Diffusive Gradients in Thin Films (DGT): a novel technique to predict plant response to nutrient availability

M.Sc. Thesis

Benjamin Hill

FACULTY OF AGRICULTURE, UNIVERSITÄT HOHENHEIM

INSTITUTE FOR SOIL RESEARCH, UNIVERSITÄT FÜR BODENKULTUR

SUPERVISOR: DR. WALTER W. WENZEL

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List of Abbreviations

A	area of the DGT sampler
AEM	anion exchange membrane
AGES	Österreichische Agentur für Gesundheit und Ernährungssicherheit
ATP	Adenosine triphosphate
C_{DGT}	time averaged concentration at interface of DGT sampler and soil solution
CAL	calcium acetate lactate
Δd	thickness of the diffusive layer
D	diffusion coefficient
DGT	diffusive gradients in thin films
DMT-HFO	dialysis membrane tube – hydrous ferric oxide
ECM	ectomychorrizal fungi
EDTA	ethylenediaminetetraacetic acid
GS	growth stage
HQ	high quality
ICPMS	inductively coupled mass spectrometry
LA-ICPMS	laser ablation inductively coupled mass specrometry
LK NÖ	Landwirtschaftskammer Niederösterreich
MES	2-(*N*-morpholino)ethanesulfonic acid
SSP	single superphosphate
TEMED	tetramethylethylenediamine
WHC	water holding capacity
t	time

Abstract

Phosphorus (P) is an essential macronutrient for plant growth and therefore one of the most broadly applied fertilizer agents worldwide. P has a strong affinity for the soil solid phase, and its mobility is controlled by a suite of abiotic and biotic processes which make the labile portion difficult to assess. Following the documented failure of conventional batch extraction techniques to measure nutrient availability across different soil types and climate regimes, alternative techniques such as diffusive gradients in thin-films (DGT) have been proposed as viable alternatives. The objective of this study was to characterize the potential of DGT as a tool to assess nutrient availability, and to investigate the capacity of DGT to predict crop response to P and micronutrient concentrations.

119 soil samples were taken from 4 climate/soil zones throughout Austria, in attempts to represent the dominant agricultural landscapes in the country. P and micronutrient (Fe, Mn, Cu) concentrations were measured with DGT and conventional extraction protocols (EDTA, CAL). Subsequently, DGT was compared to extraction techniques in order to (1) determine the influence of soil edaphic (pH, $CaCO_3$) properties on DGT measurements and to (2) determine the capability of DGT to predict crop response to varying P and micronutrient concentrations under field conditions.

Our results indicate that DGT is less influenced by $CaCO_3$ and pH fluctuations, while extraction techniques were highly dependent on these soil physicochemical properties. Though DGT P concentrations were moderately correlated (R^2=0.50) with CAL values across all 4 sites, the relationship was strengthened after separating soil test values based on carbonate content (R^2=0.76, R^2=0.85). DGT concentrations exhibited saturating non-linear behavior in relation to relative yield of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*); although this relationship was statistically insignificant it was indicative of typical dose-response relationships which are commonly reported for macro and micronutrients. Interestingly, DGT Mn concentrations exhibited similar behavior, which highlight the control of soil edaphic properties on micronutrient availability and the relative importance of micronutrient nutrition in our study system.

1. Introduction

1.1. Phosphorus as a nutrient

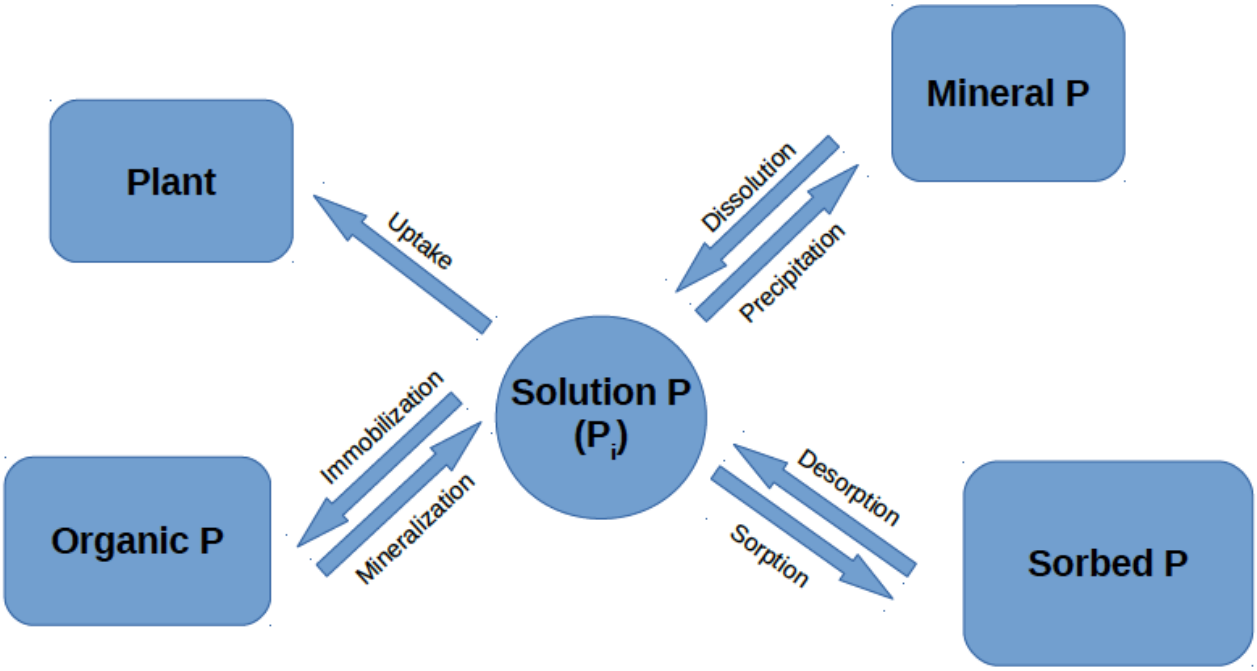
Phosphorus (P) is an essential nutrient for plant growth, and makes up around 0.2 % of a plants biomass on average (Schachtman et al., 1998). P is a primary constituent cell membranes, nucleic acids and the energy-rich molecule adenosine triphosphate (ATP), and therefore central in the biochemical processes of respiration and photosynthesis. The phosphororylation and dephosphorylation of proteins is integral for signal transduction in plants, thus a plant's P status is crucial to its growth and development (Raghothama and Karthikeyan, 2005). Maintaining an adequate P status is therefore crucial for sustaining agricultural productivity. In addition to nitrogen and potassium, P is one of the most heavily fertilized nutrients worldwide.

1.2 Phosphorus chemistry in soil

Inorganic phosphate (Pi) is the main form of P, which is taken up by plants and microorganisms, and accounts for 35-70 percent of the total P in soils (Harrison, 1987). The speciation of Pi in soil is determined by solution pH, with the orthophosphate anions $H_2PO_4^-$ and HPO_4^{2-} dominating in the range of pH generally found in agricultural soils (Lindsay, 1979). The soil solution is the main source of Pi for plants, and contains concentrations of 0.01 and 3.0 mg P L^{-1} (Frossard et al., 2000). Solution Pi concentrations are generally much lower than plant needs, as P is characterized by strong fixation and slow diffusion in soils, and thus P is often the limiting nutrient for plant growth.

Soil P can be conceptually divided into different pools that are governed by different chemical and biological transformations (Fig. 1). The mineral pool consists of both primary and secondary minerals. Primary P-containing minerals are dominated by apatites, which are relatively stable. Weathering of these minerals releases P anions into the soil, though this process is too slow for active contribution to plant requirements (Shen et al., 2011). P readily precipitates with different metal cations, forming Al/Fe phosphates in acidic soils and Ca phosphates in neutral to alkaline soils (Hinsinger, 2001). These reactions are dictated by a soil's chemical properties and pH, which determines the solubility of metal cation. Precipitation reactions are also the mechanism which controls the solubility of many concentrated P fertilizers. When concentrated granular P is applied to a soil, water initially moves into the granule and creates a solution which is super saturated with respect to P and other cations (Ca, Al, Fe); when this process is coupled with a large pH gradient in comparison to the surrounding

soil, co-precipitation leads to the formation of stable P-bearing minerals which are unavailable to plants and soil biota (Lindsay, 1979).



Organic forms of P (Po) account for anywhere from 20 to 80 percent of the total P pool (Richardson, 1994). Most Po exists in the form of phosphate mono- and diesters (i.e. phytiins, phospholipids, nucleic acids) in soil, and the inorganic form must be liberated (mineralized) in order to contribute to plant uptake (Turner, 2008). Microorganisms and plants drive the mineralization process through the production of exoenzymes (e.g. phosphatases), a process that depends on the availability of P for metabolic processes. When Pi is limited, microorganisms may stimulate the decomposition of organic matter thereby releasing Pi (Spohn et al., 2013; Heuck et al., 2015). Ectomyccorhizal fungi (ECM) are also able to access Po (Turner, 2008) via exoenzyme production and it has been suggested that ECM possibly absorb Po as an intact molecule (Rennenberg and Herschbach, 2013). Some research has shown that plants may take up organic P-containting compounds (Becquer et al., 2014), although this is of minor relevance in modern heavily fertilized agricultural systems.

The physical and/or chemical attraction of charged particles to a solid surface, or sorption, is a dominant process that controls the availability of P in soil (Sparks, 2003). As most inorganic forms of P in soil exist in anionic form, positively charged surface groups of Al- and Fe- (oxy)hydroxides are the primary compounds that bind Pi. Al/Fe- (oxy) hydroxides are positively charged in the pH ranges encountered in most soils, and thus central in P sorption in both alkaline and acidic soils (Hinsinger, 2001). Organic matter and clay minerals (i.e. kaolinite, layer silicates) have variable charged surfaces, and become positively charged at lower pH when there is a greater abundance of protons in the soil solution (Sparks, 2003). A high specific surface area also characterizes organic matter and clay minerals, and at low pH values they may serve as a significant sink for Pi.

Desorption of Pi is often initiated via a phenomenon known as ligand exchange, whereby an organic or inorganic anion replaces another anion on a solid surface or in solution. In most soils, the aforementioned surface groups (i.e. hydroxyl) of hydrous oxides are the primary players involved in these reactions (Sparks, 2003). Both plants and microorganisms may initiate desorption through the input of organic acid anions (e.g. malate, citrate, oxalate) and protons, especially in P-deficient conditions (Hinsinger et al., 2001). Organic acid anions may promote P solubilization through ligand exchange, or by ligand-promoted dissolution of Al- and Fe-oxides (Johnson and Loeppert, 2006). Plants also release protons in order to maintain internal charge balance after cation uptake (e.g. K^+, NH_4^+), thereby acidifying the rhizosphere (Marschner, 1995). The observed effect is highly dependent on a soils' mineralogy, the rate of organic acid anion or proton release, and total P concentration. For example, in calcareous soils protons may release Pi through the dissolution of calcium phosphates (Akhtar et al., 2009), where the opposite effect is seen in acidic soils, as Al/Fe-phosphates become more stable with decreasing pH (Hinsinger, 2001). Oburger et al. (2011) observed increased Pi availability upon rhizosphere acidification at low P concentrations, while the opposite effect was seen with high P concentrations.

In contrast to previously described surface reactions, inorganic P may also be occluded within minerals, rendering them unavailable to plants and microorganisms. Mineral occlusion generally takes place when either (1) hydrous oxides (mainly Al- and Fe- oxides/hydroxides) precipitate on top of P which has been previously sorbed or (2) via slow diffusion into the mineral lattice (Smeck, 1985). The first mechanism generally follows a soil's age, since weathering releases P from primary minerals

while simultaneously accrues secondary Al- and Fe- (oxy) hydroxides. Case (1) is further differentiated by the solubility of the outer mineral shell surrounding P. The protective layer may be either reductant-soluble, whereby reductive dissolution may liberate P, or P is "occluded" and considered non accessible (Smeck, 1985). The second phenomena occurs as the rapid adsorption of P onto mineral surfaces is followed by a slower diffusional process, whereby P enters lattice structure of the mineral (Barrow, 1983). Due to the slow kinetics of adsorption, this process is considered irreversible (van der Zee et al., 1989).

In order to maintain chemical equilibrium, all solid phase pools of P are reacting concomitantly with the goal of establishing a (pseudo) equilibrium with the solution phase. However, this is a simplistic view, as Pi exhibits varying affinity for solid surfaces, which affects the kinetics of mobilization and immobilization. P mobilization from solid phase pools is governed by the processes of mineral (co-) dissolution, desorption, and enzymatic breakdown; these transformations occur simultaneously, though their rates are controlled by both biotic and abiotic factors. This results in differential contribution of each solid phase pool to the pool which is available for plant uptake. Given the spatially and temporally heterogeneous nature of soil, the labile pool is inherently difficult to assess, and has been the subject of decades of research.

1.3 Soil P Testing

As the total amount of an element in soil bears little relation to the fraction which may be available for plant uptake, it is useful to characterize the bioavailable pool. It is first important to distinguish between the terms "labile" and "bioavailable". By definition, labile species have the ability to contribute to the solution phase either through dissociation, desorption, mineralization or dissolution. In contrast, species are considered inert if they are incapable of phase change on a relevant timescale, which may happen via mineral occlusion or in cases of extremely low solubility. Bioavailability takes into account the supply of an element to an organism as well as it's uptake mechanisms, and thus is species specific (Zhang and Davison, 2015). Therefore, labile species are only bioavailable if an organism's uptake is rapid.

Attempts to characterize the labile pool have given rise to two methodologies; batch extraction techniques and infinite-sink techniques. Batch extraction attempts to establish a (pseudo)

equilibrium between the P sorbed on the solid phase and the dissolved P in the extract solution. These techniques involve initiating the release of an element from the solid phase through one or mechanisms: (1) depleting the concentration of P in solution, which induces resupply from the solid phase (2) altering the solution pH (3) introducing chelating agents which have the propensity to complex or precipitate analyte cations (4) introducing elements which desorb P and prevent re-adsorption (Sibbesen, 1983). Consequently, batch extraction techniques accumulate P in the solution phase, whereby dissolved P may interact with the reagents used for extraction (i.e. precipitation, complexation) or re-adsorb onto solid phase particles. In this way, a soil test reading is strongly determined it's parameters, including solution extract pH, soil:solution ratio, temperature, and concentration of reagents in solution (Sibbesen, 1983).

For the measurement of labile P, batch extraction techniques are the most commonly used methods due to their simplicity and affordability. A suitable P test should provide an accurate measure of the P status of a soil, and correlate with plant uptake or yield across different soil types and fertilization strategies (Six et al., 2014). Batch extraction protocols are based on empirical evidence, and vary widely in their composition, soil to solution ratios, and extraction time. In Austria and Germany, the calcium acetate lactate (CAL) protocol is standard. In this method, an extracting solution consisting of calcium lactate, calcium acetate and acetic acid is prepared, and 100 mL of this solution is added to 5 g soil (Schüller, 1969). Denmark, Italy, and the UK use the biocarbonate Olsen-P method (0.5 M $NaHCO_3$, 1:20, 30 min) (Mason et al., 2013). Modifications of this method are utilized in Australia and New Zealand, particularly the Colwell-P method, which differs only in soil to solution ratio and extraction time (1:100, 16 h). In the United States, different methods are deployed depending on the soil type, with Olsen-P dominating with alkaline soil types, while Bray (0.03 M NH_4F, 0.1 M HCl 0.2 M CH_3COOH, 0.25 M NH_4NO_3, 1:7, 40s) and Mehlich (0.015 M NH_4F, 0.013 M HNO_3, 0.001 M EDTA, 1:10, 5 min) methods are used for acidic soils (Mason et al., 2013; Tandy et al., 2011).

Soil edaphic properties, such as pH, mineralogy (e.g. presence of Al- and Fe- oxides), clay and carbonate content have the potential to strongly shape the results of an extraction protocol. In particular, $CaCO_3$ strongly interacts with common soil extractants (i.e. P, micronutrient) through two primary mechanisms: (1) strong buffering capacity (2) strong interaction between Ca and P. Schüller (1969) was aware of these interactions during his development of the CAL method, as he

recommended the test for use only on calcareous soil since the extraction neglected the P of apatitic phosphates which are exchangeable only on acidic soils. Following this logic, it is expected that the CAL method would underestimate the labile P pool in acidic soils (Schüller, 1969). On the contrary, the double lactate (DL) method was developed for use in acidic soils, since the acid extract became neutralized in soil with high $CaCO_3$ content (Riehm, 1947). Similarly, Wenzel and Blum (1998) observed divergence of metal extraction yields based on the operational pH of the extract. They observed increase in the extract pH from 4 to around ≥6 during EDTA extraction on calcareous soils, thus greatly reducing the extraction yield.

