McGechan & Lewis, 2002).

As the P concentrations of soil solution centrifugates were only determined in one replication, the interpretation of these results is limited since no statistical investigation can be performed. However, the measured concentrations confirm again the trend which was pointed out by the DGT measurements. They indicate a sharp decrease of bioavailable P concentration during the first two weeks and a subsequent stabilisation (Figure 13). These soil solution centrifugates only take into account P that is dissolved in the soil solution. Already at the very beginning of the experiment sorption processes were presumably present, since the fully fertilized treatments show again higher proportional gains than would have been expected due to the amount of P applied compared to the control. However, the extent to which the obtained soil solution concentrations increased are even higher as was seen in the DGT results (up to 300 % for SSP2). This has to be attributed to the high amount of water soluble P that was added to the substrate and which influences the soil solution concentration to a higher extent than the C_{DGT} values, since the DGT technique also takes into consideration the resupply of P from the soil solid phase (Zhang et al., 2001).

The dynamics of calculated R values (Figure 14) showed an inversion of order of treatments compared to CAL-extractable and DGT-available P. Fully fertilized treatments showed in average significantly lower R values than half fertilized ones. DGT measurements include all labile species of P in soil solution except large colloidal forms and kinetically inert complexes, thus the soil solution concentrations which are used in R calculations, should ideally only include the same species of P (Harper et al., 1998). However, the determination of speciation in soil solution would have gone beyond the scope of this thesis and are assumed to be similar to the DGT applications. The clear

distinction between fully and half fertilized treatments cannot be made any more with the same accuracy as seen for the CAL and DGT results. High R values would be desirable, which indicate a relatively higher and presumably more sustainable supply of plant available P from the solid phase of the substrate. Though lower R values indicate high amounts of P in soil solution, the majority of plant's P supply has to come from the solid phase of the soil since common soil solution concentrations cannot satisfy the P need of plants. In the present case, the inversion of order shows that the contribution of the P pool not dissolved in soil solution to the measured C_{DGT} values differed according to the amount of fertilizer applied. Whereas the C_{DGT} values of the fully fertilized treatments were relatively more dependent on the P concentration in soil solution at a given time point, it was the opposite case for the half fertilized treatments, where the measured C_{DGT} values were more dependent on the P species that were initially not dissolved in the soil solution, but can be dissolved via desorption processes. Compared to the DGT measurements, the smaller gap between the two fertilizer application levels shows that additional amounts of applied fertilizers do not show linear increases in potential plant availability and when higher amounts of fertilizers are applied, relatively higher amounts undergo retention and are subsequently shifted to less readily-available P pools.

6. Conclusion and Prospects

Neither the plant growth experiment nor the incubation experiment revealed any significant differences between the two formulations of P fertilizer. None of the three independent soil tests (CAL extraction; soil solution centrifugation; DGT deployment) demonstrated differences in behaviour over time of the two formulations. This leads us to the conclusion that the fertilizer with the alternative formulation (ALT) for allegedly better plant availability shows the same properties concerning P plant availability as the standard SSP fertilizer for the particular experimental designs used in the two experiments. Although all three soil tests depicted the applied fertilizer levels (none, half amount and full amount) well, the DGT technique gave a better separation of the levels and higher statistical significances of the results. Furthermore, differences concerning the resolution of depicting plant available P over time for the soil tests could be demonstrated. Whereas for the CAL extraction no significant decline in plant P availability could be shown, DGT and soil solution extraction depicted the expected decline in plant available P during the incubation experiment well. As a consequence it might be concluded that CAL extraction is not an appropriate mean to investigate fertilizer efficiencies and the DGT technique appears to be the method of choice which is further supported by numerous studies that underline the superior power of the DGT method.

Unfortunately, DGT applications are more time consuming than traditional soil extraction methods at the moment and further improvements concerning sample handling and standardisation have to be made to increase the sample throughput for a successful commercial usage of this technique in the laboratory and in the field. However, possibilities are already available to shorten time required for gel casting and assembling of the devices by purchasing ready-to-use DGT devices. Furthermore, there is a need to introduce a classification scheme comparable to the CAL result categorization (Table 1) for being able to give comprehensible recommendations concerning fertilizer applications and long-term fertilizer strategies.

