CRANFIELD UNIVERSITY

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Evaluating phosphorus availability in soils receiving organic amendment application using the Diffusive Gradients in Thin-films (DGT) technique

School of Applied Sciences

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Supervisors:

Dr Ruben Sakrabani

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ABSTRACT

Phosphorus is a resource in finite supply. Use of organic amendments in agriculture can be a sustainable alternative to inorganic P, provided it can meet crop requirements. However a lack of consistent knowledge of plant P availability following application of organic amendments, limits its potential. Studies suggest chemical extraction procedures, may not reflect plant available P. The Diffusive Gradients in Thin-films (DGT) technique is based on natural diffusion of P via a hydrogel and sorption to a ferrihydrite binding layer; which should accurately represent soil P (C_{DGT}) in a plant available form. The aim of this research was to evaluate changes in soil P availability, following the addition of organic amendments, cattle farmyard manure (FYM), green waste compost (GW), cattle slurry (SLRY) and superphosphate (SP) using Olsen P and DGT. The research included incubation, and glasshouse studies, using ryegrass (*Lolium perenne L.*). Soils with a history of application of the aforementioned organic amendments were used (Gleadthorpe), as well as a soil deficient in P (Kincraigie). The hypotheses were as follows **H1** A build-up of P available by diffusive supply, from historic treatment additions and subsequent availability from fresh treatment additions will be demonstrated by DGT. **H2** Historical treatment additions are more important at determining yield and P uptake than fresh additions. **H3** DGT can detect changes in P available by diffusive supply following addition of different treatments and subsequently following lysis of microbial cells on a soil deficient in P. **H4** DGT will provide a more accurate indication of plant P availability than organic amendments in a soil deficient in P. **H5** P measurements using DGT will be lower from organic amendments than superphosphate.**H6** DIFS simulations of soil kinetic parameters will provide additional information about how treatments influence P resupply from solid phase to solution following DGT deployment. DGT provides a more accurate indication of dry matter yield (DMY) (R^2=0.8) than Olsen P (R^2=0.71), and total P uptake (TP_{uptake}) (R^2=0.72) than Olsen P (R^2=0.52). There is a strong relationship between C_{DGT} and DMY for roots (R^2=0.59) and shoots (R^2=0.73). Similarly there is a strong relationship between C_{DGT} and TP_{uptake} for roots (R^2=0.53) and shoots (R^2=0.77). Gleadthorpe studies demonstrated that organic amendment addition to meet crop N demands causes a build-up in C_{DGT} by between 66 and 131 % as the P status increases. Combining Olsen P and DGT provides information about readily available P and the potential for resupply. At the lower range of soil P (Kincraigie), 14 µg l^{-1} is available by diffusive supply, with a potential resupply of 3.6 mg kg^{-1}. At the upper range (Gleadthorpe) there is 548 µg l^{-1} available by diffusive supply with a potential resupply of 65 mg kg^{-1}. Simulation of the resupply time (T_c) between P measured by solid phase and soil solution, following uptake by DGT suggests, treatment addition, reduces

(T_c) from 97 minutes to between 40 and 80 minutes in Kincraigie soils and from 4 to 2 hours in Gleadthorpe soils. This study elucidates understanding of P availability following organic amendment addition to soil, showing a good relationship between soil P available by diffusive supply following treatment additions, and its influence on root and shoot DMY and TP_{uptake}.

Keywords: DGT, Phosphorus, Soil, Organic amendments, Ryegrass.

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A	The exposure area of the gel
AEM	Anion exchange membrane
Al	Aluminium
$AlPO_4 \cdot 2H_2O$	Aluminium phosphate
ANOVA	Analysis of variance
AVP	Available P
$AVP_{treatment}$	Available P content of the treatment
C	Carbon
C:N	Carbon to nitrogen ratio
C:P	Carbon to phosphorus ratio
$C:P_{treatment}$	C to P ratio of the treatment applied.
C_1	Concentration of ion in soil solution
Ca^{2+}	Calcium
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
CAL	Calcium lactate/lactic acid
C_{DGT}	DGT measured concentration
C_e	The P concentration measured by the spectrophotometer
Cl^-	Chloride
C_{ls}	Solid phase P concentration
cm	Centimetre
$cm^2\ s^{-2}$	Centimetres squared per second
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate
C_s	Concentration of ions in the solid phase and solution
C_{soln}	Concentration of P in the soil solution
Cu	Copper
D	Diffusion layer
D	Diffusion coefficient of P in the diffusive layer
D_1	Effective diffusion coefficient in water
D_e	Effective diffusion coefficient in soil
DGT	Diffusive Gradients in Thin-films
DIFS	Diffusion Induced Fluxes in Soils
DM	Dry matter
DMY	Dry matter yield
DNA	Deoxyribonucleic acid
D_o	Diffusion coefficient of P in water
Domsize	Initial domain size
d_p	Particle density
D_s	Soil diffusion coefficient
DTPA	Diethylene triamine pentaacetic acid
f	The impedance or tortuosity factor
ϕ	Porosity
F^-	Fluoride
Fe	Iron

f_e	Elution factor for P
$Fe(NO_3)_3 \cdot 9H_2O$	Iron nitrate
$FePO_4 \cdot 2H_20$	Iron- Phosphate
FYM	Cattle farmyard manure
g	grams
g cm^{-2}	Grams per centimeter squared
g pot^{-1}	Grams per pot
GW	Green waste compost
$H^+ + Al(OH)_2$	
H_2PO_4	Freshly preciptated hydroxylphosphates
H_2O	Water (Dihydrogen monoxide)
H_2PO_4	Dihydrogen phosphate
H_2SO_4	Sulphuric acid
ha	Hectare
ha^{-1}	Per hectare
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate
$HPO4^{2-}$	Monohydrogen phosphate
hrs	Hours
I	Intensity factor
K	Potassium
$k_{1+}k_{-1}$	Sorption and desorption rate constants
K_d	distribution coefficient for labil phosphorus
kg	Kilograms
kg ha^{-1}	Kilograms per hectare
KH_2PO_4	Monopotassium phosphate
K_{sp}	Solubility product of the mineral
l	Litre
M	Mass of P on the Fe oxide gel
m	Metre
min	Minutes
m s^{-1}	Metres per second
m^2	Metre squared
MAP	Monoammonium phosphate
MBP	Microbial biomass phosphorus
mg	Milligrams
Mg	Magnesium
Mg ha^{-1}	Tonnes per hectare
mg kg^{-1}	Milligrams per kilogram
mg l^{-1}	Milligrams per litre
mm	Millimetre
mol	Mole
mol s^{-1} m^{-3}	Moles per second per meter cubed
MQ	Milli-Q
MS_{ha}	Mass of soil in a ha
MS_{pot}	Mass of soil in the pot
MSW	Municipal solid waste

MWHC	Maximum water holding capacity
n	Number of replicates
N	Nitrogen
N:P	Nitrogen to phosphorus ratio
$NaHCO_3$	Sodium hydrogen carbonate
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance
NO_3^-	Nitrate
NPK	Nitrogen phosphorus potassium fertiliser
OC	Organic carbon
OM	Organic matter
P	Phosphorus
P_c	Particle concentration
P_i	Inorganic P
PM	Poultry manure
P_o	Organic P
Q	Quantity factor
θ^2	Tortuosity
R	Ratio of the DGT measured concentration to soil solution concentration
RA	Application rate of the treatment
$Rtol$	Relative tolerance of the ordinary differential equations solver
sec	Seconds
SLRY	Cattle slurry
SMC	Spent mushroom compost
SO_4^2	Sulphate
SP	Superphosphate
SSBW	Source separated bio-waste
STP	Soil test phosphorus
t	Deployment time
t	Time
t ha^{-1}	Tonnes per hectare
T_c	Response time
TEMED	Tetramethylethylenediamine
TP	Total phosphorus
TP_{plant}	Total phosphorus in plant material
$TP_{treatment}$	Total P content of the treatment
TP_{uptake}	Total phosphorus uptake
V_{acid}	Volume of H_2SO_4 used for elution
V_{gel}	The volume of the Fe oxide gel
V_s	Volume of matrix solids
V_t	Total soil volume
V_v	Volume of void spaces

w w^{-1}	weight over weight
WHC	Water holding capacity
yr	Year
Zn	Zinc
Δg	Diffusion layer thickness
ΔI	Change in intensity
ΔQ	Change in quantity
Θf	Volumetric water content of the soil
% PRR	% of P recovered from treatment
℃	Degrees Celsius
µg	Micrograms
µm	Micrometre
µM	Micromolar
µl	Microliter

1 Introduction and Literature review

1.1 Introduction

This chapter aims to explore the main aspects related to soil phosphorus (P) dynamics and its availability following incorporation of organic amendments and inorganic fertilisers to soil. The literature review summarises organic amendments used in this study, soil nutrient plant root interactions, the P cycle, effects of different organic amendments on P dynamics, techniques for measuring available P in soil, and a section on operating principals of Diffusive Gradients in Thin-films (DGT) and previous studies employing this method. Finally a list of knowledge gaps established within the review is also presented here.

1.2 Background

The four major plant nutrients required to grow crops for food, feed, and fibre are nitrogen (N), phosphorus (P), potassium (K) and sulphur (S). In terms of global resource base, P is the least abundant. Over 85% of the phosphate rock (PR) mined each year is used in agriculture as fertiliser to grow crops and as additives to animal feeds (Hilton *et al*, 2010). Phosphorus is a key component of every living cell, however it is a resource which is in finite supply, therefore as it is exploited it becomes increasingly depleted (Hilton *et al* 2010). There are various estimates of the global phosphate resource, but the true figure is largely unknown. Partly because of the sensitive nature of the information, and partly because there may be PR yet to be discovered (Hilton *et al*, 2010). The global phosphate resource debate highlights that it is a diminishing resource which must be managed more sustainably (Smil, 2000; Steen, 1998; Smit *et al*, 2009; Hilton, 2010). At current rates of use Hilton *et al*, (2010) estimate that phosphate reserves will last about 100-150 years, and the resource will last a further 300-350 years. However Cordell *et al*, (2009) believe the reserves can be exhausted within the next 50-100 years. Responses to common resource scarcity problems include price increases, more efficient use of the resource, introduction of alternatives and recovery of the resource after use (Cordell *et al*, 2009). As world population increases, demand for phosphate to grow crops to support the increased population will ensue. Efficient and sustainable management of the world's finite P resources is in the best interests of all

who use and rely on it. Improving the efficiency of nutrient availability to crops can go some way to managing P resources.

In addition, the application of the extracted P as inorganic fertiliser to soil poses environmental risks. Over application or poor management of P can lead to P losses and cause eutrophication of aquatic systems. Eutrophication is caused by P enrichment of water bodies, and is detrimental to ecosystems. It results from increased growth of undesirable algae and aquatic weeds, resulting in oxygen consumption from their senescence and decomposition (Tunney *et al*, 1997). This is worse where P has accumulated in soils from high P inputs, and exacerbated by situations, which facilitate soil erosion (Grossl *et al*, 2009).