Criticisms of batch extraction have given rise to alternative methods, such as infinite sink techniques. These methods employ the application of a high-affinity resin to a soil paste or slurry, which depletes the P solution concentration and results in continual release of solid phase P into the solution pool (Santner et al., 2014). Early application of infinite sink methodology included use of anion exchange membranes (AEM) (Schoenau and Huang, 1991) and Fe-oxide coated paper strips (van der Zee et al., 1987). These techniques provided some advantages to traditional extraction approaches, since they rely on a binding mechanism similar to plant roots, therefore avoiding the accumulation of Pi in solution. Preliminary methods were not without shortcomings, such as the attachment of soil particles onto the Fe-oxide strips; this process included up to 40 percent additional P that would be unavailable to plant roots (Uusitalo and Yli-Halla, 1999). To address these issues, researchers filled dialysis tube membranes with with hydrous ferric oxide (DTM-HFO), an approach which avoided accumulation of soil particles on the membrane through the small pore size of the dialysis tube membrane (Freese et al., 1995; Santner et al., 2014). Similar to Fe-oxide strips, the AEM method has been criticized for it's lack of representing diffusion limitation of P acquisition by plants (Degryse et al., 2009) as well as incidence of anionic interference with the resin layer (Mason et al., 2008). These methods (Fe-oxide strips, Resin P, AEM, DTM-FO) have shown some success in predicting plant available P (Sibbesen, 1977; van der Zee et al., 1987; Schoenau and Huang, 1991; McBeath et al., 2007; Mason et al., 2010), although similar to extraction methods, one test has not proved reliable across a broad range of soil types and agricultural systems.

1.4 Soil Micronutrient Testing

In addition to P and other macronutrients, other elements are required for the growth and development of higher plants. The elements Fe, Mn, Zn, Cu, B, Mo, Cl, Co are considered micronutrients since they are required only in trace amounts for adequate growth (Sillanpää, 1982). Like P, these elements often interact with soil particles, organic matter and other dissolved species which may render them inaccessible to plants and microorganisms. Therefore, in soil testing, it is important to characterize the labile pool which is available to plants throughout the growing season.

Most soil micronutrient testing is founded on basic chemical processes of dissolution, chelation, desorption, and reduction/oxidation. Primary methods include chemical extracts (neutral salts, mild acids, organic extractants) and resin based techniques where variable success has been reported for estimating the plant available fraction (McLaughlin et al., 2000; Menzies et al., 2007). Before the advancement of analytical equipment, which allowed researchers to detect elements at low solution concentrations, soil micronutrient testing preferred the use of strong extractants that would release substantial amounts of elements from the solid phase. The most commonly used chemical extractants were chelating agents, such as ethylenediamine-N', N, N', N' – tetraacetate (EDTA) or diethylenetriaminepentaacetate (DPTA) or strong acids (e.g. HNO_3, HCl). A wealth of other procedures followed, as researchers adjusted chemical mixtures to suit the particular element of interest.

DPTA (0.005 M DTPA, 0.1 M triethanolamine (TEA), 0.01 M $CaCl_2$, 1:2, 2 hr) was initially developed to measure Mn, Fe, Cu and Zn availability, particularly on calcareous soils (Lindsay and Norvell, 1978). Similarly, the ammonium acetate EDTA extraction (0.5 M CH_3COONH_4, 0.5 M CH_3COOH, 0.02 M Na_2EDTA, 1:10, 1 hr) was developed to measure available micronutrient (Mn, Fe, Cu and Zn) concentration and performed well on calcareous soils due to it's lower sensitivity to carbonate buffering. EDTA and DPTA are widely used (e.g. Austria, Denmark, France, Finland, Hungary, Ireland, Norway and Portugal) still, even as technological advances have significantly decreased limits of detection, subsequently allowing for the use of weaker extracts (e.g. 0.01M $CaCl_2$, 1.0M NH_4N0_3) (McLaughlin et al., 2000). In spite of this, a substantial body of work suggests that they are poor predictors of plant tissue concentrations across different soil types (Menzies et al., 2007).

Various single and sequential extraction protocols are used to assess other trace elements; some of

which include the ammonium oxalate – oxalic acid extraction (249 g ammonium oxalate, 126 g oxalic acid, 10 L H_20, 1:10, 16 hr) for Mo, and the hot water soluble soil test for B. The latter test was modified from an earlier method (Sippola and Erviö, 1977) where 25 mL soil sample is mixed with 50 mL H_20 and 5 mL activated charcoal and boiled for 5 min. Subsequently, the filtrate (2 mL) is mixed with 4 mL of a buffer masking agent and 4 mL of azomethine reagent, after which the sample develops color and is measured photometrically (Sillanpää, 1982).

There has been no consensus on a superior soil test for assessing the plant available micronutrient fraction, as extreme variation in extraction pH, soil to solution ratio and other agitation time have lead to inconsistent results. In order to address the shortcomings of batch extraction techniques, infinite sink methods have recently been applied to assess available micronutrients in soil. The most promising techniques include ion exchange resins and diffusive gradients in thin films (DGT).

1.5 DGT Principles

DGT is a sampling technique which was originally developed to measure labile species in aquatic systems (Zhang and Davison, 1995), and has also proved successful in sampling both soils and sediments. When applied to soils and sediments, a DGT device depletes a particular element from the solution phase, inducing a resupply from the solid phase. The rate of resupply is determined by the extent to which the solution is depleted and the rate of resupply (i.e. desorption, dissociation). The extent to which a DGT device depletes an element from solution depends on how well buffered the element is; stronger solution depletion is observed for poorly buffered elements, which results when either the solid phase pool is small or the kinetics of desorption are slow (Degryse et al., 2009). Therefore, the mass of an element that accumulates in a DGT device during deployment is a reflection of the initial concentration of that element in porewater and the kinetics of desorption and diffusion towards plant roots (Davison et al., 2007; Zhang et al., 2001). This process is mechanistically similar to plant uptake under conditions of diffusion limitation, an extreme case of which occurs when a plant's demand for an element is high and its solution concentration is low; it follows that strong correlations have been observed between element concentrations in plants and those assessed with DGT (Zhang et al., 2001; Song et al., 2004; Zhang et al., 2004; Koster et al., 2005; Nolan et al., 2005).

A DGT device consists of a binding layer (resin gel) overlain by a hydrogel and a filter membrane. The binding layer has a high affinity for a class of elements (i.e. cations, anions) and acts as a zero sink,

inducing diffusional flux of the element through the diffusion layer (Degryse et al., 2009). The analyte is then bound by the resin and accumulates throughout the exposure period. The sampling duration is generally selected based on expected concentrations of the element in the sample and the thickness of the binding layer, as to prevent the saturation of binding sites (Zhang and Davison, 1995). In theory, DGT can measure any dissolved species for which a binding agent can be identified, such as Cd^{2+}, Mn^{2+}, Al^{3+}, PO_4^{3-}, Zn^{2+}, Ni^{2+}, and K^{+}.

Due to a defined geometry, and established diffusion rate coefficients, Fick's first law of diffusion can be applied to calculate the flux of the particular element through the sampler (Zhang et al., 1998). Results are generally expressed as the time averaged concentration at the interface between the solution (C_{DGT}) and the sampler by the equation,

$$C = \frac{M\Delta d}{DAt}$$

where M is the mass of the analyte in the eluent, Δd is the thickness of the diffusion layer, D is the diffusional coefficient in the diffusion layer, A is the area of the DGT window, and t is time (Zhang et al., 1998). The concentration of analyte in the eluent is measured and using the equation,

$$M = C_e\left(V_{gel} + V_{acid}\right)$$

the M of the analyte in the binding layer is calculated. Since C_{DGT} is a reflection of solution concentration as well as labile complexes and reversibly sorbed ions, the DGT technique can be used as an elemental speciation tool. If C_{DGT} is lower than the bulk concentration of an element, it is an indication of complexes which are non-labile on the timescale of measurement. Modelled results have depicted a corresponding increase in C_{DGT} with increasing concentration and dissociation rate of a complex, which provides evidence for the contribution of labile complexes to the diffusive flux (Tusseau-Vuillemin et al., 2003). If only free ions may be bound by the resin layer, it follows that the contribution of complexes to DGT flux is controlled by the dissocation rate of that complex, that is faster dissociating complexes will contribute more toward diffusional flux. However, it is important to note that more dissociation of complexes has been recorded during a typical DGT deployment than corresponding plant uptake, which can be explained theoretically since complexes may diffuse into

the resin layer and then strongly promoted to dissociate, since the resin acts as a zero sink (Degryse et al., 2009).

1.6. DGT Application

Two decades after inception, DGT has found successful application as a research tool in a variety of environments, most notably soils and sediments. DGT was originally developed as an in-situ method to measure labile metal species in aqueous systems (Zhang and Davison, 1995). Dynamic systems, such as marine sediments and open waters, are notoriously difficult to sample, as strong spatial and temporal gradients make accurate measurement cumbersome. Many variables contribute to the heterogeneity of element availability, including pH, redox status, edaphic qualities (e.g. texture, aggregation) environmental conditions (e.g. temperature, precipitation) and biotic interactions (e.g. microbial interaction, root exudation). DGT simplifies measurements in highly dynamic systems, as the device is deployed for anywhere from 1 h to a few days, and does not require constant monitoring. These characteristics have led to DGT's success as a research tool in a multitude studies involving chemical speciation (Huynh et al., 2015; Zhang et al., 2015), bioavailability (Zhang et al., 2001; Tandy et al., 2011; Mundus et al., 2012; Six et al., 2013; Zhang et al., 2014), kinetics (Scally et al., 2003; Levy et al., 2011; Santner et al., 2014), and chemical imaging (Santner et al., 2012; Höfer et al., 2015; Santner et al., 2015.

Initial experiments confirmed DGT's simplicity and robustness when applied to sediments and open waters. This success led to its application in assessing elemental cycling and bioavailability in soils and sediments. In both sediments and soils, fluctuating redox status strongly influences spatial and temporal distribution of metal availability. Van der Geerst et al. (2008) used DGT coupled with redox sensors to assess oxygen's influence on copper availability in historically polluted floodplains. They observed strong gradients in redox status throughout the sediment profile, which exerted strong influence on metal availability. Furthermore, the researchers observed a significant correlation between available copper (C_{DGT}) and copper concentrations in benthic organisms (van der Geerst et al., 2008). In a similar vein, Mundus et al. (2012) used DGT in to study the effect of fluctuating redox conditions on Mn mobility in soil. The study additionally utilized X-ray absorption near edge spectroscopy (XANES) to assess the speciation of Mn as changing redox conditions were simulated through the addition of glucose. The study confirmed DGT's ability to monitor redox conditions in soil,

especially in conjunction with other methods of speciation assessment.

Following repeated success in assessing elemental lability and bioavailability, DGT has been more recently applied in soil systems to characterize labile element pools and examine the relationship between DGT concentration and plant uptake. Plant nutrient uptake is quantified in several approaches and depends on the model or technique that is chosen. Mechanistic models generally characterize plant uptake as a root area-based flux (mol cm^{-2} s^{-1}), while experimental based approaches quantify uptake via internalization, thus the tissue concentration of an element (mol g^{-1} DM). Moreover, both tissue concentrations and root area fluxes are related and take into account both the transport of an element to the root and the biochemical process of uptake. Traditional models for solute transport, such as the free ion activity model (FIAM) and the biotic ligand model (BLM), consider uptake to be rate limiting. Although both models have predicted plant metal uptake from solution (Hough et al., 2005; Thakali et al., 2006), BLM and FIAM fail to describe plant uptake during diffusion limitation (Degryse et al., 2009). Under conditions of diffusion limitation, a plant's demand for a nutrient is high, while it's solution concentration is low, therefore the rate of uptake is dependent on the diffusional supply.

Similar to plant uptake under diffusion limitation, DGT deployment perturbs the solid-solution equilibrium, thus measuring the diffusive supply on element as well as the resupply from the solid phase. While many studies have reported strong correlations between uptake and DGT, the findings are still not conclusive. For example, Sun et al. (2014) compared DGT to traditional extraction techniques for predicting Zn uptake by wheat (*Triticum aestivum* L.) and Maize (*Zea mays*). They found that DGT was strongly correlated with shoot and root Zn in both crops (R^2=0.994, R^2=0.993, R^2=0.990, R^2=0.994 for wheat and maize respectively), and was a superior method for predicting plant uptake compared to EDTA, $CaCl_2$, and other methods. Similarly, Zhang et al. (2001) observed similar behavior for Cu. When compared to free Cu^{2+} activitiy, soil solution concentration, and EDTA concentrations, DGT was most strongly correlated with tissue concentrations and explained 98 percent of the variance. In contrast, Agbenin et al. (2012) found poor correlation (R^2=0.47) between Zn tissue concentrations and DGT under field conditions differing results under field versus greenhouse conditions. Tandy et al. (2012) found poor correlation (R^2=0.25) between plant Mn uptake and DGT concentration under aerobic conditions, and Nolan et al. (2005) found poor

correlation between shoot Cu concentration and DGT. Only a few studies have investigated the relationship between uptake and DGT P concentration and have also produced conflicting findings. While Tandy et al. (2011) observed a relatively strong correlation between DGT P and uptake in barley (*Hordeum vulgare*) (R^2=0.72), Mason et al. (2013) found a weak relationship between DGT and P uptake in wheat (*Triticum aestivum*). These discrepancies may be attributed to biotic factors which are not included by DGT (root exudation, microbial activity), variability in total nutrient concentration, species differences in nutrient uptake, and variability in soil water content, among others. More research is required to probe the mechanistic relationship between DGT and plant uptake, and to establish when DGT might accurately predict plant uptake.

1.7 Calibration of DGT for soil nutrient testing

More recently, DGT has been applied as a tool to predict yield response and assess fertilizer requirements in agricultural systems. While the technique has not yet been used to assess micronutrient deficiencies, experimental evidence has indicated DGT might be a useful tool to predict P deficiency and yield responses to P fertilization.

Menzies et al. (2005) evaluated the suitability of DGT to predict yield response of tomato (*Lycopersicon esculentum*) to P fertilization. The study included 24 soils throughout Australia, some of which were heavily fertilized. The yield data was described using a Mitscherlich dose-response model, where relative yield is a function of the soil test P value. After classification of the test soils as responsive or non-responsive, a critical soil test value of 2.13 µg per sampler was established. They found that DGT was accurate in discriminating between soils which did and did not demonstrate yield increase in response to P fertilization, and provided a significant improvement over the Colwell P method. McBeath et al. (2007) performed a similar study which aimed to characterize the response of wheat (*Triticum aestivum* L.) to liquid and granular fertilizers in Australian soils using different soil tests (DGT, Resin P, Bray, P-E value, Colwell). DGT provided reasonable estimation of yield response to fertilizer application, though Resin P explained slightly more of the variation for both liquid and granular fertilizers (R^2=0.88 v. R^2=0.82, R^2=0.78 v. R^2=0.74 for liquid and granular, respectively). In contrast to the aforementioned results, Mason et al. (2010) found that DGT provided a much superior prediction of yield response when compared to Resin P.

Two studies were carried out by Six et al. (2013; 2013) to compare the ability of soil tests to predict yield following fertilizer application in tropical soils. The first experiment compared DGT to existing soil test methods (AEM, Olsen, $CaCl_2$, Bray, Mehlich, Colwell) in predicting the response of maize (*Zea mays*) and rice (*Oryza sativa*) to P application. Using a Mitscherlich equation, hey found that DGT was superior to existing protocols for predicting yield response for maize (R^2=0.77). In contrast, rice yield was better predicted with existing protocols, such as Olsen, Bray or Mehlich (R^2=0.73), suggesting that P availability for rice is not only governed by diffusion of P as assessed by DGT. The later experiment examined the suitability of DGT to predict yield response of maize following amendment of organic materials. DGT displayed a greater ability to predict yield response to P application, based on R^2 assessment.