For further investigation of the allegedly better plant availability of the alternative P fertilizer formulation, additional experiments concerning plant growth and development as well as soil analysis will need to be conducted, preferably including various soil types and plant species in a wide range of pH values.

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8. List of figures

9. List of tables

10. Appendix

Appendix 1 Dry matter (DM) of leaves, stems and roots of maize eight (a) and 12 (b) weeks after sowing. Mean values of four replicates. Mean values within one column with different superscripted letters are significantly different at p < 0.05; SE are standard errors

a)	DM Leaves [g]		DM Stems [g]		DM Roots [g]	
	Mean	SE	Mean	SE	Mean	SE
ALT1	11,63[a]	0,676	9,59[a]	1,157	6,05[a]	0,643
ALT2	11,05[a]	0,599	8,76[a]	0,593	4,68[a]	0,789
SSP1	11,48[a]	0,475	8,91[a]	0,553	4,28[a]	0,832
SSP2	11,70[a]	0,397	9,28[a]	0,674	5,29[a]	0,763
C	10,97[a]	0,282	8,01[a]	0,562	4,30[a]	0,835

b)	DM Green Leaves [g]		DM Senescent Leaves [g]		DM Stems [g]		DM Roots [g]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ALT1	12,31[a]	0,77	8,46[a]	0,46	37,59[a]	0,786	10,48[a]	0,812
ALT2	11,26[a]	0,75	9,76[a]	0,40	39,47[a]	0,473	8,48[a]	1,567
SSP1	12,49[a]	0,59	8,80[a]	0,68	40,37[a]	1,562	10,90[a]	1,594
SSP2	12,15[a]	0,71	8,78[a]	0,55	39,77[a]	0,145	8,37[a]	1,812
C	11,95[a]	0,12	9,36[a]	0,21	39,98[a]	0,633	8,75[a]	0,325

Appendix 2 N concentration in leaves, stems and roots of maize eight (a) and 12 (b) weeks after sowing. Mean values of four replicates. Mean values within one column with different superscripted letters are significantly different at p < 0.05; SE are standard errors

a)	N concentration [%] Leaves		N concentration [%] Stems		N concentration [%] Roots	
	Mean	SE	Mean	SE	Mean	SE
ALT1	1,50[a]	0,137	2,67[ab]	0,281	1,30[a]	0,052
ALT2	1,77[a]	0,191	3,07[a]	0,147	1,33[a]	0,031
SSP1	1,75[a]	0,225	2,25[b]	0,123	1,34[a]	0,031
SSP2	1,66[a]	0,066	2,21[b]	0,081	1,19[a]	0,086
C	1,98[a]	0,093	2,38[ab]	0,125	1,39[a]	0,060

b)	N concentration [%] Green Leaves		N concentration [%] Senescent Leaves		N concentration [%] Stems		N concentration [%] Roots	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ALT1	1,20[b]	0,05	0,97[a]	0,02	0,48[a]	0,04	0,79[a]	0,03
ALT2	1,39[a]	0,04	0,85[a]	0,06	0,53[a]	0,03	0,79[a]	0,03
SSP1	1,14[bc]	0,04	0,64[b]	0,03	0,41[a]	0,02	0,79[a]	0,02
SSP2	1,02[c]	0,03	0,59[b]	0,01	0,45[a]	0,01	0,76[a]	0,04
C	1,21[b]	0,03	0,66[b]	0,02	0,46[a]	0,03	0,81[a]	0,02

Appendix 3 P concentration in leaves, stems and roots of maize eight (a) and 12 (b) weeks after sowing. Mean values of four replicates. Mean values within one column with different superscripted letters are significantly different at p < 0.05; SE are standard errors

a)	Leaves [mg g^{-1}]		Stems [mg g^{-1}]		Roots [mg g^{-1}]	
	Mean	SE	Mean	SE	Mean	SE
ALT1	1,41[b]	0,04	1,51[a]	0,02	1,68[a]	0,07
ALT2	1,50[b]	0,04	1,61[a]	0,06	1,71[a]	0,05
SSP1	2,09[a]	0,21	1,62[a]	0,10	1,65[a]	0,04
SSP2	1,76[ab]	0,14	1,59[a]	0,07	1,67[a]	0,10
C	2,22[a]	0,15	1,66[a]	0,05	1,81[a]	0,08