1.3 Use of organic amendments in agriculture

Organic amendment incorporation can improve soil conditions and increase availability of P (Fuentes *et al*, 2006). Thus, its use in agriculture is a sustainable alternative to inorganic P sources, provided it can meet crop requirements. However P in the waste matrix forms organic and inorganic compounds which have different bioavailabilities (Fuentes *et al*, 2006). As a result, there is a lack of consistent knowledge of plant P availability, and its controls following application to soil (Prasad, 2009). The characteristics of the residue applied are important in determining P availability, through their influence on soil characteristics which determine P adsorption strength (Pypers *et al*, 2005). P adsorption is influenced by; organic matter mineralisation, orthophosphate release (Fuentes *et al*, 2006), humic substance and organic acid release, (Mkhabela and Warman 2005). Influences on soil pH also significantly influence P availability (Waldrip *et al*, 2011).

Studies have been conducted to compare the availability of P from organic amendments in soils compared to inorganic fertilisers (Sharpley and Sisak, 1997, Eghball *et al*, 2005 Loria and Sawyer 2005). However there remains a lack of understanding, mainly due to the variability of the different treatments added and different characteristics of soils they are added to. In addition to the benefits of incorporation to the soil, organic amendments are renewable resources. Therefore improved understanding of the transformations between nutrient content of the residue and its availability in soil can help to improve efficiency of resource utilisation. This in turn can help provide a sustainable resolution

to the environmental and economic impacts associated with application of inorganic fertilisers described above.

The organic amendments related to this project are FYM, SLRY and GW. This provides a range of organic amendments, which can be compared for their effects on P dynamics in soil based on their individual properties. The following is a summary of the composition of each based on current knowledge.

1.3.1 Cattle manure and slurry

Manure and slurry have traditionally been applied to agricultural land as soil conditioners and fertilisers (He *et al*, 2004). They are generally relatively immature. Intensified agricultural production has increased production of FYM. This has implications for safe disposal as they are a diffuse source of pollution to water bodies (He *et al*, 2004). The P content of FYM varies depending on animal physiology, species, age, composition of diet, duration of storage, moisture content and type of bedding material (McDowell and Stewart, 2005). A range of total P contents of different dairy manures measured in different studies are displayed in Table 1-1. Factors such as climate and soil characteristics influence the availability of P in the soil (Atia and Mallarino, 2002). Contents of dry matter, total P and % availability for cattle FYM and SLRY is provided in Table 1-2 (Defra, 2010).

Table 1-1: Total P contents of different dairy manures from a range of studies.

	Total P (g kg^{-1})	Reference
Dairy manure	4.35	Griffin *et al*, 2003
	4.1-18.3	He *et al*, 2004
	3.5-9.8	Sharpley *et al*, 2004
	11	Hansen *et al*, 2004
	23.21	Leinwebber *et al*, 1997
	21	Mokolobate and Haynes, 2002
	0.05-1.12 (lagoon)	Hansen *et al*, 2005

Adapted from Fuentes *et al*, (2006).

1.3.2 Green waste compost

Composting is an aerobic process whereby biological exothermic oxidation of organic matter is converted into a stable humified product (Fuentes *et al*, 2006). The product is formed by a succession of microbial populations. The three main methods of compost

production are in static piles, rows or reactors. The main conditions required for composting are: pH between 5 and 11, C: N 30 and 40 and humidity between 40% and 65% (Garrido *et al*, 2002). The P content of compost is quantified as total P, however within this total, neither the quantities nor the P species available are clear (Fuentes *et al*, 2006).

Frossard *et al*, (2002) established that the P, which can be extracted from composts (green waste and bio waste), varied from 3% of total P when extracted with water or up to 98% when extracted with strong acids. The slowly or non- exchangeable phosphate was bound to calcium in the form of apatites or octacalcium phosphates. These studies involved 16 different composts, where extractable P ranged from 54.6% to 95.1%, however not all of these were plant available. Sequential extraction determined water and bicarbonate extractable P were rapidly available to the plant, sodium hydroxide (NaOH) extractable P were bound to Fe or Al oxides or to organic substances and hydrochloric acid (HCl) extractable P was only sparingly soluble in this study.

Adler (2005) found that 70-95% of total P was in the inorganic fraction in various green waste composts, but the water extractable fraction ranged from a low 1-12% to a high 15-40%. Contents of dry matter, total P and % availability for green waste compost is provided in Table 1-2 (Defra, 2010).

Table 1-2: General characteristics of treatments used in this study

	Dry matter%	Total P (kg t^{-1})	Availability %	Available P (kg m^{-3})
Cattle FYM	25	3.2	60	1.9
Cattle Slurry	2	0.6	50	0.3
	6	1.2	50	0.6
	10	1.8	50	0.9
Green waste compost	60	3	50	1.5

Adapted from DEFRA RB209, fertiliser recommendations, (Defra, 2010)

1.4 Soil nutrient and plant root interactions

Knowledge of the mobility of plant nutrients in soil is important for understanding their plant availability following application of fertilisers and organic amendments. There are three principal components involved in the movement of mineral elements to the root

surface of plants in soil (Figure 1-1). (1) Root interception: soil volume displaced by root volume (2) Mass flow: transport of bulk soil solution along the water potential gradient (driven by transpiration). (3) Diffusion: nutrient transport along the concentration gradient (Marschner, 2012).

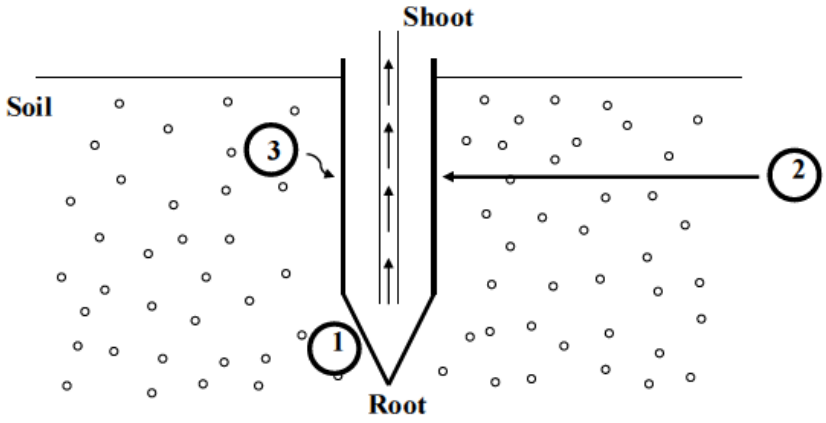


Figure 1-1: Mineral elements movement to the root surface of soil-grown plants. (1) Root interception (2) Mass Flow (3) Diffusion (o) Available nutrients (Adapted from Marschner, (1995)).

1.4.1 Root interception

As roots extend through the profile they enter spaces formerly occupied by soil, which contain available nutrients, which are intercepted by the root (Barber, 1995; Lynch 1995; Richardson *et al*, 2009). The quantity of nutrients which can be intercepted by roots is based on (a) the amounts of available nutrients in the soil volume occupied by roots; (b) root volume as a percentage of total soil volume (c) the percentage of the total soil volume occupied by pores (Marschner, 2012). Generally only a small portion of the total nutrient requirement can be met by root interception.

1.4.2 Mass flow

Mass flow is the convective transport of nutrients dissolved in the soil solution from the bulk of the soil to the root surface. When soil water content is high (field capacity) mass flow is unrestricted and maintains a similar root potential at the root surface. With decreasing water content, uptake by roots can exceed supply by mass flow, which may

result in drying soil at the soil-root interface, this can occur particularly when transpiration rates are high (Tinker and Nye, 2000). The term apparent mass flow is often used instead of mass flow, to define the amount of solutes transported to the root by mass flow since mass flow and diffusion to the root surface usually occur simultaneously, and it is difficult to separate such processes (Tinker and Nye, 2000).

1.4.3 Diffusion

Diffusion is the main mechanism for phosphorus movement to the root surface. The driving force in soil grown plants is a concentration gradient, which is formed between the adjacent soil and the root surface when the uptake rate of ions exceeds the supply by mass flow. Over time depletion profiles develop, and their shape is determined mainly by the balance between uptake by roots, replenishment from soil and mobility of ions by diffusion (Marschner, 2012). The mobility of ions can be described in terms of the diffusion coefficient. However in soils which are non-homogeneous porous mediums diffusion coefficients are orders of magnitude lower than homogeneous media such as water (Marschner, 2012). Therefore the term effective diffusion coefficient, *D*e has been introduced by Tinker and Nye (2000) for describing the diffusion of ions in soils (Equation 1-1).

$$D_e = D_1 \Theta \frac{1}{f} \frac{dCI}{dCs} \qquad \textbf{Equation 1-1}$$

Where D_e is the effective diffusion coefficient in the soil ($m^2\ s^{-1}$); D_1 is the diffusion coefficient in water ($m^2\ s^{-1}$); Θ is the volumetric water content of the soil ($m^3\ m^{-3}$); f is the impedance (or tortuosity) factor which takes into account the tortuous pathway of ions and other solutes through water-filled soil pores, increasing the path length and thus decreasing the concentration gradient. D_e is defined as the reciprocal of impedance, i.e., becomes smaller when the soil water content falls; and d*CI*/d*Cs* is the reciprocal of the soil buffer power for the ion concerned; C_1 is the concentration of the ion in the soil solution and C_s is the sum of both ions in the soil solution and those which can be released from the solid phase. Soils with a high adsorption capacity therefore have a high buffer power and thus a low d*CI*/d*Cs* value (Marschner, 2012).

Estimates of H_2PO_4- diffusion coefficient in water is 0.9 x 10^{-9} m^2 s^{-1} and between 10^{-12}-10^{-15} m^2 s^{-1} in soil. The mean diffusion coefficient in soil is 1x10^{-13} m^2 s^{-1}. Movement in soil is estimated to be 0.13mm day^{-1} (Lungk, 1991; Marschner, 2012).

1.4.4 Association with microorganisms

Microbial associations with plant roots can enhance nutrient uptake by the plant through a number of mechanisms. These are (a) increase the surface area of the roots by extension of existing root systems (mycorrhizae) (b) enhancement of root growth with branching or root hair development (rhizobacteria) (c) nitrogen fixation (rhizobia and diazotrophs) or by stimulation of metabolic processes which mobilise nutrients from poorly available sources (organic anions) (d) displacement of sorption equilibrium that results in increased net transfer of nutrients into solution (e) turnover of microbial biomass within the rhizosphere (Gyaneshwar *et al*, 2002; Jakobsen *et al*, 2005; Kucey *et al*, 1989; Richardson *et al*, 2007; Tinker 1980; Richardson 2009).

Mycorrhizal symbioses are found in the majority of ecosystems and can enhance plant growth through a number of mechanisms, including increased nutrient uptake, improved plant establishment, protection against stress (biotic and abiotic) and improved soil structure. Mycorrhizal colonisation of roots increases the effective volume of soil which can be exploited for P (Buscot 2005; Smith and Read, 2008, Richardson, 2009). They colonise the root cortex biotrophically and develop external hyphae, which connect the root with the surroundings of the soil. The majority of vascular plant species can associate with mychrorrhizal fungi (Richardson, 2009).