1.8 Aims and objectives

Conventional batch extraction techniques have failed to consistently predict plant response to P and micronutrients (McBeath et al., 2005; Menzies et al., 2007; Mason et al., 2010), and provide estimates of the available nutrient pool which are highly dependent on site characteristics (Schüller, 1969; Lindsay and Norvell, 1978; Wenzel and Blum, 1998; Feng et al., 2005; Wünscher et al., 2015). These methods are empirically based and target a specific nutrient pool which is hypothetically available to plants. However, a significant body of research has indicated that extractions often consider P sources which are unavailable to plants (Six et al., 2012; Moody et al., 2013; Mason et al., 2013). DGT has been suggested as an alternative to chemical extraction, since it is mechanistically more sound for estimating nutrient availability under conditions of diffusion limitation (Degryse et al., 2009). However, DGT has produced inconsistent results regarding nutrient accessibility to plants and the subsequent response of plants to fertilizer applications. In addition, few studies (Menzies et al., 2007; McBeath et al., 2007, Mason et al., 2010; Six et al., 2013) have aimed to calibrate DGT as a tool to assess fertilizer requirements in agricultural systems, and these attempts have been undertaken primarily in non-field conditions. Therefore, the findings of these studies represent only a few climatic regions and soil types and thus are not broadly applicable.

The goal of this study is to characterize the suitability of DGT as a tool to assess nutrient availability and predict yield response in agricultural systems. This study seeks to further the work of Mason et al. (2010) in order to characterize the effectiveness of the method across different soil types in Austria.

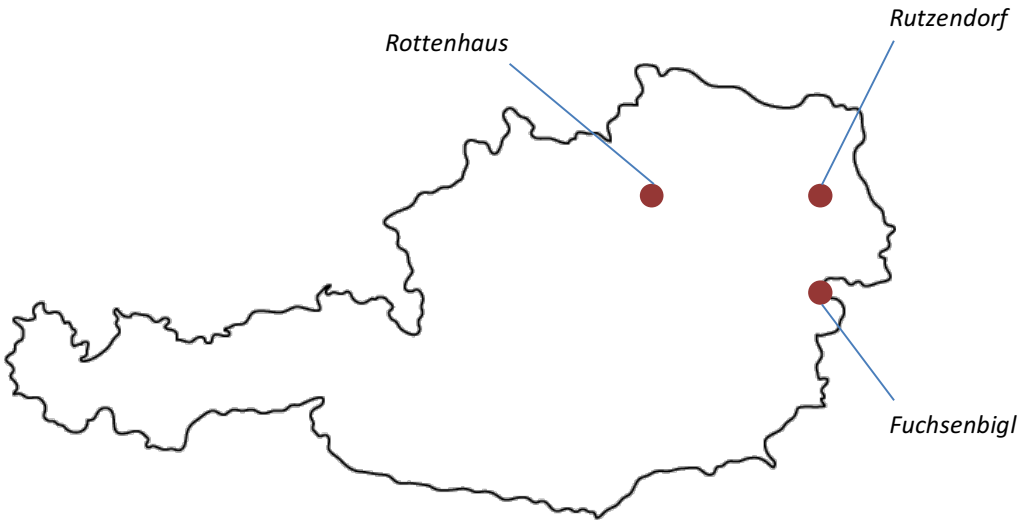
We will apply the technique to range of soils which emulate the dominant conditions in agricultural systems throughout Austria, and test DGT's ability to provide unbiased data on the pool of P and micronutrients which are available to crops. This study collaborated with the Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES) to obtain soils from long-term agricultural experiment stations throughout Austria. Thereafter, we characterized available P and micronutrient pool with DGT and compared these results to those of the existing extraction protocols. Afterward, we will compare soil test values to plant tissue concentrations and yield data for each site.

In comparison to the existing protocols, we hypothesize that DGT provides a mechanistically stronger assessment of the labile pool of both P and micronutrients in soil. In contrast to CAL and EDTA, which are highly influenced by fluctuations in carbonate and pH, we predict that DGT will give precise readings independent of these parameters. This hypothesis will be tested through correlation analysis, whereby we will compare soil edaphic properties to soil test values. Due to high ambient P and micronutrient concentrations, we do not expect strong correlations between DGT and plant tissue concentrations. This hypothesis is founded upon the principle that DGT is mechanistically similar to plant uptake, but only under diffusion limitation. Lastly, we expect DGT will provide reasonable prediction of yield by exhibiting a saturation type nonlinear response to increasing soil P concentration.

2. Materials and Methods

2.1 Site and soil characterization

Soil were sampled from four different sites in attempts to represent different soil types and climatic zones throughout Austria. The first site (Fuchsenbigl) is located near Haringsee in eastern Austria, with mean annual precipitation of 631 mm and average temperature of 9.9° C. The site sites on calcareous alluvial sediments, with the dominant soil type Sandy-Loamy Haplic Chernozem (FAO soil classification). The second site is located near Rottenhaus, with mean annual precipitation of 750 mm and average temperature of 9.0° C. The dominant soil type is Orthic Luvisol (FAO soil classification). The third site, Rutzendorf, is located in the fertile agricultural lands of the Marchfeld, ~18 km east of Vienna. The mean annual precipitation is 630 mm and average temperature is 10° C. The dominant soil type is Calcic Chernozem.



2.2 Field Trials

2.2.1 Experiment 1

Experiment 1 is part of a long-term P fertilization study, initiated in 1956 by the Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES). The experiment consisted of two experimental plots, one in Fuchsenbigl (Marchfeld) and the other in Rottenhaus (Alpenvorland). Utilizing randomized block design, both sites included five replicates of five different P fertilization strategies: no P addition, single superphosphate (SSP) and 'basic slag' at P application amounts of 44 kg ha^{-1} a^{-1} and 175 kg ha^{-1} a^{-1}. The plots were fertilized continuously from 1956 to 2004, and again

from 2012 to 2014. Both surface soil samples (0-25 cm depth) and winter wheat (*Triticum aestivum* L.) data (i.e. elemental concentrations and yield) for the year 2013 were provided by AGES for analysis.

2.2.2 Experiment 2

Experiment 2 is a long term P fertilization study, which investigates different crop residue management strategies. Commenced in 1982, the study is comprised of one experimental site in both the Marchfeld and Alpenvorland regions of Austria. Plots were fertilized with SSP at rates of 0, 75, 150, 300 kg P_2O_5 ha^{-1} a^{-1}. The fertilization rates were duplicated for both residue incorporation and residue removal treatments. A total of four replicates were produced for each treatment. Yield data from 2014 was provided by AGES for spring barley (*Hordeum vulgare*), as well as 32 surface soil samples (0-25 cm) from the same year.

Table 1. Soil physicochemical properties of experimental sites. (sf) refers to sites receiving slag fertilizer treatment (cr) refers to sites receiving crop residue treatments. Superscripts denote significance grouping (p≤0.05).

Site	*Treatment*	*pH*	*$CaCO_3$*	*TOC*	*P (CAL)*	*K (CAL)*	*Fe (EDTA)*	*Mn (EDTA)*	*Cu (EDTA)*
			%	*%*	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*
Fuchesenbigl	sf	7.5^{a}	13.2	1.7^{a}	161	161	52.3	88.4	4.35
Rottenhaus	sf	6.5^{b}	0.0	1.9^{b}	61	132	502	469	4.87
Rutzendorf	cr	7.6^{c}	na	2.1^{c}	155	213	na	na	na
Rottenhaus	cr	5.5^{d}	na	0.9^{d}	85	116	na	na	na

2.3 Plant and soil analysis

2.3.1 Soil properties

Basic soil physicochemical measurements (Table 1) were performed by AGES and follow the Austrian standard procedures for soil testing. The pH was determined in H_2O extract as well as a 0.5 M KCl solution with soil to solution ratio of 1:2.5 (v/v) (ÖNORM S 2122-1, 2004). Total organic carbon (TOC) was determined through dry combustion at 605° C (ÖNORM L 1080, 1999) and $CaCO_3$ content was determined according to the Scheibler method (ÖNORM L 1084, 2006).

The H_2O extraction method for determining P was carried out according to the Austrian standard (OENORM S 2122-1, 2004). H_2O is added to soil samples to its saturation point (soil specific), as

described in ÖNORM L 1092 (2005). In short, 300 g of fresh soil was sieved and subsequently moistened while stirring until saturation. The mixture was left to equilibrate for one hour, after which the saturation level was assessed. At this point, if samples were beyond saturation level (i.e. a film of H_2O had formed on the surface), more soil was added. Conversely, if the soil had not yet reached its saturation point, more H_2O was added. After saturation had been reached, each sample was weighed and then allowed to equilibrate overnight at room temperature. The following day, each sample was centrifuged (2500 g x 15 min) and subsequently filtered. P in the extract was determined photometrically using the molybdate blue method (Zhang et al., 1998).

The Calcium Acetate Lactate (CAL) procedure for assessing soil P was performed according to the Austrian standard procedure (ÖNORM L 1087, 2006). Briefly, 2.5 g of air dried and sieved (<2mm) soil was mixed with 50 mL extracting solution (0.05 M $C_5H_{10}CaO_6$ x $5H_2O$, 0.05 M $(CH_3COO)_2Ca$ x H_2O, 0.3 M CH_3COOH). Samples were shaken end-over-end for 2 hours, after which they were filtered and measured photometrically using the molybdate blue procedure, as described in Zhang (1998).

The Ethylenediaminetetraacetic Acid (EDTA) procedure for determining available metal concentrations in soil was performed according to the Austrian standard (ÖNORM L 1089, 2005). Briefly, 10 g of air dried and sieved (<2mm) soil was mixed with 100 mL of extracting solution (0.05 M Ethylenediaminetetraacetic Acid (EDTA)) and shaken end-over-end for 2 hours and subsequently filtered and immediately measured by inductively coupled mass spectrometry (ICPMS) (Elan 9000 DRCe, Perkin Elmer).

2.3.2. Plant digestion

In order to determine tissue concentrations of both P and micronutrients, *Triticum aestivum* (Experiment 1) and *Hordeum vulgare* (Experiment 2) was harvested at growth stage (GS) 31[†] and dried overnight at 105° C. Digestion was performed with a Multiwave 3000 (Anton Paar Ltd., Hertford Herts, UK) microwave digestion instrument using the 16 vessel rotor (16MF 100/HF 100). After drying, 0.2 of each sample was weighed into a liner. 5 mL HNO_3 (69 %, Merck Millipore, Darmstadt, Germany), 1 mL H_2O_2 (30%, Merck Millipore, Darmstadt, GER), and one drop of Iso-Octanole were

[†] Growth stage (GS) 31 refers to the Zadok's scale of cereal development. GS 31 is the beginning of stem elongation or jointing when the first node is detectable.

mixed. In each digestion, 2 liners were filled with 0.2 g of reference plant material and 2 blanks. Digestion was carried out according to setting listed in Table 2.

Table 2. Microwave digestion settings

	Digestion program (1200 Watt)	Cleaning program (1300 Watt)
Ramp time (min)	20	15
Hold time (min)	30	20
Cooling (min)	15	30

After digestion, the rotor was placed in a fume hood and the vessels were vented by opening the venting screws with the supplied key. A funnel rack was also prepared in the same fume hood, and was stacked with acid washed funnels and paper filters (Munktell folded filters, Muntell & Filtrak GmbH, Bärenstein, Germany), and 100 mL acid washed vials were placed underneath. The immersion tube and seal were rinsed with HQ H_2O, and the diluted digest was filtered. The vials were weighed once more, in order to record the accurate amount of digest obtained. Samples were then measured via ICPMS (metals) or photometrically (P).

2.4 DGT

2.4.1 Gel preparation

A 0.8 mm thick polyacrylamide hydrogel was used throughout laboratory experiments as the diffusive layer. The gel solution consisted of 15% by volume polyacrylamide and 0.3% by volume agarose derived cross-linker (DGT Research Ltd, UK). Diffusive gels were cast by mixing the gel solution with amonnium persulphate (10%) (AnalR Normapur, VWR Prolabo) and TEMED catalyst (N, N, N', N'-Tetramethylethylenediamine, 99%, Sigma Aldrich) in proportions determined empirically by Zhang et. al. (1995). This mixture was pipetted into glass plates separated by plastic spacers, and then placed in an oven at ~ 43°C for one hour in order to polymerize. The glass plates were then separated and gels removed, whereby they were washed in 1L HQ water (18 MΩ cm, prepared by a Millipore Elix 3 water purification system) two to three times in order to rinse of excess reagents. Gels were then stored refrigerated in 0.01M $NaNO_3$ (≥99% ACS Reagent, Sigma Aldrich).

Resin gels for binding P were produced by precipitating ferrihydrite into a 0.4 mm thick polyacrylamide hydrogel. The hydrogel was produced in the same fashion as for the diffusive layer. A solution containing 2.7 g $FeCl_3$ $6H_20$ (97% ACS Reagent, Sigma Aldrich) and 40 g HQ water was prepared, whereby a maximum of three gels were placed inside. The solution was filled with HQ water to an approximate weight of 100 g. Gels were allowed to soak for a minimum of 2 hours. Concurrently, a 0.05M 2-(*N*-morpholino)ethanesulfonic acid (MES) (≥99.5%, Analar grade reagent, VWR BDH Prolabo) buffer was prepared, and adjusted to a pH of 6.7 by adding 1M NaOH (97%, Merck Millipore) drop-wise. After soaking, gels were transferred separately to 100 mL MES whereby the solution was stirred for ±5 minutes to ensure a homogenous precipitation of ferrihydrite. Gels were allowed to equilibrate for 30 minutes. The finished ferrihydrite gels were washed in 1 L HQ water 2-3 times, and stored refrigerated in 0.03M $NaNO_3$.

Resin gels for binding metal cations were produced utilizing the ion-exchange resin Chelex 100 (Bio-Rad, Hercules, CA). Ratios of Chelex 100, gel solution and catalysts were mixed according to previous literature whereby the ratios of TEMED catalyst and ammonium persulphate were adjusted in order to slow polymerization, thus allowing the Chelex 100 resin to settle to one side of the gel (Zhang et al., 1995). Chelex 100 gels were washed and stored refrigerated in HQ water. Two to three DGT blanks were prepared for each deployment, and were assembled identically as samplers measuring either P or cations. Calculated values of analytes were adjusted based on blank concentrations.

2.4.2 DGT assembly

Samplers were assembled using plastic housing materials (Quernmore, Lancaster, U.K., www.dgtresearch.com) consisting of a backing cylinder and a cap with a 1.7 cm diameter exposure window. Cation samplers consisted of the backing plate, followed by a 0.4 mm Chelex 100 gel disc, a 0.8 mm diffusive gel disc, and a cellulose nitrate protective membrane (pore size 0.45 μm, thickness 130 μm; Supor, Pall GmbH, Dreieich, GER). A plastic housing was mounted with screws on top of the DGT assembly; this helps to stabilize the soil paste in the exposure window and ensure relatively similar amounts of soil are applied to each DGT sampler. Anion samplers were assembled in a similar fashion, with a 0.4 mm ferrihydrite gel, polycarbonate membrane (pore size 0.2 μm, thickness 10 μm; Nuclepore, GE Healthcare, Freiburg, GER), 0.8 mm diffusive gel disc, and a 0.13 cellulose nitrate filter disc. The polycarbonate membrane negligibly affects the diffusion process (Zhang, 1995)

and prevents the ferrihydrite gel from sticking to the diffusive gel.

2.4.3 DGT deployment

In order to reduce the effects of varying water contents on diffusional flux, the DGT method is generally carried out on soil samples at 80 to 100 percent of the maximum water holding capacity (WHC). For each soil type, a pooled subsample was taken, and 100 percent WHC was determined by visual inspection by wetting soil until a paste formed and the surface was glistening.

A soil paste was created by adjusting samples to 0.9 maximum WHC and allowing to equilibrate for 24 h at 20°C. Soil pastes were then applied to the DGT samplers and incubated 20°C for an additional 24 h. After exposure, samplers were carefully disassembled and gels rinsed with HQ water to remove any remaining soil particles. Ferrihydrite and Chelex 100 gels were then eluted in 5 mL of 0.25M H_2SO^4 (96% ACS Reagent, Sigma Aldrich) and 1M HNO_3 respectively. Samples were left on a horizontal shaker overnight to elute.