b)	Green Leaves [mg g^{-1}]		Senescent Leaves [mg g^{-1}]		Stems [mg g^{-1}]		Roots [mg g^{-1}]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ALT1	1,37[a]	0,05	0,37[a]	0,03	1,08[a]	0,04	1,09[a]	0,04
ALT2	1,32[a]	0,04	0,31[a]	0,01	1,06[a]	0,08	0,95[b]	0,02
SSP1	1,30[a]	0,04	0,28[a]	0,01	0,92[a]	0,04	1,05[ab]	0,02
SSP2	1,26[a]	0,05	0,28[a]	0,02	1,12[a]	0,05	1,01[ab]	0,02
C	1,34[a]	0,03	0,33[a]	0,03	1,00[a]	0,02	1,05[ab]	0,02

Appendix 4 K concentration in leaves, stems and roots of maize eight (a) and 12 (b) weeks after sowing. Mean values of four replicates. Mean values within one column with different superscripted letters are significantly different at p < 0.05; SE are standard errors

a)	Leaves [mg g^{-1}]		Stems [mg g^{-1}]		Roots [mg g^{-1}]	
	Mean	SE	Mean	SE	Mean	SE
ALT1	35,36[a]	1,04	64,04[a]	3,70	11,94[a]	2,03
ALT2	35,77[a]	0,45	65,25[a]	3,39	11,76[a]	1,47
SSP1	34,34[a]	0,44	69,38[a]	6,30	11,06[a]	2,08
SSP2	35,64[a]	0,96	70,20[a]	3,84	12,74[a]	1,47
C	35,55[a]	0,40	73,38[a]	4,04	15,73[a]	3,07

b)	Green Leaves [mg g^{-1}]		Senescent Leaves [mg g^{-1}]		Stems [mg g^{-1}]		Roots [mg g^{-1}]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ALT1	19,29[a]	0,38	24,05[a]	1,66	20,61[a]	20,61	10,70[a]	1,28
ALT2	19,41[a]	0,36	25,04[a]	1,41	22,51[a]	22,51	10,31[a]	0,79
SSP1	20,59[a]	0,86	25,26[a]	1,16	20,72[a]	20,72	10,00[a]	0,82
SSP2	19,59[a]	0,72	25,68[a]	0,12	26,18[a]	26,18	10,50[a]	0,24
C	20,24[a]	0,68	25,41[a]	0,81	24,10[a]	24,10	13,53[a]	0,97

Appendix 5 CAL extractable P and C_{DGT} values eight (H1) weeks and 12 (H2) weeks after sowing. Mean values of four replicates. Mean values within one column with different superscripted letters are significantly different at p < 0.05; SE are standard errors

	CAL extractable P [mg kg^{-1}] H1		CAL extractable P [mg kg^{-1}] H2		C_{DGT} [mg l^{-1}] H1		C_{DGT} [mg l^{-1}] H2	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ALT1	27,30^{b}	0,44	27,76^{c}	0,88	0,120^{b}	0,011	0,114^{b}	0,012
ALT2	35,06^{a}	2,34	33,96^{a}	1,87	0,176^{a}	0,004	0,167^{a}	0,014
SSP1	27,30^{b}	1,46	27,84^{bc}	0,80	0,122^{b}	0,010	0,111^{b}	0,003
SSP2	29,28^{ab}	1,33	32,65^{ab}	0,26	0,154^{a}	0,003	0,164^{a}	0,008
C	21,13^{c}	0,65	23,16^{c}	0,54	0,063^{c}	0,006	0,072^{b}	0,004

Appendix 6 a) Mean CAL extractable P b) mean C_{DGT} values at the six scheduled sampling dates indicated with days after sowing (DAS). Mean values of three replicates. Mean values within one column with different superscripted letters are significantly different at p < 0.05; SE are standard errors; c) soil solution P concentration of the incubation experiment