Mycorrhizal fungi have similar access to soil solution P as is available to plants, however arbuscular mycorrhizas (AM) and ectomycorrhizal fungi can access less available sources of P (Casarin *et al*, 2004; Richardson *et al*, 2009). For example exudates from fungal hyphae can solubilise more P than from root exudates alone (Twaraya *et al*, 2006). In addition extra radical mycelium of AM fungi can increase efficiency of P acquisition by developing into the soil allowing P access from the soil solution several cm from the plant root (Jakobsen *et al*, 1992). A high density of mycorrhizal fungi increases surface area for absorption of orthophosphate, and can exploit soil pores and nutrient patches not available to plants (Tibbett and Sanders 2002; Jacobsen et al, 2005).

It can therefore be seen that mycorrhizal fungi can play an important role in the mobilisation and supply of nutrients (particularly P) to plants. Although determining its role in understanding P dynamics is highly important, it will not be a major focus of research in this study. However when an experiment is conducted which involves plant roots, it is important to consider how mycorrhizal fungi may be influencing the behaviour of P in the soil, plant and rhizosphere.

1.5 The P cycle

An in depth knowledge of the P cycle (Figure 1-2), described below, is fundamental to understanding how organic amendments and inorganic fertilisers effect soil P dynamics. This section will also synthesise previous studies which consider how organic amendments influence each aspect of the P cycle, and the influence on plants where appropriate.

Organic P undergoes a mineralisation process to form inorganic P. Inorganic P in soil solution can be absorbed by plant roots, immobilised by microorganisms, adsorbed to mineral surfaces or it can be precipitated which is also known as secondary P (Halvin *et al*, 1999). P fixation refers to surface adsorption and precipitation reactions and is dependent on many factors, but mainly soil pH. Precipitation and adsorption is an ongoing process for P retention. Adsorption is the main process when soil solution P is low. However when concentration of P and its associated cations is higher than the solubility product (K_{sp}) of the mineral, then precipitation is the main process (Brady and Weil, 2002). The fundamental stages of the cycle will now be described.

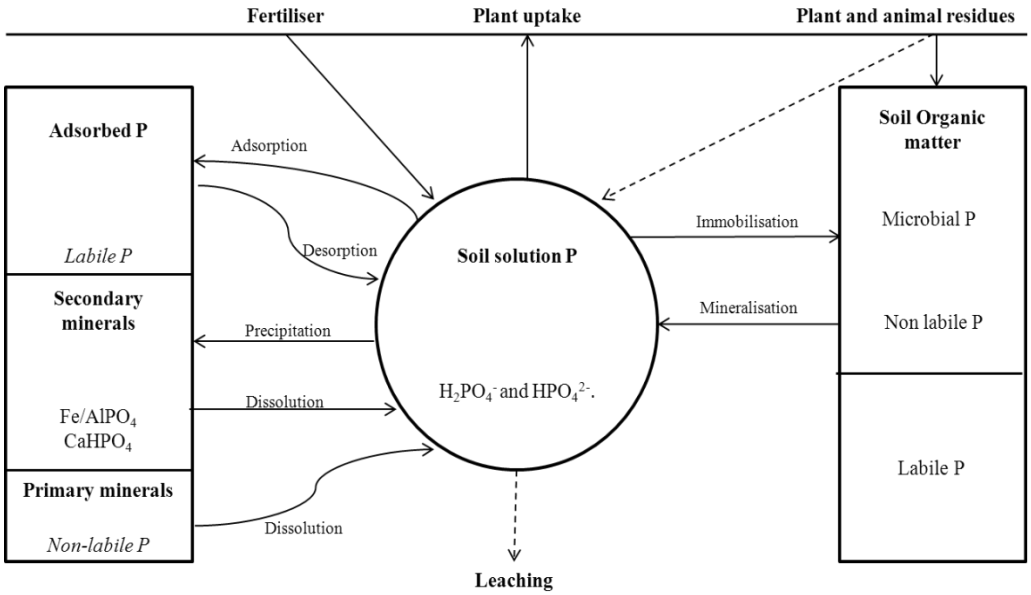


Figure 1-2: The P Cycle in soil, adapted from (Halvin *et al*, 1999).

1.5.1 P pools

In the concept outlined in (Figure 1-3) Syers *et al*, (2008) explain that P exists in soils in four separate pools. The P in each pool is related to differences in bonding energy for P between sites both on surfaces and within soil constituents. This concept explains that soil solution P is immediately available for plant uptake. The surface adsorbed pool is readily extractable and ready available, in equilibrium with P in the soil solution. The P pool, which is strongly bonded or absorbed to soil components, is less readily extractable; however it can become plant available with time. The final pool, which is very strongly bonded, or inaccessible or mineral or precipitated P is only very slowly plant available, or not available at all.

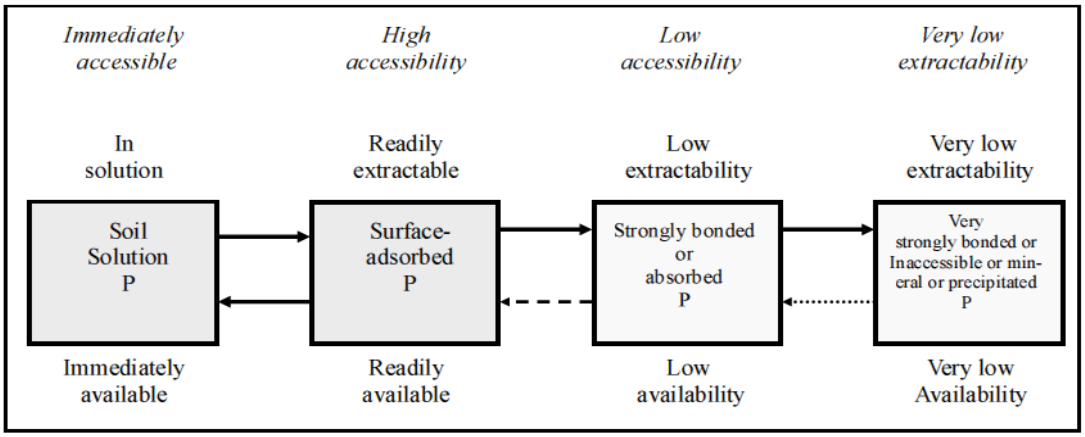


Figure 1-3: The forms of inorganic phosphorus in soil categorised in terms of accessibility, extractability and pant availability adapted from (Syers *et al,* 2008). The key feature addressed in the concept of soil P pools is the reversible transfer between the soil solution, the surface absorbed P pool and the strongly bonded or absorbed P pool.

1.5.2 Solution P

P forms found in solution are negatively charged primary and secondary orthophosphate ions $H_2PO_4^-$ and HPO_4^{2-}. $H_2PO_4^-$ is associated mainly with acid soils and HPO_4^{2-} is associated with more basic soils. These are the phosphorus forms, which are absorbed by plants, thus influence growth and yield. In most soils concentration of orthophosphate in soil solution is low (between 1-5 µM) (Bieleski, 1973; Richardson *et al*, 2008) and therefore it must be replenished from other pools to meet plant requirements. Other studies have suggested ranges between 0.1 and 10µM (Raghothama, 1999; Frossard *et al*, 2000; Hinsinger, 2001). Stevenson and Cole (1999) report that the minimum soil solution P concentration required for maximum crop yield ranges from 0.01 to 0.3 mg P l^{-1}.

However following application of P crop utilisation is low, and rarely exceeds 20%, (Damodar Reddy *et al*, 1999; Subba Rao *et al*, 1996), P availability in years after organic amendment application are determined by their transformations amongst inorganic and organic soil constituents, however it is generally accepted that P release from organic amendments is in a slow release form (Prasad, 2009)

1.5.3 Organic P (P_o)

It has been estimated that between 15 – 80 % of P in soils occurs in organic forms (Brady and Weil, 2002; Shen *et al*, 2011; Schachtman *et al*, 1998). However it is dependent on the nature of the soil and its composition. Three main groups of organic phosphorus compounds are known to exist in soils, most are believed to have been synthesised by microorganisms. The main groups can be categorised as (a) inositol phosphates (b) nucleic acids (c) phospholipids. Other phosphorus compounds can be found in soils such as phosphoproteins and metabolic phosphates; however amounts of these are less well understood. From the groups mentioned, inositol phosphates are the most abundant organic phosphorus compounds, making up approximately 10-50% of the total organic phosphorus. Phospholipids are thought to make between 1-5% of organic phosphorus in most soils (Brady and Weil, 2002; Stevenson and Cole, 1999). Nucleic acids are 0.2-2.5%, and phosphoproteins and metabolic phosphates are also found but in trace amounts (Stevenson and Cole, 1999).

1.5.4 Soil microbial biomass phosphorus (MBP)

Microbial biomass contains 0.4–2.5% of total P in cropped soils and up to 7.5% in grassland soils. It can play an important role in P cycling (Oberson and Joner 2005). The main forms of microbial P are nucleic acids and phospholipids (together 60%), cytoplasmic inorganic P (10%), cytoplasmic organic P (10%), and polyphosphate (20%) (Bunemann, 2011).

Addition of C provides a substrate for stimulation of microbial processes, which can result in immobilisation of soil nutrients, reducing availability (Fuentes *et al*, 2006). Microbial uptake of P and its subsequent release and redistribution significantly affect P availability to plants, especially following addition of organic amendments (Oberson and Joner, 2005). Zhang *et al,* (2005) explained that soil microbial biomass is related to several factors, such as organic C and N limitation, residue and nutrient management, differences in plant species, soil texture, soil moisture and temperature

Organic amendment incorporation into the soil effects the composition and enhancement of microbial biomass, which results in changes in enzyme activity (Speir

et al, 2004). It was found that organically managed soils had higher microbial P than conventionally managed soils and non-fertilised soils (Marinari *et al*, 2006).

Organic waste decomposition by microbial degradation has a significant effect on the P adsorption desorption dynamics (Iyamuremye *et al*, 1996c) and it is likely that this is the main mechanism in the reduction of P adsorption (Fuentes *et al*, 2006). The organic anions compete with orthophosphate for sites on soil surfaces and can replace P bound to soil surfaces increasing P availability (Pypers *et al*, 2005).

The biological stability of the product being applied to the soil is an important mechanism influencing soil microbial biomass production (Smith and Hughes, 2004). Immature organic amendments contain substantial amounts of easily degradable organic compounds, such as organic acids, and a higher microbial biomass and therefore enzyme activity which decreases upon compost reaching maturity and stabilising.

The act of composting leads to the formation of a more stable product over time. A more mature product leads to a reduction in easily degradable organic compounds such as organic acids (Smith and Hughes, 2004) and a reduction in microbial biomass, and thus enzymatic activity. Scherer (2004) conducted a greenhouse experiment to investigate compost made from source separated bio waste (SSBW) within increasing stability on growth and P uptake of ryegrass (*Lolium perenne cv. Turilo*). Compost application resulted in a significant yield increase compared to the control. Increased yields and P uptake resulted from more stable compost. P increases ranged between 8.5% and 104% in the first year of compost application.