2.5 Chemical analysis

Phosphorus concentration in the eluates was determined through molybdate blue procedure (Zhang et al., 1998) on a Hitachi U-2000 UV/VIS spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan). In this procedure, the staining reagent was prepared by mixing 10 mL HQ water, 3 mLof 0.009M ammonium heptamolybdate (99%, Merck Millipore), and 1 mL of 0.004M potassium antimony (III) tartrate hydrate (99.95%, Sigma Aldrich). To produce the color reaction, 1 mL of sample, 0.14 mL of the staining reagent, and 0.06 mL 0.1M ascorbic acid (≥99% ACS Reagent, Sigma Aldrich) were mixed. After 15 to 20 minutes, the color reaction had finished and the samples were immediately measured at 881 nm with the spectrophotometer.

Mn, Cu, Fe, Zn in the eluates were measured with inductively-coupled-mass-spectrometry (ICP-MS) (Elan 9000 DRCe, Perkin Elmer). To obtain proper matrix concentration, eluates were diluted to 1% HNO_3, after which internal reference standards were added at 10% of the total sample volume. The analytical process was validated by processing internal plant reference standards, chemical blanks, and eluates simultaneously.

2.6 Statistical Analysis

Correlation analysis was performed between soil properties ($CaCO_3$, pH, TOC) and soil test (EDTA, CAL, DGT) values for both P and micronutrients, and strength of correlation was reported using the coefficient of determination (R^2). Analysis of variance (ANOVA) was performed to determine differences between soil test values across different experiments and soil properties (i.e. $CaCO_3$). Data did not meet the assumption for normality of residuals, thus we proceeded with Welch's F test to determine differences in means. This test was also more suitable due to unequal sample sizes across sites. Mean comparisons were performed with Games Howell post-hoc analysis. All statistical analysis was performed with the SPSS software package (IBM SPSS Statistics, Version 23, 2015).

3. Results

3.1 Extraction vs. DGT

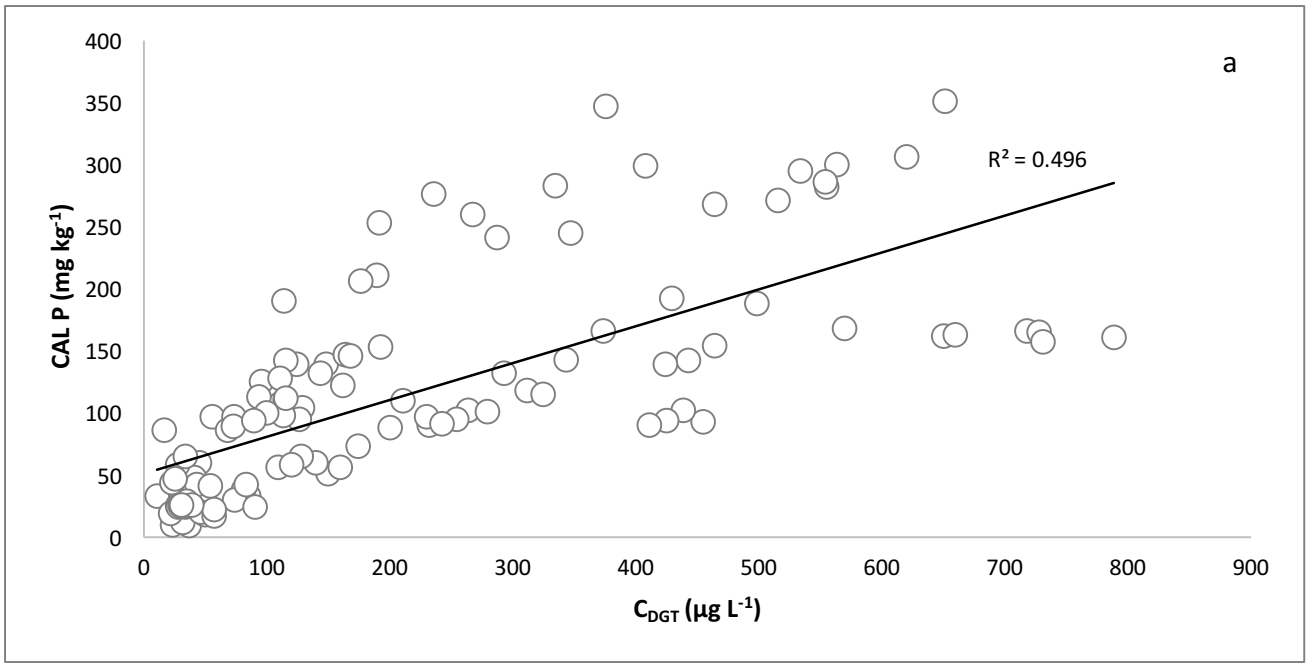
3.1.1 Phosphorus

C_{DGT} values varied greatly among experimental sites, ranging from 10.7 to 788.3 µg L^{-1}. Likewise, CAL P ranged from 9 to 351 mg kg^{-1}. The highest CAL P values were observed in calcareous soils, while maximum values reported in non-calcareous soils were below half of this (351 mg kg^{-1} and 168 mg kg^{-1} respectively). C_{DGT} P did not exhibit similar behavior, as maximum values for calcareous and non-calcareous soils were 651 and 788.3 µg L^{-1} respectively.

Across all sites, C_{DGT} and CAL P values were correlated, as 49.6 percent of the variation in CAL P was explained by C_{DGT}. The separation of data based on relative carbonate content (i.e. calcareous and non calcareous) strengthened the association between soil test values (Fig. 3.1) (R^2 = 0.762 calcareous; R^2 = 0.852 non calcareous). Further separation of data based on experimental site resulted in strong correlations across all sites (Fig 3.3) (R^2 = 0.886, R^2 = 0.954, R^2 = 0.887, and 0.871 for sites 1-1, 1-2, 2-1 and 2-2 respectively).

Table 3. Pearson correlation coefficients for edaphic properties and soil test yields. **(p≤0.01) *(p≤0.05)

	CAL P	DGT P	EDTA Cu	DGT Cu	EDTA Fe	DGT Fe	EDTA Mn	DGT Mn
pH	0.379**	-0.151**	-0.606**	-0.69**	-0.914**	0.257**	-0.901**	-0.628**
$CaCO_3$	0.566**	0.045**	0.456**	0.456**	-0.917*	0.296**	-0.986**	-0.821**
TOC	0.206**	-0.137**	0.41**	-0.817**	0.722**	0.145**	0.695**	-0.276**



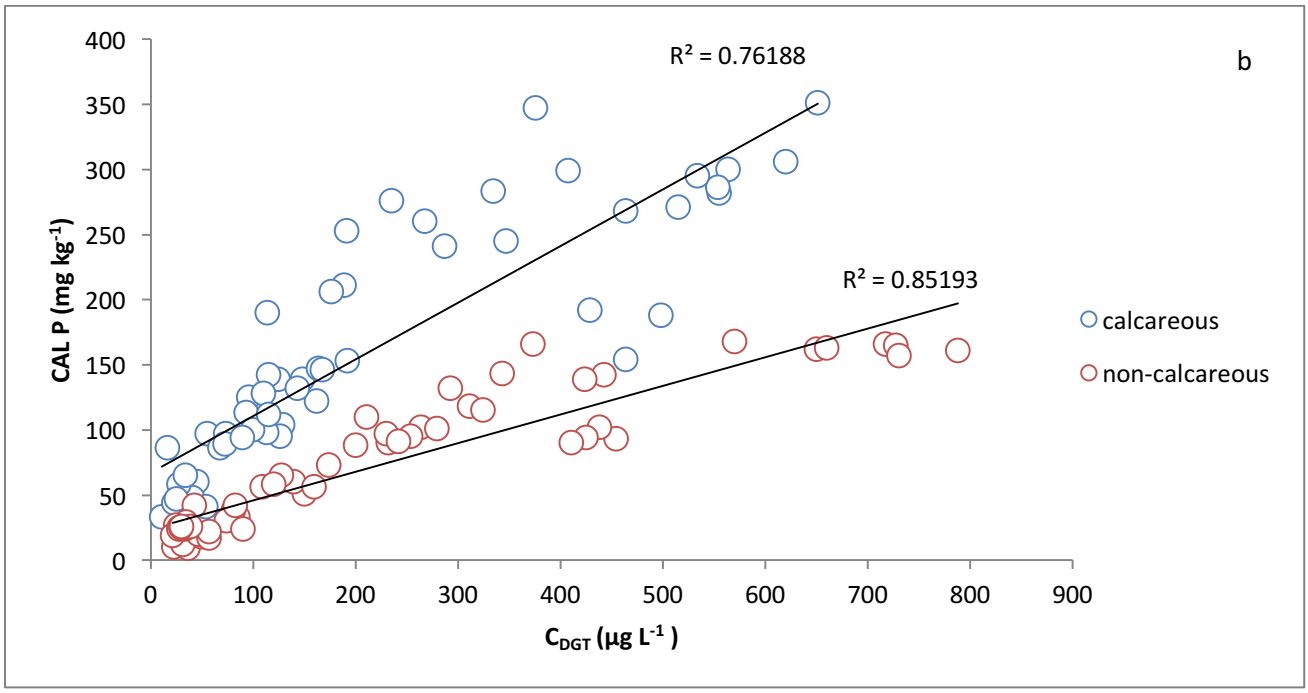


Fig 3.1 Relationship between DGT P concentration and CAL P across (a) all experimental sites or (b) separated based on carbonate content

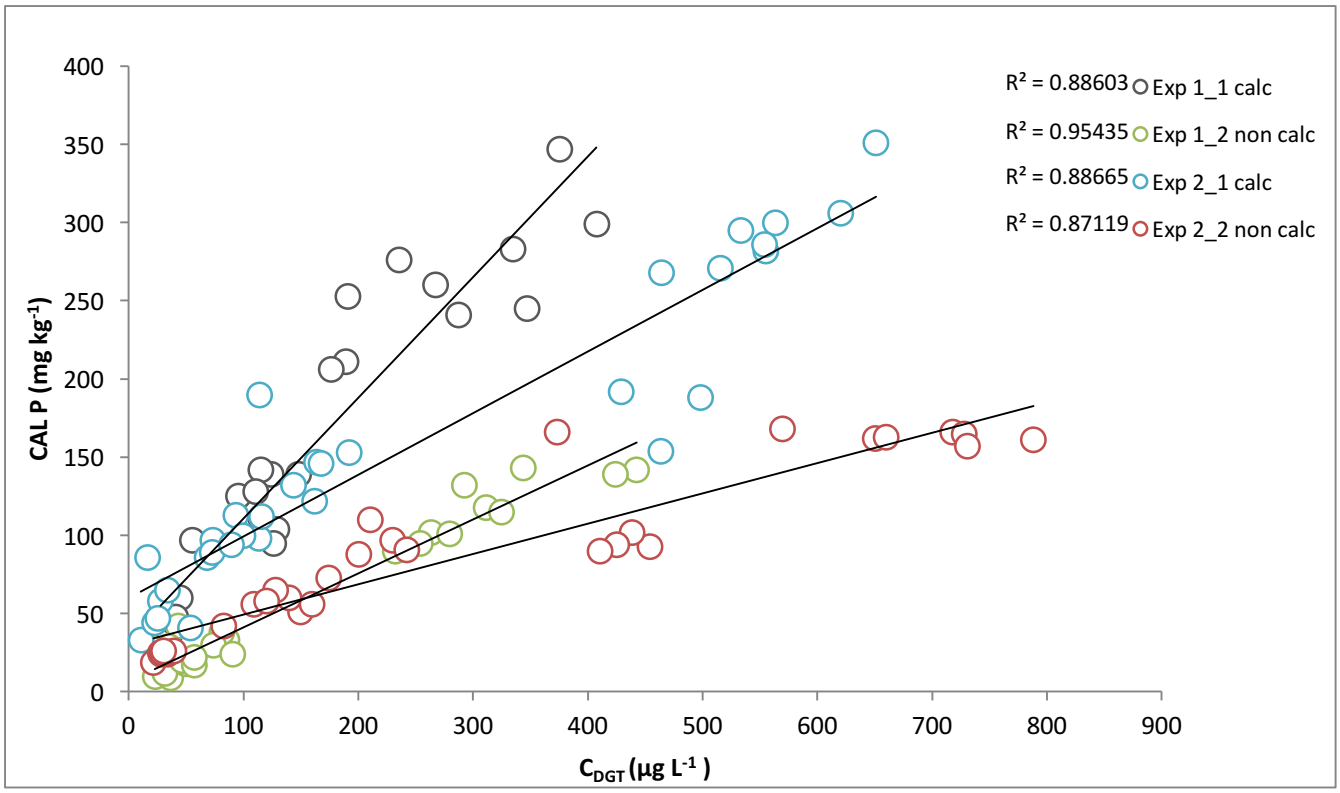


Fig. 3.2 Relationship between soil DGT P and CAL P, separated based on experimental site

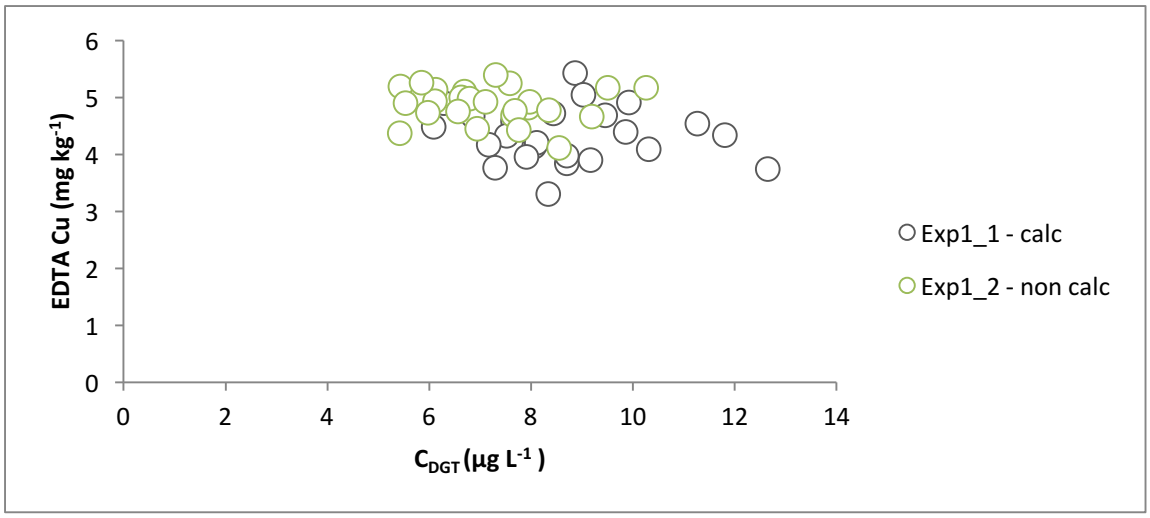
3.1.2 Micronutrients

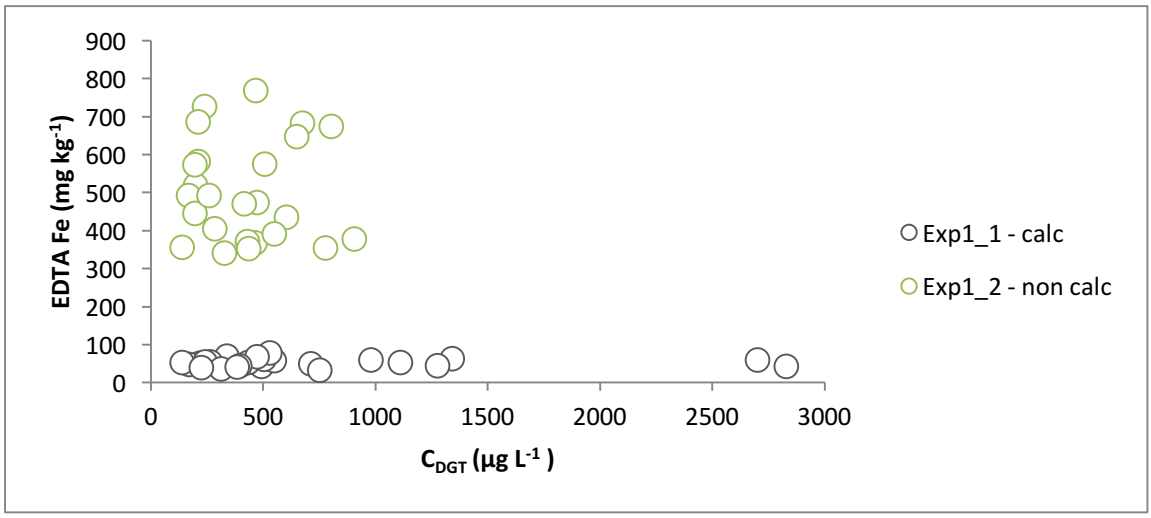
Due to extremely low concentrations, Zn data was deemed unreliable and was omitted from analysis. EDTA extractable Mn, Fe, and Cu data was not available for sites in experiment 2, therefore all comparisons of soil extractions and DGT measurements were performed for the two sites in experiment 1 only. For all micronutrients under consideration, DGT and EDTA measured values show no overall association.