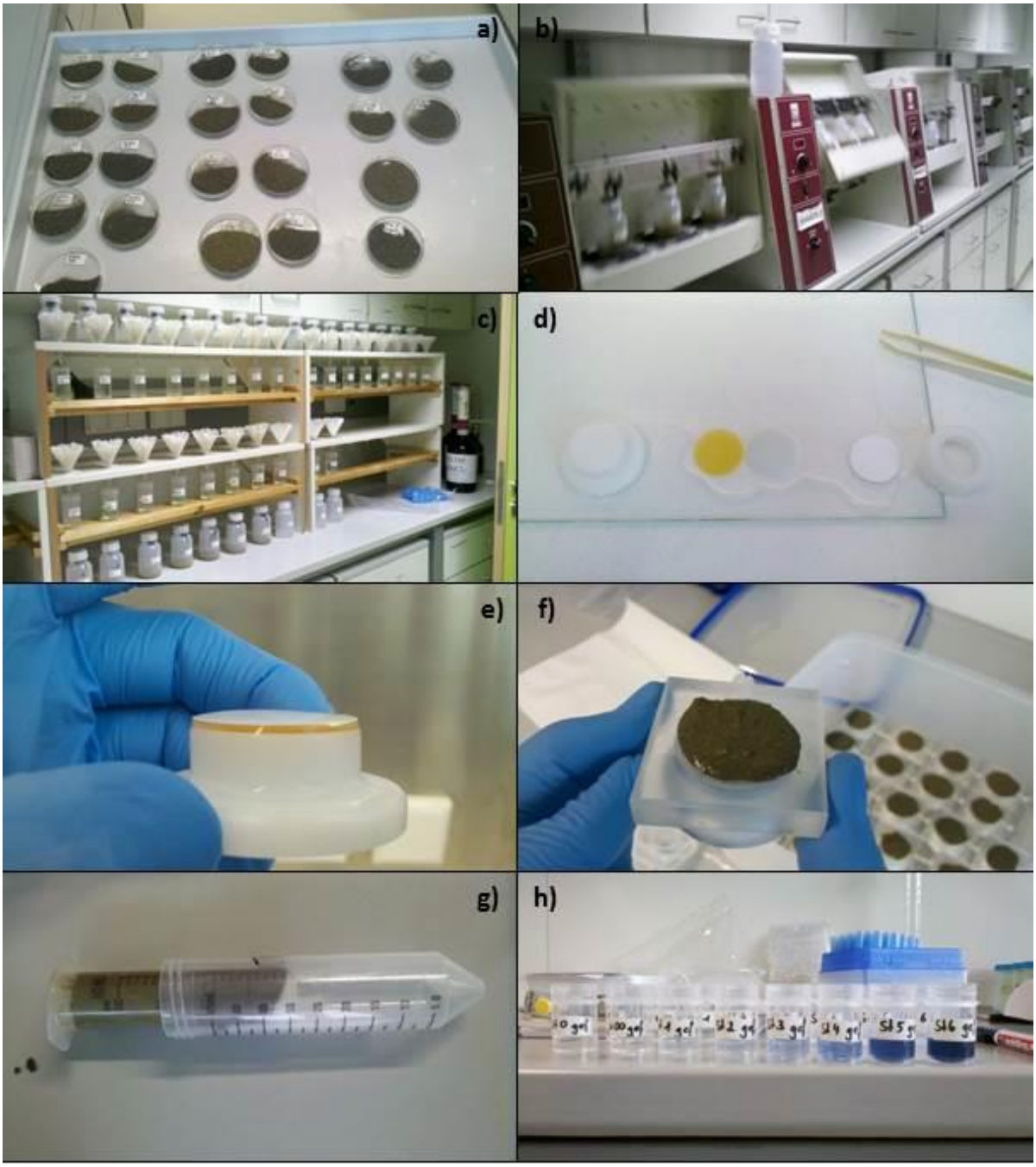
a)	CAL 1 DAS [mg kg^{-1}]		CAL 4 DAS [mg kg^{-1}]		CAL 8 DAS [mg kg^{-1}]		CAL 11 DAS [mg kg^{-1}]		CAL 30 DAS [mg kg^{-1}]		CAL 45 DAS [mg kg^{-1}]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ALT1	35,08^{b}	0,51	39,21^{b}	0,82	35,22^{b}	1,06	33,74^{b}	0,58	38,58^{ab}	0,84	32,02^{ab}	0,44
ALT2	40,56^{a}	0,68	45,15^{a}	1,24	42,41^{a}	0,76	38,84^{a}	0,87	44,19^{a}	1,26	37,94^{a}	0,95
SSP1	36,09^{b}	0,70	37,85^{b}	0,66	35,69^{b}	0,99	32,05^{b}	0,76	38,29^{ab}	1,10	30,45^{bc}	0,79
SSP2	40,50^{a}	0,26	44,42^{a}	1,33	40,39^{a}	0,52	38,14^{a}	0,54	44,79^{a}	0,25	37,24^{a}	0,20
C	29,74^{c}	0,54	33,44^{c}	1,17	29,70^{c}	0,33	28,00^{c}	0,20	34,48^{b}	0,51	27,19^{c}	0,81