1.5.5 Mineralisation/imobisation

P mineralisation is the conversion from organic P forms to soluble P, and the reverse reaction occurs for immobilisation. Mineralisation and immobilisation require microbial organisms for conversion. Organic P mineralisation rates have been measured at between 1.4 and 2.5 mg P kg^{-1} per day in arable soils (Oehl *et al*, 2004; Frossard *al*, 2011). The process can be represented by the following (Figure 1-4):

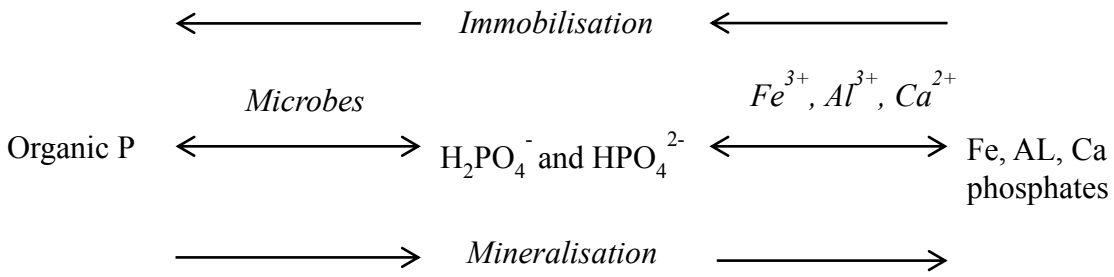


Figure 1-4: Microbial mineralisation and immobilisation of P in soil.

A number of factors determine whether P is mineralised or immobilised, however, the C:P ratio of the material undergoing decomposition by soil microorganisms is the primary determinant. The most important factors affecting P mineralisation are the amount and quality of soil OM, temperature, moisture, texture and pH. These factors are important in terms of suitability for microorganism to carry out decomposition of organic matter thus facilitating P release. The content of P in organic residues is important for regulating the quantity of soluble P in the soil (Griffin *et al*, 2003, Azeez *et al*, 2009, Miller *et al*, 2010). It was suggested by Laboski and Lamb (2003) that a critical P content of 0.2-0.3 % above which there is no net immobilisation from organic amendments. Results from Mafongoya *et al*, (2000) and Gichangi *et al*, (2009) confirm this. Gagnon and Simard (1999) proposed that the C:P ratio of the amendment can be a good indication of P availability. The C:P ratio of the amendment influences whether there will be an initial net mineralisation or an initial net immobilisation of soil P. A C:P ratio of 200 is typically used as an indication of the threshold for mineralisation and immobilisation. Several authors have suggested that a C:P> 200 will result in immobilisation and <200 will result in mineralisation (Dalal 1977; Soloman *et al*, 2002).

Other authors have found that these treatment characteristics cannot be used as an accurate predictor of P availability from organic amendments. Nwoke *et al*, (2004) highlighted that previous attempts to elucidate the relationship between treatment characteristics and soil P availability have led to inconsistent results. Fuentes *et al*, (2006), suggests that properties of manures, compost and inorganic P differ in many respects, which will result in different P transformations following application to soil.

The influence of treatment characteristics on P release to soil can subsequently influence the growth of plants. Treatment properties influence shoot dry matter yield (DMY) and total phosphorus uptake (TP_{uptake}). The carbon to phosphorus ratio of the treatment ($C:P_{treatment}$) can be used as a predictor of TP_{uptake} (when N is non limiting) (Kwabiah *et al,* 2003). However Ylivainio *et al,* (2008) and Nwoke *et al,* (2004) found that shoot DMY did not increase with increasing $TP_{treatment}$ or decreasing $C:P_{treatment}$. Umrit and Friesen (1994) stated using $C:P_{treatment}$ to predict immobilisation would be misleading and Nwoke *et al,* (2004) suggested that attempts to elucidate the relationship between C:P ratio and available P following treatment addition to soil have been inconsistent.

A number of studies have assessed the influence of organic amendments on soil P dynamics following incorporation and its subsequent effect on plant characteristics (DMY and TP_{uptake}). Read *et a*l, (2007) observed ryegrass TP_{uptake} values in the region of 11.6 to 23 kg P ha^{-1}. By adding broiler litter at between ~4.5 and 36 kg ha^{-1} /yr^{-1}, an increase in ryegrass TP_{uptake} by ~108 -333% could be expected. Ylivainio *et al,* (2008) found that increases in the range 27 and 141% for meat and bone meal and dairy manure at 25 and 100 mg P kg^{-1} respectively. Read *et al,* (2007) found annual ryegrass DMY values in the region of 5000 (control) to 14 000 (treated) kg ha^{-1} for the first year following application representing an 180% increase. Antille, (2011) found ryegrass DMY to range between 2000 (control) and 9000 (treated) kg ha^{-1}, in glasshouse pot experiments, representing a 350% increase

It was determined that studies into the effect of treatments on ryegrass yield and P uptake following treatment addition, focuss on aboveground biomass, and neglect the important effect on root yield and P uptake (Waldrip *et al,* 2011). A few studies (Chen *et al,* 2002; Pederson *et al,* 2002) investigated root uptake of P, and Waldrip *et al,* (2011) looked at the effect of an organic amendment (poultry manure (PM)) on root and shoot P uptake and total biomass production. Root P concentrations were 37% higher and total P uptake 59% higher with PM application than control. At week 16, there was 30% more labile-Pi (H2O- plus $NaHCO_3$-Pi) in the rhizosphere with PM than in the control.

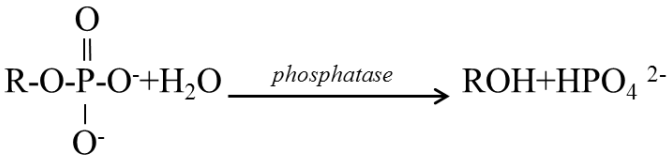
N:P ratios differ between organic amendments and depend on source material, however it has been established (Read *et al,* 2007, Evers, 2002) that the P content of organic amendments generally provide a greater proportion of the available nutrient required by the plant, than N. This result in a build-up of available P in soils which have received long term amendment application based on N demands. Read *et al,* (2007) explained that an N:P ratio of broiler litter is lower than the ratio of N and P absorbed from the soil by plant root (Bermudagrass), (2:1 vs 10:1) (Evers, 2002), this causes a build-up in soil P levels substantially greater than those required for optimum yield.

Mineralisation rates and P availability are influenced by animal physiology, species, age, composition of diet, duration of manure storage, moisture content and type of bedding material (Atia and Mallarino, 2002; McDowell and Stewart, 2005). In addition factors such as climate and soil characteristics furthermore influence the availability of P in the soil (Atia and Mallarino, 2002).

Gichangi *et al,* (2009), carried out an experiment to investigate changes to resin P, $NaHCO_3$, MBP and HCl, representing P forms in terms of availability respectively. Available P (resin P) decreased with time and was resupplied to MBP representing a gradual immobilisation before mineralisation. Mineralisation coincided with an increase in $NaHCO_3$ (Olsen P), it was suggested that this was evidence to support the theory of mineralised P being transferred to available P pools.

1.5.6 Phoshatase enzymes

Phosphatase enzymes are responsible for the final stage in the conversion of organic P to inorganic phosphate in soils. They catalyse the hydrolysis of ester–phosphate bonds, leading to the release of P, which can be taken up by plants or microorganisms (Nannipieri *et al,* 2011). The general equation for the reaction catalysed by phosphatases is displayed in Equation 1-2: (Stevenson and Cole, 1999).



Equation 1-2

Phosphatase can mobilise organic P through enzyme-catalyzed hydrolysis. The efficiency of this is determined by the availability of a substrate, interactions with microorganisms, soil pH and soil physical and chemical conditions (George *et al*, 2005; Shen *et al*, 2011). Creccio *et al*, (2004) found that application of low rates of MSW compost (12 and 24 Mg ha^{-1}) increased phosphatase enzyme activity by 9.7% (increasing from 12 to 24 Mg ha^{-1} did not induce any further increase).

1.5.7 31P NMR analysis

Solution 31P nuclear magnetic resonance (NMR) spectroscopy is the most widely used spectroscopic technique for the speciation of soil organic P **(**Doolette and. Smernik 2011**)**. Below is a review of some of the most up to date work on P in soil and organic amendments.

Hansen *et al*, (2004) investigated P forms in manure stored in solid form or in a lagoon, by means of NaOH-EDTA extraction and 31P NMR. P compounds in solid and liquid manure were similar, indicating that about 30% of total P is in organic form. The primary forms extracted from solid and lagoon manures were orthophosphate (63.3 and 58.4 %, respectively), pyrophosphate (3.5 and 7.1 %, respectively), the monoester phytic acid (15.6 and 10.8 %, respectively), other monoester (14.4 and 20.1 %, respectively) and diester as phospholipids (1.8%) and DNA (0.9 and 1.8%, respectively) and phosphonates (0.5%) (Fuentes *et al*, 2004).

Characterisation of extracted soil and sludge P using solution 31P NMR has shown the presence of both inorganic P species (orthophosphate, pyrophosphate, polyphosphate) and organic P species (phosphonates, orthophosphate monoesters and diesters) (Cade-Menun and Preston 1996).

Smith *et al*, 2004 revealed that P was mainly in the inorganic pool in three sludge samples, with the highest proportion (of the total extracted P) as inorganic P in the anaerobically digested liquid sludge. Following incorporation to soil, P was immobilised to organic species (mainly monoester-P forms, the remainder were diester P and phosphonate P).

31P NMR is therefore an important technique for determining organic P speciation in soil. It can offer a useful insight into the P forms which are available following

incorporation of organic amendments. It is a technique in its relative infancy and offers good potential for future research and development. However it is also a complex tool which requires assistance from experts in the field as well as significant training for use of the equipment and interpretation of results. Therefore use of 31 PNMR would be best suited to a study which has the intention to determine P speciation in soil.

1.5.8 Inorganic P (P_i)

The majority of inorganic phosphorus compounds fall within two groups (1) containing Ca and Mg (2) containing Fe and Al. These are affected the pH of soil solution (Hinsinger, 2001) due to variations in proton dissociation which are categorised by pKa values, which represent an important property of chemical compounds and indicate their ionisation capability (Fuentes *et al*, 2006).

As a group, ($FePO_4 \cdot 2H_2O$) and ($AlPO_4 \cdot 2H_2O$) phosphate compounds are insoluble and stable in acid soils and become more soluble as soil pH increases. They are therefore unstable in alkaline soils. In acid soils, because of the much increased solubility of Fe and Al oxides, trivalent Fe and Al can occur in large concentrations in the soil solution, whereas they will be negligible at neutral or alkaline pH (Lindsay, 1979). Calcium phosphate compounds are associated mainly with high pH where they are stable and insoluble and their solubility increases as pH decreases. In neutral and alkaline soils, Ca and, to a lesser extent Mg will be the dominant cations in soil solution (Hinsinger, 2001).

It is accepted that phosphate forms inner-sphere complexes via ligand exchange reactions between phosphate and hydroxyl groups at the surface of metal oxyhydroxides (Arai and Sparks, 2007). These mainly include bidentate binuclear (BB) and monodentate mononuclear (MM) surface complexes. Bidentate binuclear complexes form when one phosphate replaces two hydroxyl groups, whereas monodentate mononuclear complexes refer to phosphate groups that bind with a single metal (*Me*) on the mineral surface via a P–O–*Me* linkage. Formation of outer-sphere complexes may be possible (Chitraker *et al,* 2006), but is not widely supported (Arai and Sparks, 2007; Li *et al*, 2013).