EDTA extractable Cu values were tightly grouped and ranged from 3.3 to 5.43 mg kg^{-1}. C_{DGT} values were similar, ranging from 5.4 to 18 µg L^{-1}; the highest Cu values were recorded in experiment 2 where no EDTA data is available for comparison. We observed no effects of site on the availability of Cu (Fig. 4). Fe concentrations were more variable, with EDTA values ranging from 32.2 to 769 mg kg^{-1} and C_{DGT} values from 67.5 to 2830 µg L^{-1}. It should be noted that most C_{DGT} values fell below 1500 µg L^{-1}, and were variable across both sites. As depicted in Fig. 4, soil tests exhibited no clear association. EDTA Fe values were relatively low (i.e. below 100 mg kg^{-1}) in the calcareous site, and more variable in

the non-calcareous site (340.1 to 726.5 mg kg^{-1}). Available Mn, as assessed by both DGT and EDTA was highly site dependent, with lower maximum values (158.9 mg kg-1 and 429.0 µg L^{-1} for EDTA and DGT) measured in the calcareous site, and high maximum values in the non-calcareous site (550.8 mg kg^{-1} and 3230.3 µg L^{-1} for EDTA and DGT) (Fig. 4).

Strong correlations were observed between soil test values and edaphic properties, in particular pH and $CaCO_3$ (Table 3). While DGT concentrations of Mn and Cu displayed reasonable correlations (r=0.296, r=0.456 respectively) with pH and $CaCO_3$, all correlations between EDTA, pH and $CaCO_3$ were significantly higher.





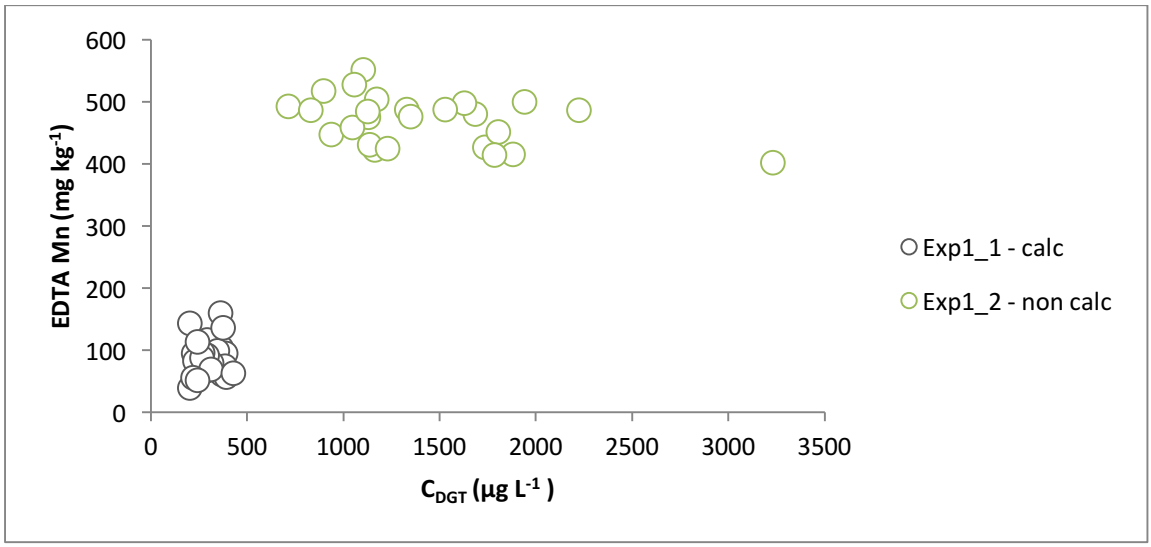


Fig. 4 Relationship between soil test for micronutrients (Cu, Fe, Mn) as assessed by DGT and EDTA

3.2. DGT & plant response

3.2.1 Uptake

We found no association between soil test values and uptake for either P or micronutrients (Figs. 4 & 5). We observed clustering of micronutrient values based on site, particularly for Mn and Fe. Clustering behavior was more pronounced with EDTA values. As in previous correlations, we observed a separation of CAL P values based on $CaCO_3$ content of the site.

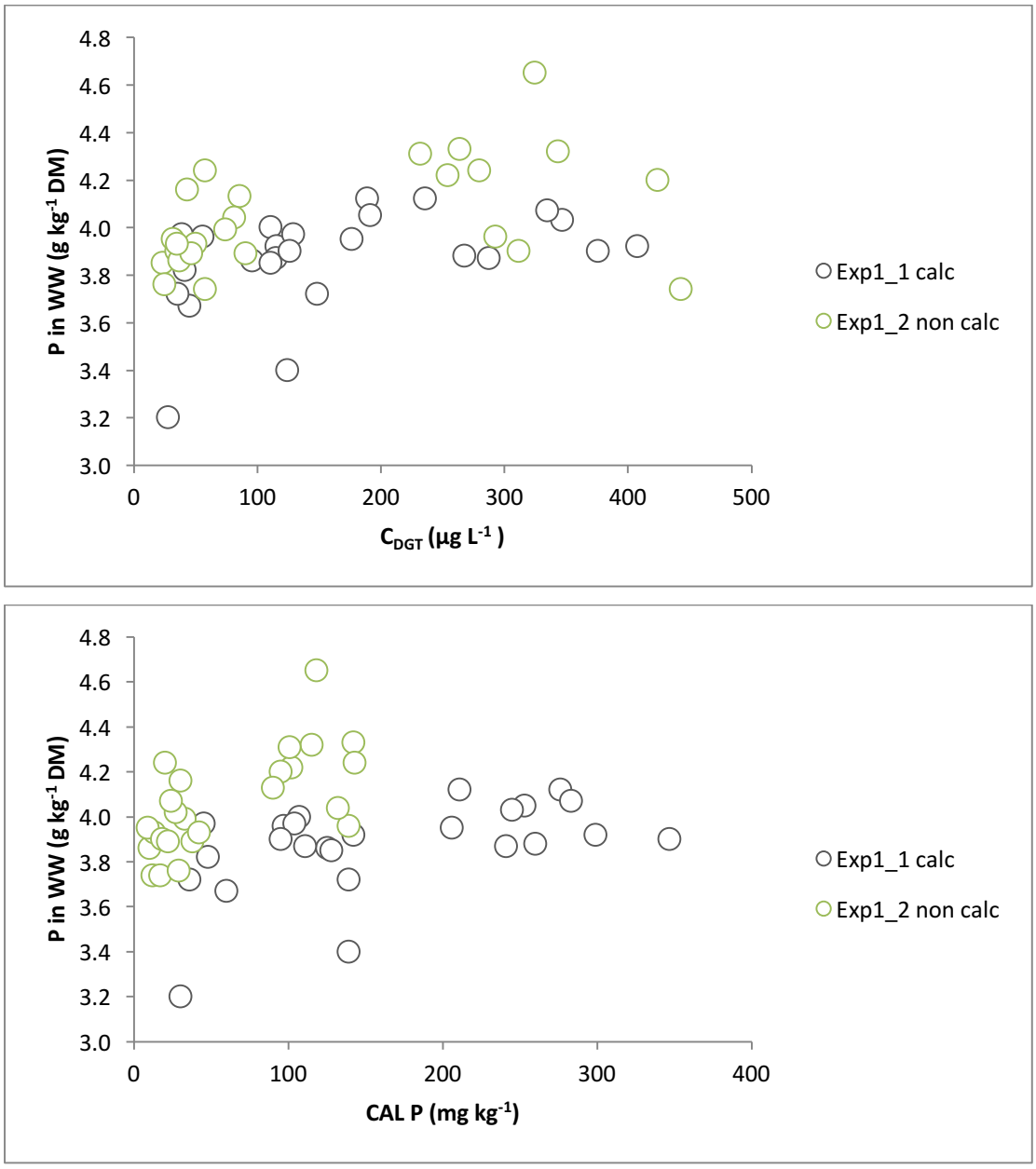


Fig. 4 Relationship between plant uptake in winter wheat (WW) and available P

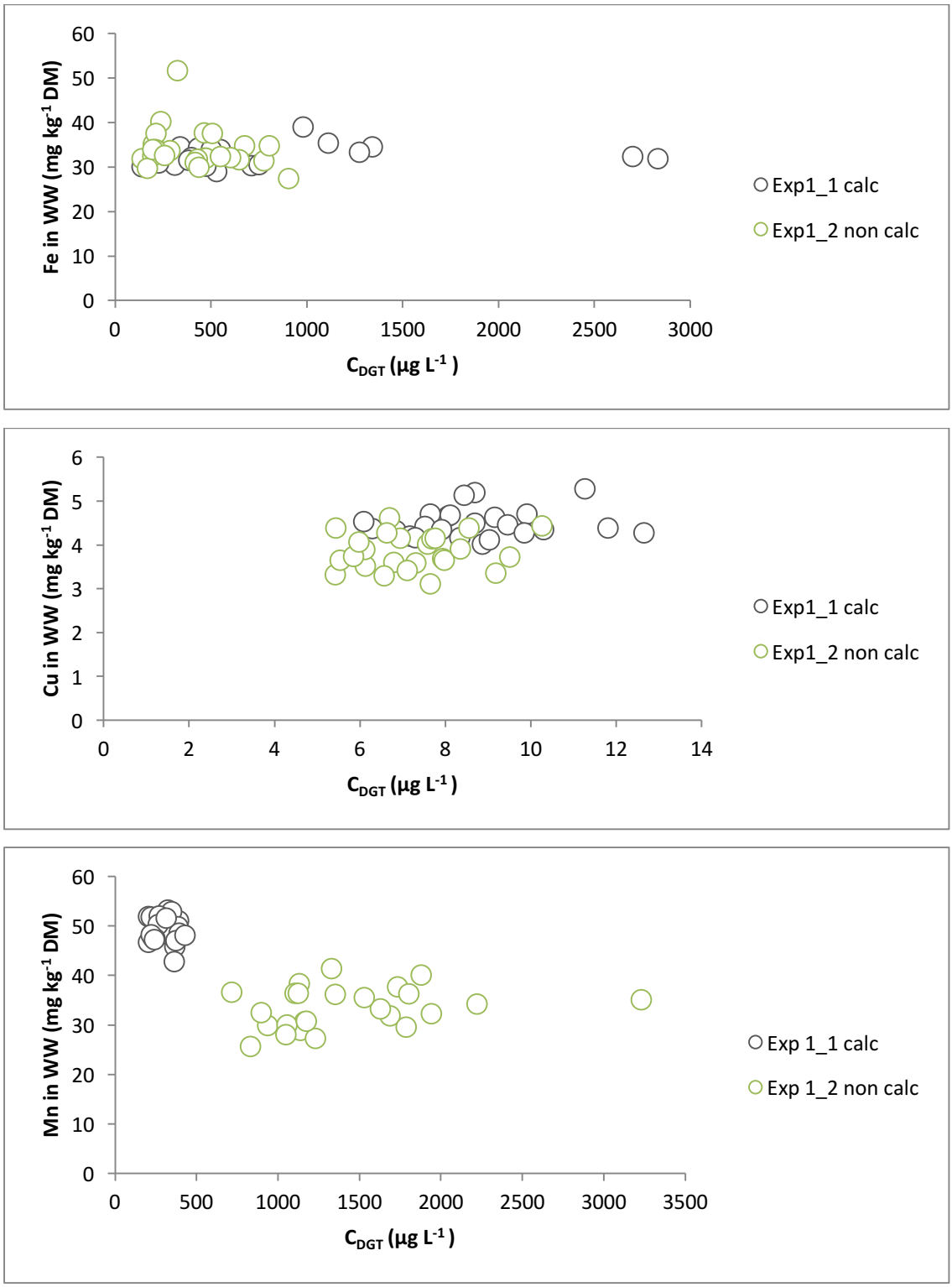
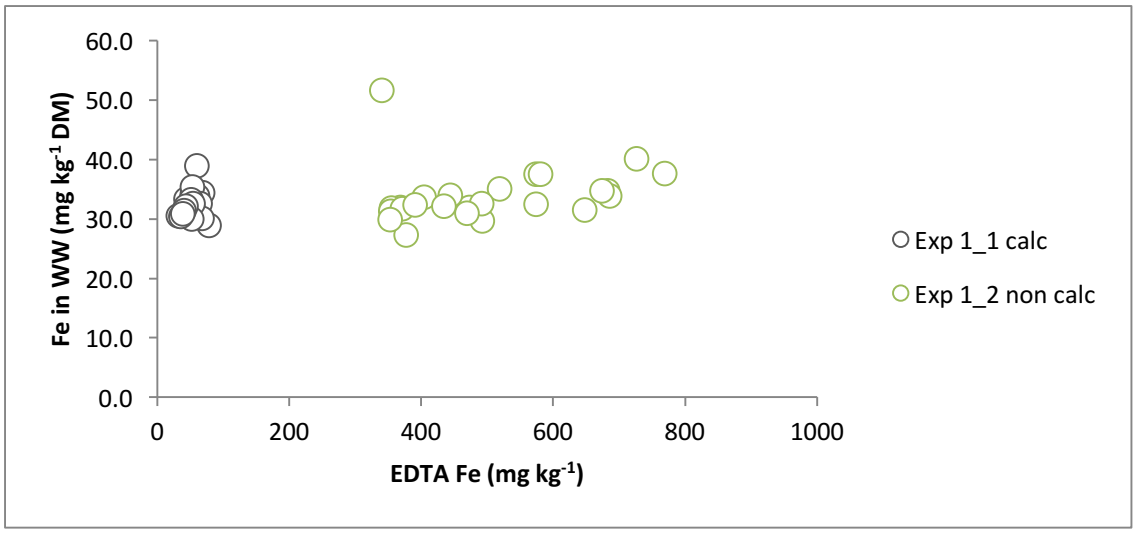
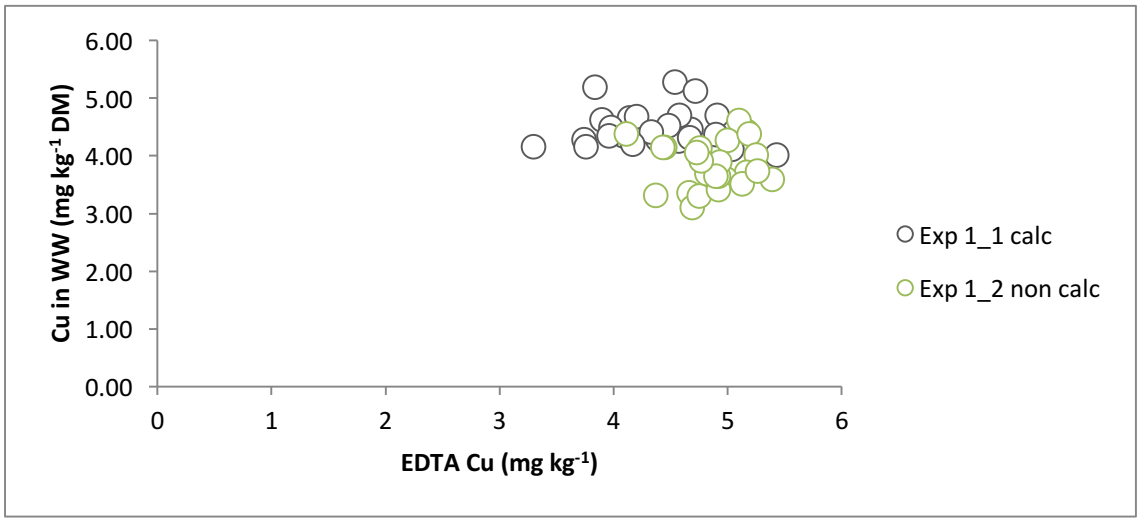


Fig. 5 The relationship between available micronutrients (C_{DGT}) and uptake by winter wheat (WW)