b)	C_{DGT} 2 DAS [mg l^{-1}]		C_{DGT} 5 DAS [mg l^{-1}]		C_{DGT} 9 DAS [mg l^{-1}]		C_{DGT} 12 DAS [mg l^{-1}]		C_{DGT} 31 DAS [mg l^{-1}]		C_{DGT} 46 DAS [mg l^{-1}]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ALT1	0,209^{b}	0,006	0,190^{b}	0,009	0,170^{b}	0,005	0,159^{b}	0,006	0,147^{b}	0,003	0,154^{c}	0,004
ALT2	0,349^{a}	0,015	0,280^{a}	0,002	0,276^{a}	0,007	0,248^{a}	0,013	0,206^{a}	0,003	0,234^{a}	0,002
SSP1	0,219^{b}	0,015	0,188^{b}	0,008	0,156^{b}	0,002	0,167^{b}	0,004	0,153^{b}	0,002	0,161^{c}	0,002
SSP2	0,344^{a}	0,008	0,304^{a}	0,015	0,262^{a}	0,005	0,233^{a}	0,004	0,214^{a}	0,006	0,196^{b}	0,003
C	0,121^{c}	0,008	0,114^{c}	0,010	0,102^{c}	0,006	0,100^{c}	0,002	0,094^{c}	0,002	0,096^{d}	0,001

c)	C_{SOL} 1 DAS [mg l^{-1}]	C_{SOL} 4 DAS [mg l^{-1}]	C_{SOL} 8 DAS [mg l^{-1}]	C_{SOL} 11 DAS [mg l^{-1}]	C_{SOL} 30 DAS [mg l^{-1}]	C_{SOL} 45 DAS [mg l^{-1}]
ALT1	0,848	0,521	0,363	0,339	0,353	0,354
ALT2	1,109	0,960	0,565	0,672	0,556	0,554
SSP1	0,634	0,522	0,259	0,424	0,341	0,373
SSP2	1,208	0,918	0,602	0,717	0,597	0,561
C	0,293	0,254	0,117	0,200	0,169	0,188

Appendix 7 a) Set up of the pot experiment 0 days after sowing (DAS); b) Pot experiment with maize plants 11 DAS prior to thinning out; c) Pot experiment with maize plants eight weeks after sowing (first harvest); d) Separately harvested plant organs 12 weeks after sowing (second harvest); e) Maize roots after washing in a root washbasin; f) Grinding of maize leaves with a knife mill; g) Open acid digestion of maize tissue samples for chemical analysis with AAS and spectrophotometry; h) Varian SpectrAA 300 Atomicabsorptionspectrometer with carrousel rack

Appendix 8 a) Air-dried and sieved (< 2 mm) soil samples prepared for soil analysis; b) Overhead shakers for the CAL extraction method; c) Filter rack for the CAL extraction method; d) Components of a single DGT device (from left: piston, ferrihydrite gel disc, Whatman Nucleopore membrane, diffusive gel disc (transparent), Pall filter membrane and ring with exposure window); e) Assembled DGT device before the ring is pulled over; f) DGT device loaded with soil paste; g) Set up for soil solution centrifugation; h) Calibration standard solutions for photometric measurement of the phosphomolybdate complex (blue method 1) for determination of P concentrations