Precipitation can be viewed in different ways: (a) formation of a new surface phase, (b) multilayer adsorption (c) formation of a solid solution Ler and Stanforth (2003). Dzombak and Morel (1990) describe surface precipitation as the formation of a different solid phase, a solid solution whose formation starts when the saturation concentration is exceeded. Alternatively, precipitation can begin when metal ions adsorb onto the adsorbed anion. The onset of precipitation is controlled by the bonding constant for the ternary complex and the dissolved metal ion concentration Ler and Stanforth (2003).

Organic matter in residues contains significant quantities of organic P and during mineralisation; orthophosphate is released into soil solution. In addition authors (Iyamuremye *et al*, 1996; Iglesias Jimenez *et al*, 1993) highlighted that organic amendments can block P adsorption sites, improving the availability of P to the plant. During decomposition of organic waste, inorganic and organic products are generated and humic substances and organic acids can be absorbed into soil surfaces. This decreases the potential P adsorption by blocking sites for the formation of complexes with Al, Fe and Ca (Fuentes *et al*, 2006; Iyamuremye *et al*, 1996a; Haynes and Mokolobate, 2001; Mkhabela and Warman, 2005).

Iyamuremye *et al*, (1996a) describes a significant increase in available P, readily mineralisable organic P and chemisorbed fractions in soil after incorporation of P rich residues. The main mechanisms involved in increasing P availability are: orthophosphate incorporation, pH increases, P solubilisation, production and release of organic anions, increased enzyme activity, incorporation of organic matter and complexation of exchangeable ions such as Al, Fe, Ca and Mg (Fuentes e*t al*, 2006).

Establishment and growth of plant roots significantly influences soil chemistry, through root exudates, Shen *et al*, (2011) explained that physiological activities in the rhizosphere, such as the exudation of organic compounds like mucilage, organic acids, phosphatases determine mobilization and acquisition of soil nutrients. Phosphorus is mobilized from the bulk soil to the rhizosphere to meet plant demand.

1.6 Soil pH

pH is of major importance when determining availability of phosphates to plants (Figure 1-5). At pH 7.2 there are approximately equal amounts of $H_2PO_4^-$ and HPO_4^{2-}.

Below this $H_2PO_4^-$ is the major form in solution, whereas HPO_4^{2-} is the main form above pH 7.2. Plant uptake of $H_2PO_4^-$ is much faster than HPO_4^{2-} (Waldrip *et al*, 2011). P in these forms is highly mobile and is available to plants and crops for uptake. Phosphate is increasingly unavailable as soil acidity increases, due to retention by Al and Fe (Bohn, 2001). In acid soils most solid-phase phosphate is associated with Fe and Al in their hydroxyoxides. In basic soils phosphate is associated with Ca in apatite-like forms (Bohn, 2001).

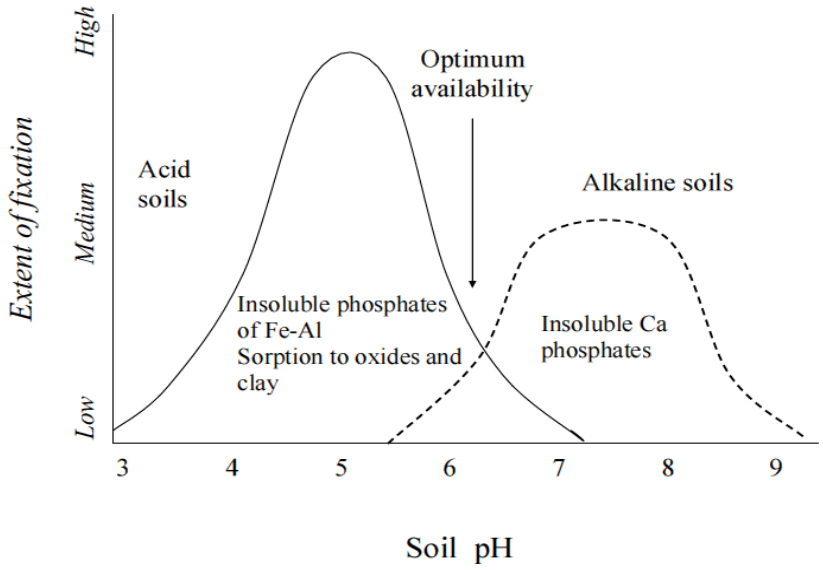


Figure 1-5: Effect of pH on phosphate forms and extent of P fixation in soil (Adapted from Stevenson and Cole 1999).

The effect of organic amendments on pH may be attributable to self-liming caused by the mineralisation of C and the release of basic cations (Hue, 1992; Iyamuremye *et al*, 1996a). Increase in pH with the addition of manure may also be a result of the production of OH^- ions by ligand exchange mechanisms which occur between organic acids and hydroxyl Fe and Al in soil (Iyamuremye *et al*, 1996a; Mokolobate and Haynes, 2002). In a study of the influence of the amount and kinds of organic and inorganic amendments on phosphorus sorption characteristics Iyamuremye *et al*, (1996a) found that wheat straw had little effect on soil pH (0.6), but manure (1) and alfalfa (1.4) caused increases in pH averaging an increase of more than 1 pH unit among the soils. Pypers *et al*, (2005) found a significant pH increase associated with green

manure residue incorporation to soil. Samples with an initial pH of 4.97 and 4.39 increased 0.48 and 0.67 pH units on the first day of application; on the third and seventh days pH had increased to 5.95 and 5.72 respectively. In addition a positive correlation existed between pH increase and reduction of exchangeable Al, which favours orthophosphate anions in soil solution.

1.7 Comparison between inorganic fertilisers and organic amendments

Previous authors have identified contradicting results when comparing of the contribution of organic amendments to P availability with inorganic P sources (Laboski and Lamb, 2003; Eghball *et al*, 2005; Sikora and Enkiri 2005; Sneller and Laboski, 2009). However, (Gracey, 1984; Griffin *et al*, 2003; Sharpley and Sisak, 1997) found inorganic sources supply more P than organic amendments. The following studies are categorised based on the scale of the experiment.

In incubation experiments, Loria and Sawyer (2005) showed Olsen, Bray 1, and Mehlich 3 levels less than fertiliser for the first 28 days after application for swine manure application rates from 0 to 50 mg P kg^{-1}. However Griffin *et al*, (2003) observed an increase in Mehlich 3 P similar to $KH_2PO_4^-$ levels when poultry manure and swine slurry were applied to a sandy loam soil. However cattle and dairy manures gave soil STP levels significantly lower than $KH_2PO_4^-$. Poultry manure increased Modified Morgan P more than other manures, which in turn were higher than $KH_2PO_4^-$. Laboski and lamb (2003) observed that swine slurry increased STP levels more than fertiliser after 1 and 9 months of incubation. It was hypothesised that because the organic P fractions in manure are composed of mainly high molecular weight compounds, such as DNA, polyphosphates and inositol phosphate. They were absorbed onto the soil surface and contribute to the release of inorganic P bound to the surface (Fuentes *et al*, 2006).

In a glasshouse study, Leytem and Westermann (2005) found that, solid swine manure, swine slurry, and dairy slurry increased barley P uptake and dry matter (DM) yield more than fertiliser, however beef manure and composted dairy manure increased P uptake and yield less than fertiliser. However inorganic fertiliser increased P uptake and pasture yield more than pig beef and sheep manures (Gracey, 1984).

From the glasshouse and incubation studies it is difficult to make an informed comparison about the value of organic amendments compared to inorganic P sources due to the variation in materials, soils and rates in each study.

Field studies have shown inorganic sources release more P than organic amendments. Montevallo *et al,* (1989) found soil with Bray 1-P levels ranging from 17 to 82 mg P $kg^{-1,}$ average apparent recovery of manure P in corn biomass was 14%. However fertiliser P recovery was 23% suggesting manure was 60% as available as fertiliser P with regard to crop utilisation. Eghball and Power (1999) studied a soil with a very high Bray 1-P level of 69 mg P kg^{-1} and found fertiliser P use efficiency was 40% greater when compared with raw and composted beef feedlot manure at various application rates (12–25%) for corn. Sharpley *et al,* (1996) suggested greater release of P from inorganic fertilisers was because the P in them is more water-soluble than organic amendments.

However other studies have found manure P to be as available as fertiliser P. Paschold *et al,* (2008) found swine slurries applied to two sites, with a Bray 1-P level of 7.7 mg kg^{-1}, resulted in a similar corn yield to fertiliser. Bergström and Kirchmann, (2006) reported swine slurry applied at 120 kg P ha^{-1} resulted in barley P uptake similar to fertiliser applied at 40kg P ha^{-1}. Lower slurry application rates resulted in lower P uptakes. However this reduced uptake is as a result of inefficient N supply to the crop at lower application rates. Maher, (2005) assessed the effects of spent mushroom compost (SMC) and GW on the performance of onions. The soil P level was increased by 0.28mg for SMC and 0.04 mg for GW per ton of compost. Plant P uptake from GW compared to SMC was approximately 80% at 25 t ha^{-1}, and 79% at 50 t ha^{-1}, and 59% at 250 t ha^{-1}, and was 84% in relation to superphosphate.

Kluge (2003) reported that supplying 6-10 t ha^{-1} $year^{-1}$ of biocompost gives an absolute supply of P at around 13-17kg of P. The efficiency of this is 30-50%. In the application year the efficiency of the compost P is 15-20%, however the efficiency is 40-50% over the next 10-20 years. A good correlation was found between compost P supply and P available in the soil. It was concluded that P supply in the year of application from compost is less than that of mineral fertilisers.

From the information outlined above it is difficult to draw any meaningful conclusions about the value of organic amendments compared to inorganic sources as the

experiments vary in so many aspects and there is no standard by which to compare them. However what is evident is that organic amendments offer a useful resource for recycling nutrients in agricultural systems, and it is well worthwhile investigating this further in order to improve understanding about the availability of P following addition to soil, and its influence on plant yield and uptake.

1.8 RB209 P index

The RB209 fertiliser manual (Defra, 2010), outlines a range of target N, P and K and Mg indexes which are a recommended index for how much fertiliser to apply in order to achieve the optimum nutrient status for growth of a required crop. The P index is based on Olsen P values (mg P l^{-1}) between 0 and >280 and the K index is based on Ammonium nitrate values (mg K l^{-1}) between 0 and >3600. Indexes range between 0 and 9 respectively (Table 1-3).