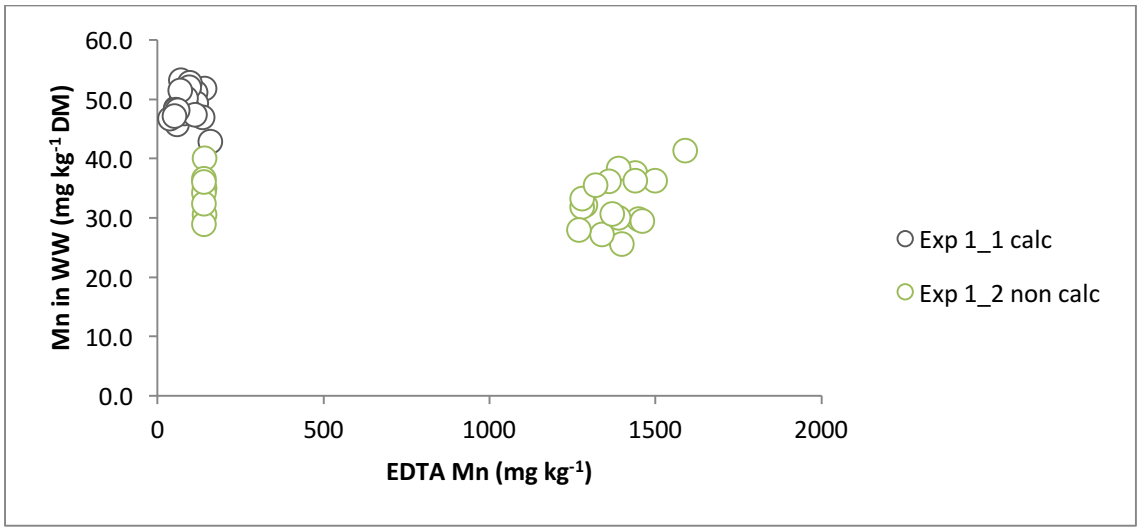
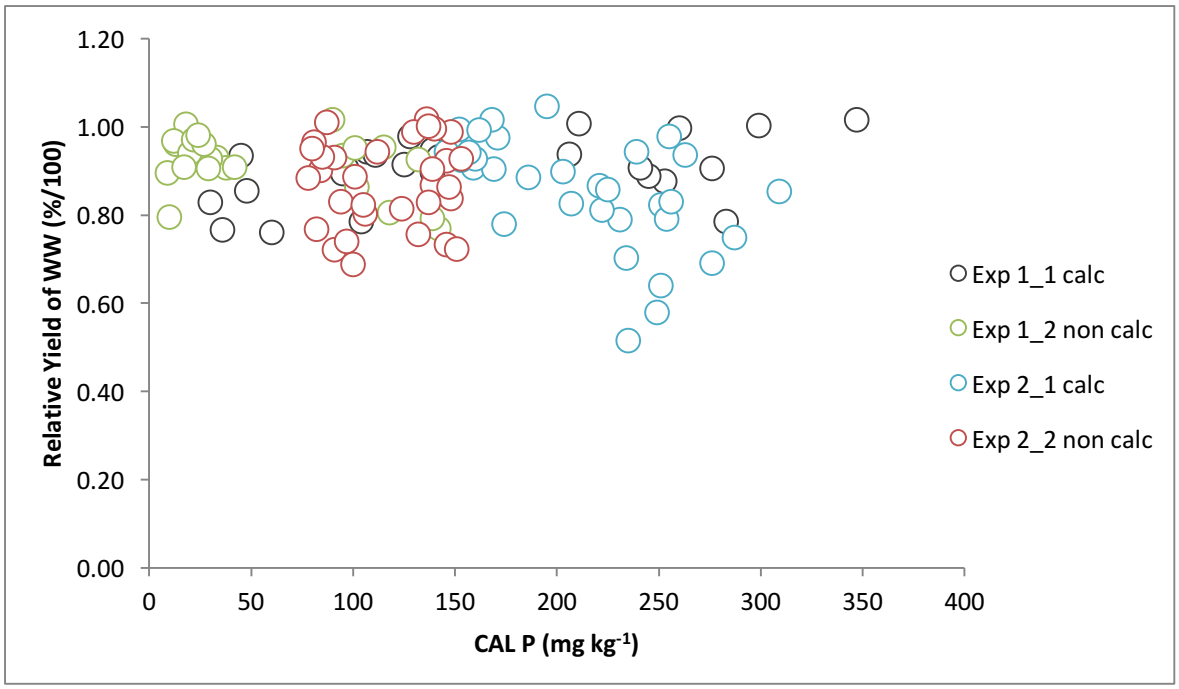


Fig 6. The relationship between available micronutrients (EDTA) and uptake by winter wheat (WW)



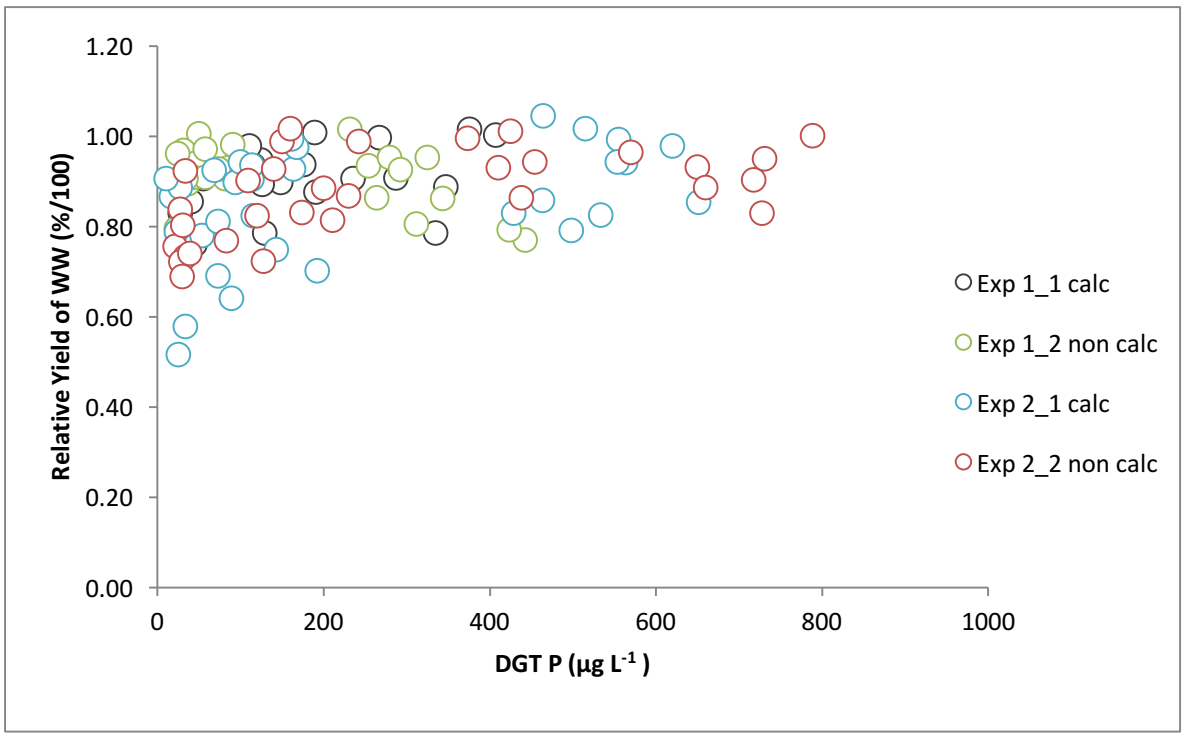
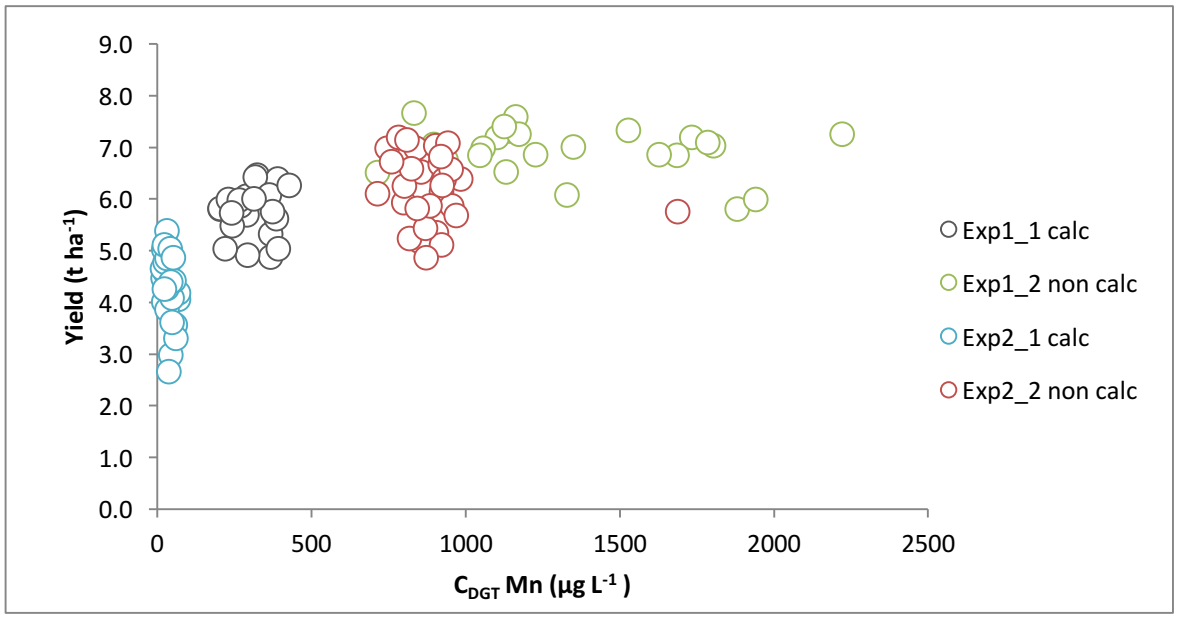


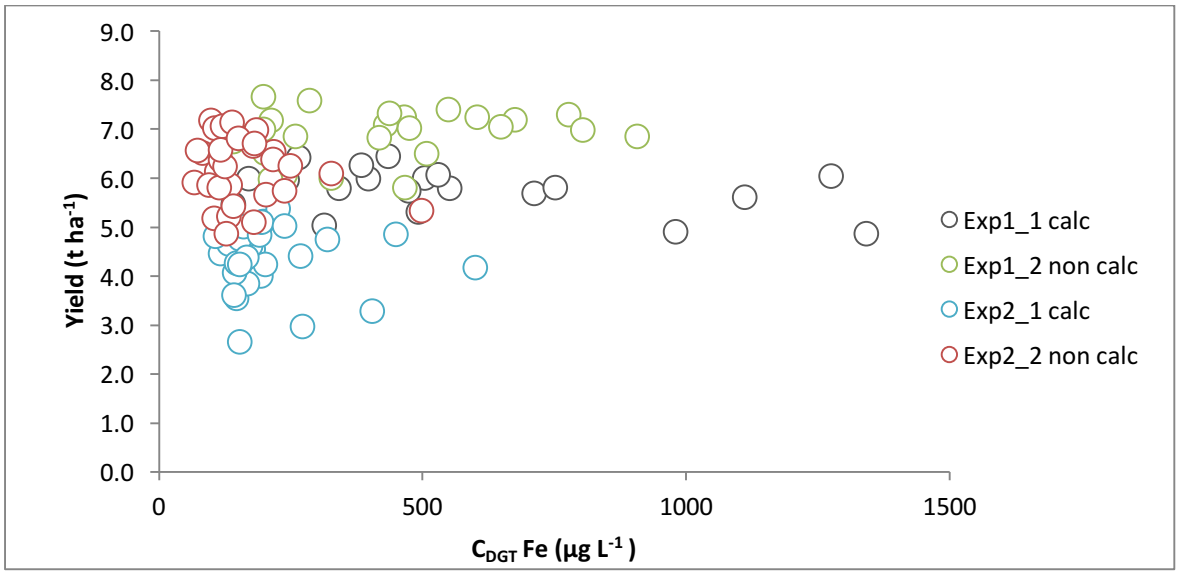
Fig. 6 Relationship between available P and relative yield of winter wheat (WW)

3.2.2 Yield

We tested the suitability of DGT as a predictor for fertilization response through the comparison of relative yield and soil test values. Across all sites (Exp. 1 & 2), both CAL and DGT P measurements show no correlation with relative yield. Relative yield values ranged from 0.52 to 1.05 (%/100). Although no significant correlation was observed, we saw markedly different behavior in CAL versus DGT data (Fig. 6). C_{DGT} values were more homogenously distributed, and no distinct clustering was detected.

In order to detect any effects of micronutrients on yield, we also compared C_{DGT} micronutrient concentrations with observed yield. When data are plotted together, no overall correlation is present for Cu or Fe, although data suggests increasing yields with higher DGT Mn concentration (Fig. 7). After separating the soil test values based on site, distinct grouping was observed. Site effects were most pronounced for Mn, which exhibits tight clustering across the experimental sites.





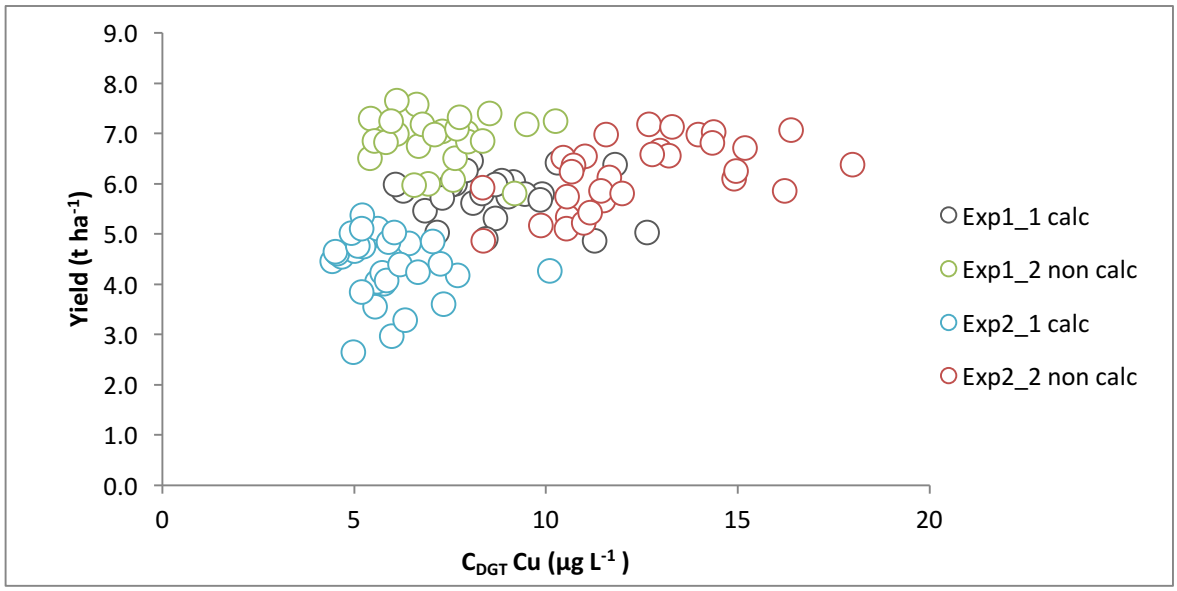


Fig. 7 Relationship between C_{DGT} micronutrients and absolute yield

4. Discussion

4.1. Extraction vs. DGT

4.1.1 Phosphorus

The objective of this study was to assess the suitability of DGT as an alternative method to assess nutrient requirements for agricultural crops and their subsequent response following fertilizer application. We hypothesized that DGT would be less influenced by edaphic conditions and other soil parameters, therefore serving as a more precise technique for use across different soil types. Our data supports this hypothesis. Figs. 3.1 and 3.2 display DGT values which are similar across all experimental sites, whereas CAL values are strongly affected by soil conditions. Specifically, when we separate soil test values based on the relative carbonate content (i.e. calcareous vs. non-calcareous), maximum CAL values on the calcareous sites exceed those on non-calcareous sites by a factor of 2. When the data is further separated by experimental site, the correlation between DGT and CAL strengthens, suggesting other soil properties also influence soil test readings. Our study confirms others (Menzies et al., 2005; Menzies et al., 2007; Mason et al., 2010; Six et al., 2012, Wünscher et al., 2015), which have shown that CAL and other extraction procedures are highly variable, and provide unreliable estimates of bioavailable P across soil types. The relationship between both soil P tests clearly depict the differential behavior of CAL on calcareous soils (Fig 3.2). By neglecting the P in apatitic structures, CAL grossly underestimates the labile P pool in acidic soils (Schüller, 1969). Following this logic, our results indicate that DGT is less affected by soil edaphic properties, in particular $CaCO_3$ content.