11. Abstract

The essentiality of phosphorus (P) for every living cell lies in its pivotal role in cell metabolism. With its use as fertilizer in agricultural production systems and rapidly decreasing world reserves, P use efficiency (PUE) turns into the spotlight of public interest and scientific community as well as commercial research. In this study two P fertilizer formulations (single super-phosphate (SSP) and an alternative P fertilizer formulation (ALT)) were tested for their supply of plant available P. Experiments consisted of a greenhouse and an incubation experiment. In both trials P supply was established with two different fertilizer levels and a control treatment which received no additional P supply. The greenhouse pot experiment with maize was set up to assess the direct effect of the fertilizer formulations on plant growth, plant development and elemental constitution of the harvested plant material eight and 12 weeks after sowing. In the 44-day incubation experiment the same substrates and treatments were used. Analysis of soil samples included calcium lactate - calcium acetate (CAL) extractable P, diffusive gradient in thin films (DGT) and soil pore water extraction by centrifugation. In the pot experiment other stress factors than P deficiency influenced plant growth to a large extent. Therefore, no differences between treatments could be observed. These results extend to some extent to the incubation experiment where no beneficial effects of the ALT treatments could be determined in this particular substrate. However, between the applied soil analysis methods variations in depiction of dynamics of plant available P could be monitored. CAL extraction did not show a significant decrease in plant available P after the fertilizer application and therefore expressiveness for short-term fertilization strategies might be limited. The DGT technique and the soil solution extraction depicted the expected decrease in plant available P after an initial application of fertilizer, followed by a subsequent levelling out at significantly lower concentrations.

12. Kurzzusammenfassung

Die Essentialität von Phosphor (P) für alle lebenden Organismen beruht in der zentralen Rolle, die P im Stoffwechsel von Zellen trägt. Die Nutzung als Düngemittel in der Landwirtschaft und die rapide schrumpfenden weltweiten Reserven rücken die effiziente Verwendung von P in landwirtschaftlichen Produktionssystemen in das öffentliche Interesse und weiters in das Interesse der Forschung. Für die vorliegende Arbeit wurden zwei verschiedene P Düngemittelformulierungen verwendet (Single Super-Phosphate (SSP) und eine alternative P Formulierung (ALT)), um Unterschiede bezüglich der Pflanzenverfügbarkeit aufzuzeigen. Beide Düngerformulierungen wurden in zwei unterschiedlichen Aufwandmengen getestet und zusätzlich wurde eine Kontrollvariante ohne zusätzliche P-Düngung mitgeführt. Neben einem 12-wöchigen Glashausexperiment mit zwei Erntetermine (acht und 12 Wochen nach der Aussaat), in dem der Einfluss auf die Pflanzenentwicklung von Mais, Biomasseproduktion und Zusammensetzung des Pflanzenmaterials untersucht wurde, wurde ein 44 Tage Inkubationsexperiment mit denselben Behandlungen durchgeführt, um das Verhalten des hinzugefügten P im Boden zu evaluieren. Die Bodenanalysen umfassten: Calciumlactat-Calciumacetat (CAL) extrahierbarer P, diffusiver Gradient in Dünnfilmen (DGT) und Extraktion der Bodenlösung durch Zentrifugieren. Während des Versuches im Glashaus traten für die Pflanzen starke negative externe Umwelteinflüsse ein, welche mögliche Auswirkungen der Düngerformulierung eventuell überdeckten. Jedoch wurden auch im Inkubationsversuch keine statistisch signifikanten Differenzen zwischen den Düngerformulierungen nachgewiesen, da diese nahezu exakt dasselbe Inkubationsverhalten zeigten mit einer starken Abnahme der Pflanzenverfügbarkeit des hinzugegebenen P innerhalb der ersten 14 Tage gefolgt von einem Einpendeln der gemessenen Konzentrationen auf deutlich niedrigerem Niveau. Zusätzlich konnten zwischen den drei Bodenanalysemethoden große Unterschiede, bezüglich deren Fähigkeit Sorptionsprozesse (und folglich abnehmende Pflanzenverfügbarkeit) darzustellen, festgestellt werden. Hierbei zeigte vor allem die DGT Technik große Vorteile bezüglich der Darstellung rasch ablaufender Adorptions- und Desorptionsprozesse.