Table 1-3: Details of the RB209 P and K index

Index	Phosphorus (Olsen P (mg l^{-1}))	Potassium (Ammonium nitrate (mg l^{-1}))
0	0-9	0-60
1	10-15	61-120
2	16-25	121-240
3	26-45	241-400
4	46-70	401-600
5	71-100	601-900
6	101-140	901-1500
7	141-200	1501-2400
8	201-280	2401-3600
9	> 280	> 3600

Adapted from RB209 fertiliser manual (Defra, 2010)

1.9 Techniques for measuring soil P availability

Numerous soil tests are available to measure P availability in soils. Different tests exist to suit different soil types, and each has its own limitations. It has been documented that a description of soil P must include an intensity factor (*I*) a quantity factor (*Q*) and a capacity factor ($\Delta Q/ \Delta I$), as well as rate and diffusion factors (Dalal and Hallsworth 1976). (*I*) is solution P; (*Q*) is the labile portion of the solid phase, which can be

estimated by soil test extraction techniques. However Stevenson and Cole, (1999) highlight a key limitation of extraction techniques. The capacity of the soil system to maintain P concentration in the solution phase as P is removed by plants ($\Delta Q/ \Delta I$) is not determined, nor is the rate of soil solution replenishment from solid phase forms. As highlighted above the forms of P in the soil solution include organic and inorganic P, and within the inorganic P range, P forms are mainly associated with Al, Fe and Ca, the distribution between these forms is heavily dependent on pH. As a result of this the extraction technique used to measure plant available P will be determined by pH. A summary of soil tests is highlighted below.

1.9.1 Chemical extraction procedures

Olsen P - An extractant of 0.5M sodium bicarbonate ($NaHCO_3$) solution at pH 8.5 is used (Olsen *et al*, 1954). The solubility of calcium phosphate is increased because of precipitation of calcium (Ca^{2+}) as calcium carbonate ($CaCO_3$), in calcareous, alkaline or neutral soils (Hanlon *et al*, 1999). In acid soils P concentration in solution increases when Al and Fe phosphates such as variscite and strengite are present (Lindsay and Moreno, 1960). Secondary precipitation reactions are reduced in acid and calcareous soils because iron (Fe), aluminium (Al) and Calcium (Ca) concentrations remain low in the extract (Olsen and Dean, 1965). The 0.5M ($NaHCO_3$) can also lead to solubilisation of a portion of the soil organic P, which is regarded as a quantitative measure of the potential contribution of soil organic P to plant uptake (Stevenson and Cole, 1999). A limitation of this technique is that it was initially developed for alkaline soils, therefore on acidic soils pH <5.5, the test can give a less accurate assessment than on alkaline soils overestimating plant available P.

Mehllich extraction-Various forms of Mehlich extraction have been developed. Mehlich 1 (Mehlich, 1953) extraction is primarily used for soils, which have exchange capacities of less than 10 milliequivalents per 100 grams; it is used on acid soils and is unsuitable in alkaline soils. A reagent of 0.05 N HCl and 0.025 N H_2SO_4 is used. Mehlich 3 is an adaptation of Mehlich 2 (Mehlich, 1984), and is designed to be used across a wide range of soil properties, from acid to basic (Hanlon *et al*, 1999) a reagent of (0.2 N CH_3COOH—0.25N NH_4NO_3-0.015N NH_4F-0.013N HNO_3-0.001 M EDTA) is used.

Morgan extraction -Was developed by Morgan (1941); it was used primarily for determining P content in acid soils with cation exchange capacities of less than 200 milliequivalents per 100 grams. The extracting reagent is (0.72 N NaOAc + 0.52 N CH_3 COOH) well buffered at pH 4.8 and when used in conjunction with activated carbon yields clear and colourless extracts (Hanlon *et al*, 1999).

Bray P1extraction- The Bray P1 extraction technique (Bray and Kurtz, 1945) (0.025 N HCl: 0.03 N NH_4 F) is designed to remove easily acid-soluble P forms, mainly Ca phosphates, with a portion of Fe and Al phosphates. The technique is based on hydrogen (H^+) ions solubilising soil P, and the ability of the fluoride (F^-) ion to lower the activity of aluminium (Al^{3+}), and to a lesser extent calcium (Ca^{2+}) and iron (Fe^{3+}). Limitations of this procedure are in that the method is normally limited to soils with a pH value less than 6.6 when the texture is silty clay loam or finer, as when these soils are calcareous or have a high degree of base saturation the solubilising ability of the extractant is lowered (Hanlon *et al*, 1999). Low estimates are obtained with calcareous soils due to neutralisation of the acid by $CaCO_3$ (Stevenson and Cole, 1999).

Ammonium Bicarbonate –DTPA extraction- The reagent for extraction is 1M ammonium bicarbonate (NH_4HCO_3) in 0.05M DTPA adjusted to a pH of 7.6 (Stevenson and Cole, 1991) as the solution is shaken the pH increases due to carbon dioxide (CO_2) evolution consequently a fraction of the bicarbonate (HCO_3^-) changes to carbonate (CO_3^{2-}). The CO_3^{2-} ions precipitate calcium from the labile calcium phosphates which in 15 minutes of shaking dissolves labile phosphorus.

1.9.2 Other tests

Anion exchange membrane (AEM) - Resin P -AEM strips are soaked in 0.5M HCl for 2 days rinsed in deionised water, and then transferred to 0.5 M $NaHCO_3$ to convert to HCO_3^- form. The AEM is then placed in a soil solution which has been air dried and sieved (<2 mm). 1g of soil in 40 ml deionised water is shaken for a range of hours (2-65) with the AEM strips in HCO_3^- form. Strips are then rinsed with deionised water prior to elution with 0.1 *M* H_2SO_4 for 15 minutes on a reciprocating shaker (Stevenson and Cole, 1999)

1.9.3 Limitations of soil P tests

Currently the most common and widely used soil tests to measure soil P are chemical extraction procedures. This is mainly due to their simplicity. However authors have reported that there are problems associated with these.

There is no one test which is universally applicable to all soil types, different tests are used for different soils. Stevenson and Cole, (1999) explain that when selecting a method, discretion must be exercised as a given soil property (notably pH) can negatively affect the performance of the test. Bates (1999) reported that in a comparison of five tests, correlations between extractable P and P uptake by corn were highly variable and strongly affected by pH. Menzies *et al*, (2005) reported that extraction solutions are used to solubilise P pools, which are available to plants. However the presence of an ion which competes with P for adsorption sites on the soil can result in displacement of adsorbed P and prevents re-adsorption of solubilised P. F^- accomplishes this in Bray and Mehlich extraction where low pH enhances the competitive ability of the anion (Hingston, 1972). A solution containing bicarbonate can extract relatively stable forms of P which are not plant available. Menon, (1990) and Menzies *et al*, (2005) have explained that the bicarbonate extraction methods, such as Olsen P and Colwell P, were originally developed for calcareous soils, however they are suitable for use on both acid and alkaline soils. However Schuman, *et al*, (1988) reports that Olsen P is less effective than acidic extractants for predicting P response on acid soils. In calcareous soils Colwell P values can be relatively high (>2% $CaCO_3$) despite such soils having a poor P nutritional status. Extractant solutions such as Colwell, and resin P tests use a wide range of soil to extractant (or water in the case of resin). These ratios are not representative of the soil: water ratios found in field conditions. Soil responds to diluting solutions by replenishing the solution with P from the solid phase (Mason *et al*, 2008).

Incomplete or inaccurate understanding of the P content of soil which is in a plant available form can lead to P application practices which are inefficient, and do not fully utilise resources to their full potential. This can cause both economic and environmental damage. It is therefore important to improve efficiency of resource use in order to reduce these negative impacts.

An accurate method of determining plant available P in soil can therefore improve understanding of the potential of the resource applied, and can thus help to provide a resolution to the environmental and economic issues outlined above.

1.10 DGT

The Diffusive Gradients in Thin-films (DGT) technique has been described by previous authors (Menzies *et al*, 2005; Mason *et al*, 2010; Mcbeath *et al*, 2007) as accurately measuring soil P which is in a plant available form. DGT is based on the diffusional characteristics of elements through a hydrogel and the sorption to the binding layer (Zhang and Davison, 1995). Specifically, the hydrogel layer is utilised to control the transport of elements in solution, by diffusion to a binding layer, which is a Fe oxide gel for P analysis**.** The technique was initially developed to measure labile species in freshwater and marine systems (Davison and Zhang, 1994) and was later developed for deployment in soils (Harper *et al*, 1998). DGT induces diffusion of ions, which are continuously accumulated in proportion to their bulk concentration in soil solution in the DGT device. The total amount of ions accumulated in a given time is measured after retrieval of the DGT device and used to calculate the concentration of labile species present in bulk solution during its deployment (Zhang *et al*, 1995). The DGT device utilises a three layer system (Figure 1-6): 1) a Fe oxide gel layer, 2) a diffusive gel layer 3) a filter membrane. The purpose of the Fe oxide gel is to act as a sink for the labile species which diffuse through the diffusive gel layer. The filter membrane protects the diffusive gel layer from particles. The gel layers are arranged so that transport of ions is solely by molecular diffusion. Ions diffuse from the soil solution, through the filter membrane and the diffusive gel layer. The diffusive and Fe oxide gel layer are made from polyacrylamide, which is a hydrous polymer consisting of acrylamide-polymer chains (Chramback, 1985). It contains 95% water, and its matrix is open to movement at the molecular scale, thus creating an environment where transport of ions can take place through diffusion (Zhang and Davison, 1999).

The induction of diffusion by the Fe oxide sink attempts to mimic P uptake by plant roots, as during plant uptake, removal of P from the soil solution, promotes resupply from the soil solid phase. The diffusive gel controls the flux of P, in a similar way to plants control P flux (Six *et al*, 2012a; Mason *et al*, 2010). The P taken up by the Fe

oxide gel is then eluted and measured. Subsequently calculations are conducted based on Fick's first law of diffusion (Zhang and Davison, 1999), to determine the DGT measured P concentration.

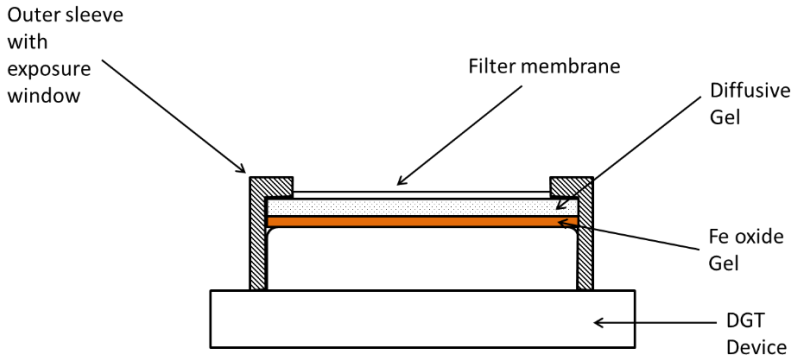


Figure 1-6: Schematic representation of the DGT assembly, demonstrating how gels are layered in the DGT device.

Most information regarding DGT for P measurement focuses on its value as a tool to predict P deficiency. Menzies (2005) tested tomato yield response to the addition of P fertiliser for 24 soils in a glasshouse experiment. DGT results gave a very good separation of soils on which tomatoes showed yield response from soils with no response. This experiment derived a critical value of 2.14 µg of P per sampler (24hr deployment, 0.8mm hydrogel) corresponding to a C_{DGT} of ~4 µM (Degryse *et al*, 2009).