4.1.2 Micronutrients

Across all experimental sites, no correlation between EDTA extractable micronutrients (Cu, Mn, Zn) and C_{DGT} was observed. There are several mechanisms that may explain the contrasting behavior of soil tests on different experimental sites; these relate to background soil chemistry, mode of action of the particular soil test, or underlying treatment structure.

In general, a soil test that measures the bioavailable metal fraction falls into one of three categories: 1) acid extractant 2) neutral salt solution 3) complexing/chelating reagent. The EDTA extraction protocol

Table 4. Mean element concentrations (mean ± 1.96*S.E.) at experimental sites 1 and 2. (F) represents Fuchsenbigl (R) represents Rottenhaus (Ru) represents Rutzendorf. Superscripts denote significance grouping (p=0.05) following Games Howell post-hoc analysis.

Exp.	Fe EDTA mg kg^{-1}	Fe DGT µg L^{-1}	Mn EDTA mg kg^{-1}	Mn DGT µg L^{-1}	Cu EDTA mg kg^{-1}	Cu DGT µg L^{-1}	P CAL g kg^{-1}	P DGT µg L^{-1}
1 (F)	52.6 ± 4.4	724.9 ± 285.2^{a}	88.4 ± 11.8	307.2 ±27.2^{a}	4.3 ± 0.2	8.7 ± 0.7^{a}	161.1 ± 36.5^{a}	165.1 ± 44.6^{a}
1 (R)	502.3 ± 52.5	424.5 ± 86^{a}	469.4 ± 15.3	1427.7 ± 212.5^{b}	4.9 ± 0.1	7.2 ± 0.5^{b}	60.8 ± 19.4^{b}	157.4 ± 55.1^{a}
2 (Ru)		209.3 ± 37.3^{b}		38.5 ±5.1^{c}		5.9 ± 0.4^{c}	154.6 ± 32.2^{a}	240.8 ± 76.8^{a}
2 (R)		159.9 ± 30.1^{b}		899.9 ±57.2^{d}		12.4 ± 0.8^{d}	85.3 ± 18.3^{b}	285.4 ± 88.3^{a}

operates under the principle of chelation and combines the use of ammonium acetate and ethylenediaminetetracetic acid whereby ammonium ions displace metal cations, which are subsequently complexed by EDTA. The displacement ability of ammonium is largely controlled by soil pH, clay content, organic matter concentration, and dominant mineralogy (Menzies et al., 2007). Concomitantly, the ability of EDTA to complex an analyte is dependent on the concentration of competing cations, which are governed by the same factors/processes.

The influence of various soil parameters on DGT and EDTA measurements are apparent, as both Mn and Fe display much lower concentrations in non-calcareous soils when compared to calcareous soils (Table 3). The effect of carbonate on extraction yields has been previously documented by Wenzel and Blum (1998), who found that elevated carbonate content increased the extraction solution pH, subsequently diminishing the extraction yield on calcareous soils. Mn and Fe solubility is strongly influenced by pH, and has been shown to increase 10^4 and $10^2 – 10^3$ times with each unit decrease in soil pH, respectively (Rechcigl, 1995). The observed decrease in soil Mn and Fe in calcareous soils is expected, as pH values are significantly higher (Table 2.)

It is important to note that the sites from experiment 1 are a part of a long term P fertilizer experiment, where treatments included inorganic P fertilizers (e.g. single superphosphate) as well as steel slag. Slag is a silicate- and oxide-rich byproduct of steel production, where impure ores are smelted to produce the metal and impurities are removed (Piatak et al., 2015). The remaining

substance is rich in P, As, Cr, Cu, Fe, and Mn and has been extensively studied to determine its' suitability for reuse (i.e. construction material, fertilizer) and metal extraction, as well as potential environmental effects (Piatak et al., 2015). Several studies have reported elevated bioavailable Mn and Fe concentrations as a result of continuous slag application; this effect is attributed to increased total concentrations and elevated pH as a consequence of repeated slag application (Makabe-Sasaki et al., 2013; Hejcman et al., 2008). Due to our selection of experimental sites, we were able to compare samples from the same experimental site (i.e. Rottenhaus) with contrasting treatment structures. Our data supports the findings of Hecjman and Makabe-Sasaki, as average pH values, as well as Mn and Fe concentrations, were significantly elevated in sites treated with slag fertilizer ($p \leq 0.01$) (Table 3).

EDTA and DGT Cu concentrations were relatively similar between calcareous and non-calcareous sites. While Cu mobility is strongly affected by pH, Cu also has a strong affinity for clay minerals and organic matter and is readily complexed by organic ligands (Rechsigl, 2015). The interaction between these soil parameters could explain the similar behavior of Cu on calcareous and non-calcareous soils. Organic C content of the sites in experiment 1 ranged from 1.5 – 2 (w/w), whereas sites in experiment 2 had much lower C content, with values <1 (Table 1). As EDTA extractable Cu data is missing from site 2, we were able to compare the labile fraction through DGT measurements. Average C_{DGT} values were significantly lower in soils from experiment 1 (7.2 vs. 12.4 µg L^{-1}), which support the hypothesis that organic matter complexation results in decreased Cu availability.

4.2 DGT & Plant Response

4.2.1. Uptake

Following the principle that a mechanistically sound test will access the same pool of an element as a plant, we compared C_{DGT} values (P, Mn, Fe, Cu) with tissue concentrations in *Triticum aestivum* at growth stage 31. Soil P exhibited no correlation with plant tissue concentrations (Figs. 4 & 5). To our knowledge, no previous studies have compared plant response and DGT P under field conditions. The group of Mason performed a laboratory experiment whereby soil test measures of available P were compared to P uptake in *Triticum aestivum* using isotope dilution. They found significant ($p \leq 0.05$) correlations between both plant concentration and uptake, though DGT concentration explained only 39 and 44 percent of the variation, respectively (Mason et al., 2013). Tandy et al. (2011) found

contrasting results when comparing DGT to soil extraction procedures to predict plant available P, Cu, and Zn in agricultural soils. Their study found that effective P concentration as measured by DGT (C_e) explained 72 percent of the variability in tissue P concentration in *Hordeum vulgare* (Tandy et al., 2011). While both of these previous studies were carried out on agricultural soils, they utilized plants which were grown in controlled greenhouse conditions. The contrasting findings of these studies may be attributed to the difficulty in replicating field conditions in the laboratory, differences in growth stage at harvest, or perhaps genotypic differences in nutrient uptake.

Commonly overlooked and underrepresented in short term greenhouse studies and P availability assays is the possible contribution of organic P sources to plant nutrition (Frossard et al., 2000). It is not unlikely that over the course of a growing season plants access significant amounts of P which are made available through biological activity and subsequent mineralization. In soils, up to 50 percent of P is immobilized in microbial biomass and other forms of organic matter (Harrison et al., 1987), thus constituting a significant source which may become available for plant uptake. Since DGT deployments, are routinely carried out over the course of 24 hours, especially in areas with high P concentrations, they likely exclude mineralization of organic P. Plant P uptake from organic sources may have contributed to the disconnect we observed between plant tissue an DGT P concentrations. Future studies which seek to calibrate DGT for field conditions should account for P released through mineralization, and perhaps combining DGT with some meaningful assay of the potentially mineralizable pool.

Similary, available micronutrients (Mn, Fe, Cu) displayed no association with tissue concentration. These findings are in contrast to some previous studies, which found strong correlation between DGT concentration and tissue concentration of Cu (Zhang et al., 2001; Song et al., 2004; Zhang et al., 2004), Zn (Zhang et al., 2004; Nolan et al., 2005; Koster et al., 2005). It is important to note that these studies did not use *Triticum aestivum*, since it has been well documented that plant species and cultivars differ in nutrient uptake efficiency and partitioning (Jhanji et al., 2014; Pedas et al., 2011; Ai-Qing et al., 2011). The only known study that used *Triticum aestivum* as a model species found weak correlation between DGT concentration and tissue Cu concentrations (Nolan et al., 2005). Similary, Tandy et al. (2011) performed a study to determine the suitability of DGT to assess the availability of Mn in soils and found poor correlation between DGT concentration and tissue concentration. The

poor predictive ability of DGT is likely related to the extremely dynamic nature of Mn species in soil, which are strongly influenced by redox status, temperature, pH, organic matter content. In agreement with Tandy, the relatively brief sampling window of a DGT deployment likely does not reflect the extremely heterogeneous nature of soil, especially over the length of the growing season, and thus provides a poor prediction of Mn uptake in plants (Tandy et al., 2011).

The poor ability of DGT to predict plant tissue concentrations may also be attributed to processes of nutrient translocation in plants. In this study, tissue concentrations were taken from the shoots of *Triticum aestivum* at growth stage 31. For particular elements (e.g. Cu), shoot concentrations do not accurately reflect the nutrient status of the plant, as they have difficulties in translocating the element away from roots. In these situations, root concentration may provide a better indicator of plant uptake, and would likely correlate more strongly with DGT concentrations (Song et al., 2004). Antagonistic behavior between P, Fe, and Mn has been previously observed, whereby elevated P fertilization has inhibited Mn uptake/translocation in barley. A similar response has been recorded for Mn, whereby elevated Fe supply suppressed Mn uptake and translocation from roots to shoots (Ai-Qing et al., 2011; Ghasemi-Fasaei et al., 2008). The antagonistic interactions of these key nutrients could contribute to the lack of correlation we observed between DGT and shoot concentrations of both P and micronutrients. Further studies should additionally use root concentrations as a metric for a more accurate comparison of soil concentrations and nutrient uptake.

In addition to the interaction among trace elements, "main" cations may compete with micronutrients for root adsorption sites. According to the biotic ligand model, the first step of nutrient uptake is the adsorption of ions onto the root surface. Other cations (e.g. Mg, Ca) may interfere with interfere with the uptake of micronutrient cations, therefore disturbing the relationship between free/labile ion concentration and plant uptake (Thakali et al., 2006; Degryse et al., 2009). This mechanism might explain the disconnect observed between labile micronutrient fractions (i.e. DGT, EDTA) and plant uptake. For example, Thakali et al. (2006) observed competition between H^+ and Cu^{2+} ions for binding sites on the roots of barley (*Hordeum vulgare*), subsequently decreasing toxicity of Cu. Similary, Wang et al. (2012) noted similar behavior for Mg^{2+}, as a strong positive

relationship was observed between Mg^{2+} concentrations and $EC_{50}(Cu^{2+})$[‡] (R^2 = 0.97). Previous studies have also showed a competitive effect between Ca/Mg and Cu uptake in wheat (Kinraide et al., 2004; Luo et al., 2008). Although these studies investigated the effect of cation competition on metal toxicity in plants, it is clear that competition between cations for binding sites occurs and has implications for plant nutrition.

In a comprehensive review, DeGryse et al. (2009) suggest that DGT may provide more accurate and mechanistically sound predictions of plant concentrations in conditions where uptake is diffusion limited (i.e. high affinity and low element concentration). In our study, where soils were obtained from heavily fertilized agricultural conditions, plant uptake may become saturated and the relationship between DGT and plant concentration will break down, as DGT – in contrast to plants – continues to act as a "zero-sink" even at high elemental concentrations.

4.2.2. Yield

One of the objectives of this study was to characterize DGT's potential as an improved method for predicting crop response to bioavailable P. Across all sites, for both CAL and DGT, there was no significant relationship between soil test P and relative yield (Fig. 6). However, when comparing the two methods, it is important to note the differences in this relationship for each soil test. For CAL, there is no systematic explanation for variation in yield as a function of soil test P. This sits in contrast to the relationship depicted for C_{DGT} and yield; there is a distinctively larger fluctuation in yield response below ≤ 200 µg P L^{-1}.

The only known study that has used DGT to predict wheat response under field conditions described a similar relationship between DGT concentration and relative yield. Mason et al. (2010) compared the predictive ability of DGT and extraction protocols (Olsen, Resin P) in measuring wheat response to P application across 35 field sites in southern Australia. After obtaining results, they fit a Mitscherlich dose-response curve to describe the relationship between soil test P and relative yield. Across all sites, C_{DGT} explained 42 percent of the variance in relative yield, compared to 5 percent for the Resin P method. They found no association between Colwell P and relative yield. After refining the data to

[‡] EC50 refers to the half maximal effective concentration; at this concentration, 50 percent of inhibition is reached

exclude sites where maximum yield could not be calculated, or was deemed unreliable, C_{DGT} explained greater than 70 percent of the variation in yield. Although we were unable to fit a Mitscherlich model to our data, our data displayed a similar saturation type nonlinear relationship (Fig. 6) as reported by Mason et al. (2010). It is also important to note that the concentrations of P which were measured in our sites were drastically larger than those include in the aforementioned study, with maximum values approaching 800 µg L^{-1} compared to 232 µg L^{-1} (Mason et al., 2010). Crop response to fertilizer application is described well using the Mitscherlich model, especially when application rates are low. When plants grow in low P conditions, the response to P application is considerably higher than when P is sufficient. Since the soils used in this study had relatively high P concentrations, and it may be possible that the saturation "plateau" of a dose-response relationship had already been reached. It's possible that a stronger relationship between relative yield and DGT is observed when experimental sites also include P deficient soils. Experimental results support this hypothesis, as Six et al. (2013) found a strong relationship (R^2 = 0.74) between DGT P and relative yield of maize in weathered soils characterized by strong P fixation.

As previously mentioned, a DGT device is mechanistically similar plant uptake of a nutrient under diffusion limited conditions. Soils of low P status (i.e. diffusion limitation) should in theory generate a much higher response to increasing soil concentrations, which might strengthen the overall relationship between DGT concentration and yield. Our results contrast those of some previous studies (Menzies et al., 2007; Six et al., 2013), which described strong predictive ability of DGT to predict plant response to available nutrient concentrations. These studies incorporated a broader range of ambient P concentrations, which might support a stronger dose response relationship. Confounding this hypothesis are the findings of Mason et al. (2010), who also included a large range of ambient P concentrations, but only observed moderate relationships between DGT P and relative yield. Further field calibration studies should include soils with a large range in P concentration, as an effective soil test should predict crop response at both low and high P conditions.

The response to micronutrients has not yet been characterized by DGT, therefore no literature exists for comparison of results. Nevertheless, we were not able to ignore the striking relationship between DGT Mn and absolute yield of *Triticum aestivum.* In general, an increase in DGT Mn concentration corresponds with an increase in absolute yield (Fig. 6). Prior to investigation, and in accordance with

published literature, we expected to see this type of relationship between P and yield; though unexpected, there are several possible explanations for the observed yield increase in response to higher DGT Mn concentrations. Primarily, our observation may be attributed to the relative importance of Mn nutrition, especially in relation to other elements (P, micronutrients). It be possible that at higher available P concentrations, Mn then becomes limiting for plant growth. This could help to explain why we observe elevated yield response in non-calcareous soils, whereby Mn solubility is increased. Many studies have also witnessed interactions between Mn and P, especially in soil with high ambient P conentrations (Neilsen et al., 1992; Barben et al., 2011; Pedas et al., 2011). Due to high P concentrations in our system, it's possible that the interaction between P, Mn and soil edaphic properties (pH, $CaCO_3$) is controlling macro- and micronutrient nutrition and subsequently influencing crop yields.

An auxiliary hypothesis to describe the relationship between DGT concentrations and crop yields is related to the interaction between Mn, redox, and pH. Since pH and redox (pH + pE) exert strong controls on Mn solubility, a decrease in pH + pE drastically increases the amount of Mn(II) in the soil solution (Sillanpää, 1982). Shahandeh et al. (2003) has previously observed the ability of Mn oxides to constitute a strong P sink, especially in high Mn soils; it is important to note that the study was carried out on paddy soils, which are frequently flooded and have varying redox conditions. While these conditions are not directly comparable to agricultural soils in Austria, the findings of Shahandeh are useful due to the significant interactions which were observed between redox, pH, Mn and P. Generally, in non calcareous soils, Fe constitutes a main P sink (Lindsay, 1979). Fe follows similar redox behavior as Mn, though dissolution of Fe oxides has been known to happen at a lower pH + pE than Mn oxides. This would suggest that in high Mn soils, P solubility may instead be controlled by the dissolution of Mn oxides due to fluctuating pH + pE. Our experimental sites had elevated Mn and P concentrations, thus it is possible that with lowered pH levels, both Mn and P solubility is enhanced. While our data does not directly support this hypothesis, it may be that Mn solubility is driving P availability in the soils used in our study.

5. Conclusions

The aim of this work was to characterize the potential of DGT as a tool to assess soil nutrient status and to predict crop response to nutrient concentrations under field conditions. In comparison to

conventional batch extraction techniques (EDTA, CAL), we found that DGT was less influenced by soil edaphic properties (pH, $CaCO_3$). These findings support those in scientific literature, and suggest that DGT may be a suitable method to assess labile P and micronutrient fractions that is not influenced by ambient soil chemistry. After comparing plant and soil data, we found no association between conventional batch extraction techniques, DGT and plant tissue concentrations of both P and micronutrients. We attribute this to the interactive effect of nutrient acquisition in plants, competition between ions for root adsorption sites, and differential translocation of elements within plant tissue. Lastly, we sought to test the ability of DGT to predict crop response, in comparison with traditional extraction protocols. We did not find statistically significant relationships between any soil test and yield, though DGT concentrations were the only measure to display a saturation-type nonlinear relationship to relative yield. Interestingly, we observed a stronger trend between Mn and relative yield than for any other elements. The lack of correlation between P and yield may be attributed to the difficulty in relating soil testing to growth conditions in the field (e.g. mineralization of P from organic sources), or relative importance of micronutrient nutrition at P sufficient conditions.

For future research seeking to calibrate DGT under field conditions, we suggest the incorporation of soils with both high and low P status, as it has been suggested that DGT mimics plant uptake mechanisms under conditions of diffusion limitation (Degryse et al., 2009). In addition, more studies are needed to characterize the potential of DGT to predict micronutrient response, also including soils which represent deficient conditions. Lastly, we suggest investigating incorporation of DGT with a meaningful assay of the organic P pool, as this provides a P source which remains unaccounted for during the brief DGT deployment.

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Appendix

Sample	Location	pH	TOC %	$CaCO_3$ %	P (H_2O) mg kg^{-1}	P (CAL) mg kg^{-1}	P (C_{DGT}) µg L^{-1}	Fe (EDTA) mg kg^{-1}	Fe (C_{DGT}) µg L^{-1}	Cu (EDTA) mg kg^{-1}	Cu (C_{DGT}) µg L^{-1}	Mn (EDTA) mg kg^{-1}	Mn (C_{DGT}) µg L^{-1}
5/1099	Fuchsenbigl	7.5	1.6	13.4	2.8	60	44.9	62.6	1341.9	4.5	11.3	103.8	368.4
5/1100	Fuchsenbigl	7.5	1.6	11.3	3.8	97	55.4	69.0	341.3	4.9	9.9	142.6	204.8
5/1101	Fuchsenbigl	7.5	1.8	15.5	1.5	30	27.6	43.1	492.7	3.8	8.7	60.0	368.9
5/1102	Fuchsenbigl	7.5	1.6	14.4	6.6	107	110.6	43.6	1275.8	3.9	9.2	66.8	289.9
5/1103	Fuchsenbigl	7.6	1.7	12.4	1.8	36	35.4	60.1	980.4	4.7	8.4	115.9	293.1
5/1104	Fuchsenbigl	7.5	1.7	14.6	11.9	211	189.0	53.2	435.4	4.1	8.1	71.6	325.7
5/1105	Fuchsenbigl	7.5	1.6	12.6	17.7	260	267.4	58.9	2701.0	4.3	11.8	94.5	391.3
5/1106	Fuchsenbigl	7.5	1.7	12.4	16.9	276	235.4	56.9	551.5	4.7	9.5	94.2	223.7
5/1107	Fuchsenbigl	7.5	1.7	12.4	11.4	206	176.3	61.5	503.9	4.6	7.6	97.8	347.8
5/1108	Fuchsenbigl	7.5	1.7	14.4	11.9	253	191.1	53.3	1111.6	4.2	8.1	73.5	388.1
5/1109	Fuchsenbigl	7.5	1.5	12.2	26.1	347	375.6	64.6		4.6		116.9	
5/1110	Fuchsenbigl	7.5	1.7	14.4	24.7	299	407.5	53.9	264.9	4.1	10.3	78.6	318.4
5/1111	Fuchsenbigl	7.5	1.6	12.6	22	245	346.9	49.6	712.4	4.4	9.9	91.4	292.1
5/1112	Fuchsenbigl	7.6	1.9	14.9	16.9	241	287.1	32.2	751.8	3.3	8.3	38.0	205.3
5/1113	Fuchsenbigl	7.6	1.7	14.6	20.8	283	334.5	42.6	2830.8	3.7	12.7	56.4	394.5
5/1114	Fuchsenbigl	7.5	1.5	10.5	8.1	139	124.2	78.1	529.5	5.4	8.9	158.9	364.2
5/1115	Fuchsenbigl	7.5	1.5	12.4	9.4	139	148.0	67.3	474.4	5.0	9.0	135.8	375.5
5/1116	Fuchsenbigl	7.6	1.7	12.4	6.5	125	95.5	51.4	220.8	4.9	6.3	95.6	271.9
5/1117	Fuchsenbigl	7.6	1.8	13.8	2.3	45	38.8	48.0	170.7	4.5	6.1	81.6	230.9
5/1118	Fuchsenbigl	7.5	1.7	13.4	7.3	142	115.2	54.2	243.4	4.3	7.5	87.1	266.5
5/1119	Fuchsenbigl	7.6	1.6	12.4	2.6	48	40.9	52.3	141.3	4.7	6.9	113.0	243.9
5/1120	Fuchsenbigl	7.6	1.6	12.4	6.9	111	115.0	44.0	397.8	4.0	8.7	68.2	314.6
5/1121	Fuchsenbigl	7.6	1.8	12.6	8.6	104	128.8	35.8	313.5	4.2	7.2	55.1	221.1
5/1122	Fuchsenbigl	7.6	1.8	12.6	8.5	128	110.4	40.2	384.4	4.0	7.9	62.0	429.0
5/1123	Fuchsenbigl	7.6	1.8	15.9	7.3	95	126.1	38.7	226.9	3.8	7.3	50.9	242.3
5/919	Rottenhaus	6.8	1.7	0.0	1.9	10	23.0	340.1	327.5	4.5	6.9	401.0	3230.3
5/920	Rottenhaus	6.6	2.0	0.0	2.1	13	34.1	368.2	465.0	5.2	10.3	485.7	2223.3
5/921	Rottenhaus	6.6	1.9	0.0	1.8	9	36.6	355.3	140.8	5.1	6.7	446.3	938.1
5/922	Rottenhaus	6.2	2.0	0.0	3	18	49.8	404.5	286.0	5.0	6.6	421.9	1163.4
5/923	Rottenhaus	6.7	2.0	0.0	2.3	12	31.4	354.1	777.7	5.2	5.4	429.9	1138.1
5/924	Rottenhaus	6.2	2.1	0.0	24.7	118	311.5	726.5	239.2	5.3	7.6	487.4	1331.1
5/925	Rottenhaus	6.2	1.9	0.0	35.8	142	442.6	769.4	467.0	4.7	9.2	415.2	1881.5
5/926	Rottenhaus	6.0	2.0	0.0	21.9	115	324.4	683.5	676.1	5.2	9.5	426.2	1734.5
5/927	Rottenhaus	6.7	1.7	0.0	17.1	102	263.6	518.6	200.5	4.4	5.4	474.9	1132.4
5/928	Rottenhaus	7.0	1.8	0.8	19	143	343.4	574.7	507.9	4.7	7.6	492.5	715.8
5/929	Rottenhaus	6.4	2.1	0.0	16	95	254.0	648.2	649.1	5.4	7.3	517.1	898.1
5/930	Rottenhaus	6.5	2.0	0.0	19.8	101	279.4	581.5	213.2	5.0	6.8	550.8	1104.5
5/931	Rottenhaus	6.4	1.9	0.0	41.9	139	423.7	685.7	212.0	4.8	6.6	498.8	1942.5
5/932	Rottenhaus	6.4	2.0	0.0	18.5	90	231.9	574.1	198.6	5.1	6.1	485.9	833.4
5/933	Rottenhaus	6.6	1.9	0.1	23.5	132	292.5	673.9	804.8	4.9	7.1	527.0	1057.3
5/934	Rottenhaus	6.1	2.0	0.0	6.6	33	85.2	473.8	475.2	4.8	7.9	451.0	1805.6

Sample	Location	pH	TOC %	$CaCO_3$ %	P (H_2O) mg kg^{-1}	P (CAL) mg kg^{-1}	P (C_{DGT}) µg L^{-1}	Fe (EDTA) mg kg^{-1}	Fe (C_{DGT}) µg L^{-1}	Cu (EDTA) mg kg^{-1}	Cu (C_{DGT}) µg L^{-1}	Mn (EDTA) mg kg^{-1}	Mn (C_{DGT}) µg L^{-1}
5/935	Rottenhaus	6.1	2.0	0.0	6.8	38	81.0	493.1	169.2	4.9	8.0	480.2	1687.3
5/936	Rottenhaus	6.1	2.1	0.0	6.1	30	73.6	444.7	197.9	4.9	6.1	475.5	1351.5
5/937	Rottenhaus	6.3	2.0	0.0	4.5	20	46.2	371.4	430.6	4.8	7.7	413.5	1786.1
5/938	Rottenhaus	6.3	2.1	0.0	7.3	42	43.0	491.8	259.0	4.9	5.5	424.5	1229.3
5/939	Rottenhaus	6.9	1.8	0.0	1.9	17	57.3	377.1	907.0	4.8	8.4	497.1	1629.8
5/940	Rottenhaus	6.4	2.1	0.0	5.6	29	34.6	469.8	417.8	5.3	5.8	457.6	1048.3
5/941	Rottenhaus	7.2	1.6	0.0	1.7	22	57.3	353.0	438.1	4.4	7.8	487.0	1529.5
5/942	Rottenhaus	6.3	2.1	0.0	4.6	27	24.5	434.5	603.7	4.7	6.0	503.1	1173.5
5/943	Rottenhaus	6.8	1.8	0.0	3	24	90.5	391.1	549.7	4.1	8.5	483.7	1126.5
5/187	Rutzendorf	7.7	2.0			86	16.6		117.7		4.4		20.6
5/188	Rutzendorf	7.7	2.0			41	53.9		193.6		5.8		22.3
5/189	Rutzendorf	7.7	2.0			58	27.4		178.5		4.7		28.1
5/190	Rutzendorf	7.7	2.0			33	10.7		155.3		5.0		23.6
5/191	Rutzendorf	7.7	2.0			113	93.3		174.7		4.6		25.7
5/192	Rutzendorf	7.7	2.0			98	113.5		133.4		4.5		18.9
5/193	Rutzendorf	7.6	1.9			100	99.7		170.2		5.3		35.5
5/194	Rutzendorf	7.7	1.9			86	68.0		319.6		5.3		39.3
5/195	Rutzendorf	7.6	2.0			190	113.9		107.3		6.4		31.5
5/196	Rutzendorf	7.7	2.0			147	163.6		153.3		5.1		26.4
5/197	Rutzendorf	7.6	2.0			146	167.6		161.3		4.9		22.9
5/198	Rutzendorf	7.6	2.0			122	161.7		198.2		5.6		30.0
5/199	Rutzendorf	7.6	2.1			268	464.0		226.1		5.2		33.3
5/200	Rutzendorf	7.6	2.0			300	563.5		191.7		5.9		31.4
5/201	Rutzendorf	7.6	2.0			271	515.3						
5/202	Rutzendorf	7.6	2.1			282	555.0		194.5		5.2		25.0
5/203	Rutzendorf	7.7	2.1			97	73.3		147.4		5.5		59.2
5/204	Rutzendorf	7.7	2.2			65	33.7		272.7		6.0		45.7
5/205	Rutzendorf	7.7	2.0			44	22.7		150.6		5.6		69.4
5/206	Rutzendorf	7.6	2.2			47	25.5		153.0		5.0		37.9
5/207	Rutzendorf	7.6	2.3			132	143.3		168.4		5.2		32.7
5/208	Rutzendorf	7.6	2.1			112	115.3		202.0		5.7		51.6
5/209	Rutzendorf	7.6	2.0			89	72.5		600.5		7.7		70.0
5/210	Rutzendorf	7.6	2.2			94	89.4		404.9		6.3		61.5
5/211	Rutzendorf	7.6	2.1			188	498.2		143.3		5.9		49.5
5/212	Rutzendorf	7.6	2.1			192	428.9		148.0		10.1		32.6
5/213	Rutzendorf	7.6	2.1			154	463.7		268.3		7.3		54.5
5/214	Rutzendorf	7.6	2.1			153	192.1		143.0		7.3		50.0
5/215	Rutzendorf	7.5	2.3			351	651.1		167.4		6.2		44.5
5/216	Rutzendorf	7.5	2.2			306	620.1		238.6		6.1		43.4
5/217	Rutzendorf	7.6	2.1			286	553.8		450.0		7.1		52.4
5/218	Rutzendorf	7.5	2.2			295	533.4		153.9		6.7		24.9
5/219	Rottenhaus	5.7	0.9			19	21.5		498.7		10.6		906.5
5/220	Rottenhaus	5.5	0.9			24	33.4		83.6		10.5		856.5
5/221	Rottenhaus	5.2	0.8			25	27.4		67.5		8.4		800.6

Sample	Location	pH	TOC %	$CaCO_3$ %	P (H_2O) mg kg^{-1}	P (CAL) mg kg^{-1}	P (C_{DGT}) µg L^{-1}	Fe (EDTA) mg kg^{-1}	Fe (C_{DGT}) µg L^{-1}	Cu (EDTA) mg kg^{-1}	Cu (C_{DGT}) µg L^{-1}	Mn (EDTA) mg kg^{-1}	Mn (C_{DGT}) µg L^{-1}
5/222	Rottenhaus	5.7	0.9			25	33.9		104.9		9.9		850.2
5/223	Rottenhaus	5.6	0.9			51	149.8		115.2		11.6		746.6
5/224	Rottenhaus	5.4	0.8			60	139.8		217.8		11.0		951.5
5/225	Rottenhaus	5.4	0.9			56	159.7		98.8		12.7		784.3
5/226	Rottenhaus	5.7	0.9			65	127.9		180.1		10.5		923.7
5/227	Rottenhaus	5.6	0.9			93	454.3		179.1		13.0		919.7
5/228	Rottenhaus	5.5	0.8			97	230.0		110.0		11.7		923.9
5/229	Rottenhaus	5.5	0.9			91	242.0		185.7		14.0		841.0
5/230	Rottenhaus	5.4	0.9			102	438.4		327.5		14.9		716.4
5/231	Rottenhaus	5.5	0.8			166	373.3		106.1		14.4		904.2
5/232	Rottenhaus	5.4	1.0			166	717.6		217.0		18.0		986.0
5/233	Rottenhaus	5.4	0.9			165	727.6		135.5		16.2		957.2
5/234	Rottenhaus	5.3	0.9			161	788.3		120.4		16.4		944.6
5/235	Rottenhaus	5.5	0.8			24	27.9						
5/236	Rottenhaus	5.4	0.9			26	39.0		132.5		11.0		818.5
5/237	Rottenhaus	5.3	0.8			25	29.4		128.3		8.4		874.0
5/238	Rottenhaus	5.1	0.8			26	30.4		203.5		11.5		970.3
5/239	Rottenhaus	5.7	0.9			42	82.9		141.2		11.2		870.9
5/240	Rottenhaus	5.4	0.9			73	173.9		95.2		11.4		885.0
5/241	Rottenhaus	5.2	0.9			56	109.0		116.9		10.7		932.1
5/242	Rottenhaus	5.2	0.8			58	120.1		114.5		12.0		844.4
5/243	Rottenhaus	5.6	0.8			94	425.1		138.7		13.3		810.8
5/244	Rottenhaus	5.4	0.8			88	200.3		126.0		10.7		802.4
5/245	Rottenhaus	5.3	0.9			90	410.6		73.4		13.2		954.3
5/246	Rottenhaus	5.6	0.9			110	210.6		238.5		10.6		1689.6
5/247	Rottenhaus	5.6	0.8			162	650.1		116.7		12.8		825.0
5/248	Rottenhaus	5.6	0.9			168	569.6		150.7		14.3		920.6
5/249	Rottenhaus	5.4	0.8			157	730.6		181.9		15.2		761.7
5/250	Rottenhaus	5.5	0.9			163	659.7		249.9		15.0		924.5