In a study involving P deficiency under field conditions (Mason *et al*, 2008) found a critical C_{DGT} of ~ 3.2 µM for which no response of wheat to P application was observed in field trials above this value. Whereas for the same plant species under laboratory conditions the critical value was 1.2 µM (McBeath *et al*, 2007). This supports the theory put forward by (Degryse *et al*, 2009) that lower critical DGT concentrations are to be expected for field conditions.

Mason *et al*, (2010) carried out experiments under field conditions on 35 soils and found that the DGT method predicted plant responsiveness to applied P more accurately than Colwell P and resin P at sites where maximum yields were reached with P rates used. C_{DGT} explained 74% of the variation in response for both early dry matter and grain, compared to 7% for early dry matter and 35% for grain using the resin P method. No significant relationship could be obtained for Colwell P.

Other studies have focussed on comparing DGT to extraction procedures, Six *et al*, (2012a) conducted an isotopic dilution study to assess the performance of DGT versus conventional soil P tests in tropical soils. They assessed the requirement of DGT in comparison to Olsen, Colwell, Bray-1, Mehlich-3, ammonium oxalate, anion exchange membranes and 0.01*M* $CaCl_2$ solution. It was established here that DGT only measures P from a pool, which is plant accessible, whereas other soil P tests extract a fraction of P, which was not available to the plant (Maize (Zea mays L.).

Six *et al*, (2012b) also conducted a study to assess maize and rice response to P application and assessed the performance of DGT versus conventional soil P tests in tropical soils. Shoot dry weight increased with increasing P application by factors 2 to 90. P application required to reach 80% DMY ranged from 20 to 580 mg P kg^{-1}. DGT and $CaCl_2$ extraction explained relative yield of maize amongst soils better (R^2=0.8 and 0.69 respectively), than Olsen, Colwell, Bray and Mehlich, Ammonium oxalate and resin extractions (R^2 <0.53). However the opposite trend was found for rice. Where Olsen, Colwell, Bray and Mehlich, ammonium oxalate and resin extractions (R^2~0.7) and DGT (R^2=0.59) and$CaCl_2$ (R^2=0.12). It was therefore suggested that in tropical P deficient soils intensity based indices such as DGT and $CaCl_2$ are a better indication of maize requirements than extraction solutions. However this is not the case with rice suggesting diffusion of P as measured by DGT is not the main factor explaining rice P uptake.

Tandy *et al*, (2012) conducted a study into the use of DGT for prediction of plant available copper zinc and phosphorus in agricultural soils. DGT predicted plant uptake of P (R^2=0.72) whereas conventional extraction methods (no relationship) and soil solution (R^2=0.43) performed poorly.

Mason *et al*, (2010) conducted a study to predict wheat response to an application of phosphorus under field conditions using DGT and extraction methods (Colwell and resin). Regression analysis with early DMY and grain yield response showed that DGT measured plant responsiveness to applied P (R^2=0.74 for DMY and grain) more accurately than resin P (R^2=0.07 and 0.35 for DMY and grain respectively) and Colwell

P (no significant relationship) when maximum yield was reached. The critical DGT threshold for C_{DGT} was 255µg L^{-1} and 66µgL^{-1} for early DMY and grain respectively.

In the studies described above, comparisons between DGT and extraction techniques have generally shown a better correlation between DGT and yield response than extraction techniques. Menzies *et al,* (2005) found that P accumulation by DGT was strongly correlated with soil solution P concentration and anion exchange resin – extractable P, but showed poor correlation with Colwell or Bray 1 –extractable P. Optimum deployment time was 24 hours when a comparison was made between 4, 8, 16, 24 and 48 hours. The DGT P accumulation rate of 3.62 x 10^{-7} to 4.79 x 10^{-5} mol s^{-1} m^{-3} for the soils tested was comparable to the uptake rate of roots of tomato plants that were adequately supplied with P (2.25 x 10^{-5} mol s^{-1} m^{-3}).

However in an experiment predicting the response of wheat to liquid and granular phosphorus fertilisers in Australian soils McBeath *et al,* (2007) found that five soil test procedures (Bray, Colwell, resin, isotopically exchangeable P and DGT) all provided a reasonable prediction of dry matter responsiveness to applied P either as liquid (R^2=0.7-0.88) or granular P (R^2=0.5- 0.82), with the resin P (R^2=0.88) having a slightly greater predictive capacity on the range of soils tested.

Mason *et al*, (2008) investigated the chemical constraints to the measurement of phosphorus in soils using DGT and resin methods. It was found that exposure to ranges of anion (Cl^- (15,000 mg l^{-1}), NO_3^- (1200 mg l^{-1}), SO_4^{2-} (600 mg l^{-1}) and HCO_3^- (93 g l^{-1})) concentrations relevant to agricultural soils had minimal effect on P recoveries using DGT.

DGT performance for measuring P has been found to be unaffected by pH within the range 3-9 (Mason *et al,* 2005). This highlights that DGT performs well under acidic conditions and its versatility gives it an advantage over other tests, which are limited to a specific soil type.

Previous authors (Menzies *et al*, 2005; Mason *et al*, 2008), have stated that the advantage of DGT over extraction techniques is that it can mimic plant uptake by creating a well-defined sink for P, lowering the concentration in solution phase prompting re-supply from the solid phase. DGT uses a ferrihydrite binding agent (Fe

oxide gel), which is highly specific to P. Therefore it has an advantage over non-specific anion exchange resins, as P sorption is unaffected by anions such as sulphate, bicarbonate, nitrate and chloride, which are present in solution at concentrations relevant to agricultural soils. DGT is not based on an equilibrium process; it instead integrates solution concentrations of P with the P resupply capacity of the soil (Mason *et al*, 2008).

The information above presents DGT as an attractive technique for the measurement of plant available P in soil. However studies have been limited to soils which have received application of inorganic P, therefore testing is required to establish whether it can accurately measure plant available P in soil following addition of organic amendments. If so, it can potentially be used to enhance understanding of the contribution of such resources to plant available P forms in soil, thus improving understanding of how such resources could be best utilised in agriculture to reduce reliance on inorganic P and reduce impacts associated with its use. For clarity, in the remainder of this document extractable P will be used when referring to measurements conducted with extraction techniques. DGT measurements will be characterised as available P.

1.11 Knowledge gaps

- DGT has been successfully used on soils to measure plant available P. However it has never been used to measure P availability following addition of organic amendments.
- Most studies about addition of organic amendment to soil focuses on aboveground biomass production and P uptake, there is a lack of information on how organic amendments influence plant root growth and P uptake.
- There is a lack of information about rates of mineralisation of P in soil following addition of organic amendments.
- There has been no work conducted to understand the portion of soil P available for resupply compared to that available by diffusive supply.
- There is a lack of information about the kinetics of P release from solid phase to solution in studies into DGT measurements of P in soil.

1.12 Research Aim

To evaluate changes in soil P availability, following the addition of organic amendments, cattle farmyard manure (FYM), green waste compost (GW), cattle slurry, (SLRY) and superphosphate (SP) using the DGT technique, in two contrasting soil P indexes.

1.13 Objectives and Hypotheses

Objective 1: To produce and use DGT in a consistent and reliable manner across a range of scales, on soils used in this study.

Objective 2: To determine P availability patterns in soils which have historically received application of organic amendments, and the subsequent impact on ryegrass (*Lolium perenne L.*) yield and P uptake, with and without addition of further treatments (FYM, GW, SLRY and SP) at agronomic application rates.

Hypotheses:

H1 A build-up of P available by diffusive supply, from historic treatment additions and subsequent availability from fresh treatment additions will be demonstrated by DGT.

H2 Historical treatment additions are more important at determining yield and P uptake than fresh additions.

Objective 3: To determine P availability patterns in soils deficient in plant available P following addition of the aforementioned treatments, at agronomic application rates, and determine the impact on plant yield and P uptake.

Hypotheses:

H3 DGT can detect changes in P available by diffusive supply following addition of different treatments and subsequently following lysis of microbial cells on a soil deficient in P.

H4 DGT will provide a more accurate indication of plant P availability than organic amendments in a soil deficient in P.

Objective 4: To investigate how organic amendments perform compared to SP in the aforementioned soils.

Hypothesis:

H5 P measurements using DGT will be lower from organic amendments than superphosphate.

Objective 5: To investigate the effects of treatment addition to each of the aforementioned soils on soil kinetic parameters, in order to understand how kinetic limitations influence P supply.

Hypothesis:

H6 DIFS simulations of soil kinetic parameters will provide additional information about how treatments influence P resupply from solid phase to solution following DGT deployment.

1.14 Experimental soils

It is necessary to use two different soils with different treatment application histories, to understand changes in P availability following the addition of treatments using the DGT technique. Soils in this experiment had vastly differing P application histories and subsequently P indexes.

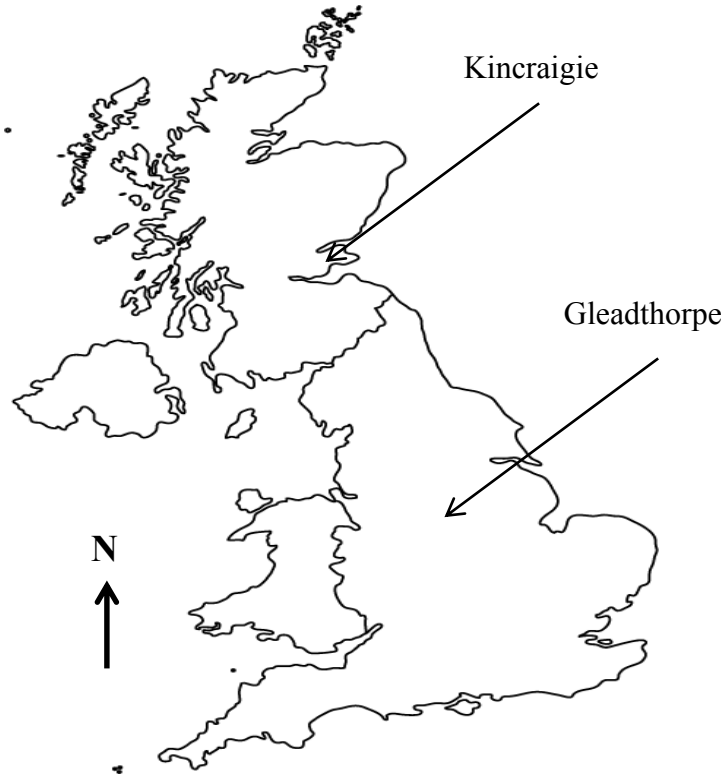


Figure 1-7: Map of the United Kingdom, highlighting sources of soils used throughout the study.

The first soils were taken from an existing field trial (ADAS –QC) (Bhogal *et al*, 2011), at Gleadthorpe farm, Nottingham, England (Figure 1-7), which had received historical application of organic amendments. Analysis of these soils was important in meeting objectives 2, 4 and 5. Soils had a well-documented application history, which made it possible to assess the influence of this on plant available P. The P index of the soils was 3-4.

The second soils were taken from Kincraigie farm, Strathmiglo, Fife, Scotland (Figure 1-7) which was initially deficient in P, with no recorded history of organic amendment applications. These conditions are necessary for an accurate investigation of how plants respond to increasing soil P following treatment addition. These soils had a P index of 0. Analysis of these soils was important in meeting objectives 3, 4 and 5.

1.15 Outline methodology

The methodology used to meet the aim and objectives of the study is outlined in Figure 1-8. It was necessary to establish a reproducible methodology for DGT deployment on

soils used in this study. The method development chapter was also intended to act as a reference point for methods used in subsequent chapters.

Incubation and glasshouse studies were used for each of the two soils used in this project. Work was divided into two sections, based on the soil used. For experiments using soils from Gleadthorpe analysis was undertaken with and without additional application of corresponding treatments. The incubation experiment was established to identify differences in P release between treatments, from the historical treatment additions, and from fresh treatment additions at two agronomically relevant application rates. Pot experiments were established to understand how repeated application of the different treatments influence plant available P in soil and plant characteristics (dry matter yield (DMY), total phosphorus uptake (TP_{uptake})).

Kincraigie soils, which are deficient in P, were used to quantify the response of ryegrass to increasing P addition from different treatments. Incubation studies established how fresh application of treatments influence C_{DGT} measurements in soil and the relationship with microbial biomass P. The pot experiment was set up to understand how fresh application of treatments influence C_{DGT} measurements and plant characteristics (root and shoot (DMY) and (TP_{uptake})).

Diffusion induced fluxes ion soils (DIFS) studies were established to understand how addition of treatments to soil affects the quantitative relationship for the distribution of P between solid and solution phases, to the DGT device. This was conducted on soils from pot experiments for both Gleadthorpe and Kincraigie soils.

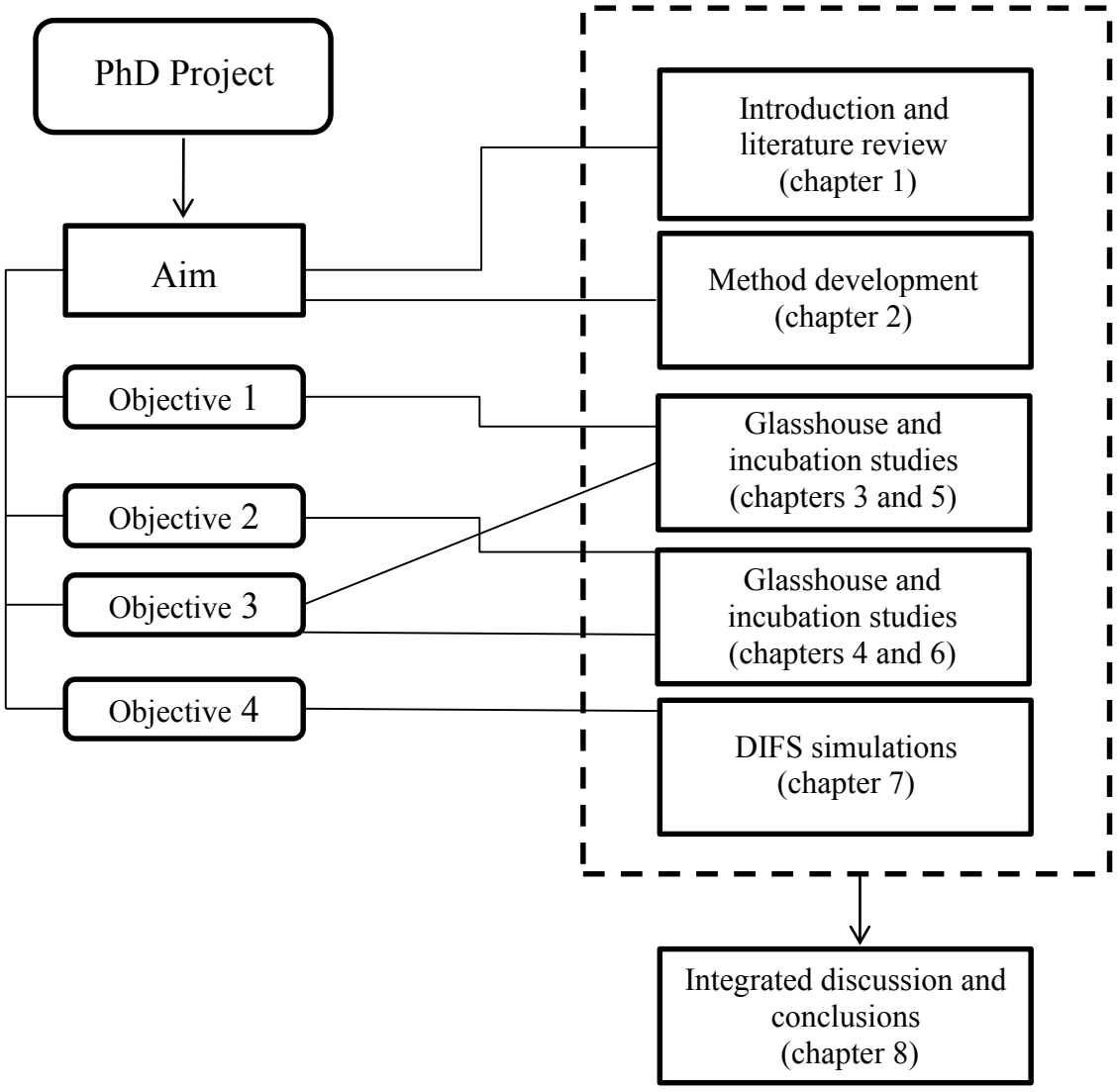


Figure 1-8: The methodology used to meet the aim and objectives of the study.

1.16 Thesis structure

Chapter 1 is a brief introduction and background providing context for the work. It is also review of the literature currently available regarding P availability following application of different treatments to soil and methods used for its measurement. **Chapter 2** outlines the methodology for deployment of DGT on soils in this experiment. **Chapters 3 and 5** are incubation and pot experiments respectively for Gleadthorpe experiments. **Chapters 4 and 6** are incubation and pot experiments for Kincraigie soils respectively. **Chapter 7** is DIFS work for Gleadthorpe and Kincraigie

pot experiment trials. **Chapter 8** is an overall analysis and integrated discussion of the main research findings from all experiments.

2 Method development

2.1 Introduction

The DGT technique has been tested extensively, on a range of soils to measure heavy metal availability (Hooda *et al*, 2001). However less work has been conducted on soils to measure P availability. Menzies *et al*, (2005) and Mason *et al*, (2008) have conducted the most relevant work on P availability in soil. Menzies *et al*, (2005) assessed the effects of exposure time, soil water content and solution P concentration on DGT sampler uptake. Soils used in this experiment vary significantly in their Olsen P content (from 6 to 60 mg P kg^{-1}); therefore it is important to determine optimum deployment conditions, for accurate P measurement.

The use of DGT as a technique for measuring P in soil is in its relative infancy. Thus, it has never been produced or used at Cranfield University. It was therefore important to establish a consistent and reproducible method of producing and testing components (DGT gels) of the DGT device, before testing on soils could be carried out.

DGT is typically deployed in soils which have been removed from their location, dried, ground and homogenised, before being re-wet. A principal advantage of DGT is that it can be deployed directly on soil, representing pH, and moisture content which is more representative of field conditions than the more commonly used extraction solutions such as Olsen P. Therefore, as DGT is based on diffusive supply from the soil to the device, it is useful to use this method as it provides information which cannot be gained using chemical extraction procedures, by simulating plant uptake by diffusion.

The overall aim of this chapter is to establish a reproducible methodology for DGT deployment in soils used in this study. The chapter is also intended to act as a reference point for methods used in subsequent chapters.

2.2 Methodology

2.2.1 Preparation of DGT devices

The DGT devices were prepared using a two stage process where an Fe-oxide and ion-permeable diffusive gels according to the standard procedures (Zhang and Davison, 1995). In stage one a DGT gel solution was made which contained, 15% by volume acrylamide (40%, Electron, Boehringer), 0.3% by volume patented agarose-derived cross linker (2%, DGT Research Ltd, Lancaster, UK) and Milli-Q (MQ) water. Following ion-permeable diffusive

gels were made using 10 ml of gel solution and mixing with 70 µl of ammonium persulphate solution (10%) and 25 µl of TEMED (*N,N,N'N'*-Tetramethylethylenediamine, 99%, Electron) as a catalyst. The gels were cast between two glass plates; the thickness (0.92 mm) was achieved by fitting a plastic spacer around three edges. Plates were placed in an oven for 1 hour at a temperature of 42~46 ºC in order to set. Gels were then hydrated in ultra-pure MQ water which was changed 3-4 times during the 24-hour hydration period. After hydration, the pH of the solution with gel was measured to make sure it was between 6.5 – 7. Gels were stored in 0.01 M $NaNO_3$ solution until use. In stage two Fe oxide gels were produced. To make these, firstly Fe-oxide (Ferrihydrite) slurry was produced. This was achieved by dissolving 8g $Fe(NO_3)_3 \cdot 9H_2O$ into 200 ml deionised water. Then separately 1M NaOH was produced. The NaOH solution was then slowly titrated into the Fe $(NO_3)_3$ whilst stirring until the pH reached 6.8. The slurry was then left to settle and excess water removed with a pipette. The slurry was then washed 2 times with MQ water and excess water removed. This was done by pipetting the slurry onto tissue paper, and scraping the slurry off with a spatula once water had transferred to tissue paper.1.5g of the slurry was then added to a sterile tube and mixed with 5ml gel solution. The solution was mixed vigorously before 30 µl of ammonium persulphate solution (10%) and then 8 µl of TEMED (*N,N,N'N'*-Tetramethylethylenediamine, 99%, Electron) was added. The gels were cast between two glass plates, the thickness (0.92 mm) was achieved by fitting a plastic spacer around three edges then placed in an oven for 1 hour at 42~46 ºC in order to set. Gels were then hydrated and stored in ultra-pure MQ water (to allow impurities within the gel to diffuse out). The DGT devices (Figure 1-6) consist of a round base, a Fe Oxide gel layer (0.25cm^3), an ion-permeable diffusive gel layer (0.92mm), an 0.45-µm hydrophilic polyethersulfone membrane (0.14mm) to stop soil particles sticking to the gels and a cap with a 2.54-cm^2 exposure window that holds all layers together (Warnken *et al*, 2004). The Fe oxide gel acts as a sink, inducing a flux of P ions from the soil through the diffusive gel.

2.2.2 Deployment and measurement

2.2.2.1 Testing gel quality and C_{DGT} calculation

This section is based on the principal outlined (Zhang *et al*, 1998). 2L of deionised water was mixed into a 3L plastic container, with 4ml of 100 ppm (KH_2PO_4) solution to make up a 200 ppb immersion solution (Figure 2-1). The container was placed on a magnetic stirrer and stirred for 1 hour. Assembled DGT devices were then fixed to a cylindrical holder and placed

in the immersion solution, with the plane of the filter vertical and parallel to container walls, facing towards the inside of the container. The solution temperature was then measured, whilst the solution was stirred, to establish the P diffusion coefficient in water. An aliquot of immersion solution was then taken for analysis of solution P concentration at the start of the experiment. After 4 hours, another aliquot of immersion solution was taken for analysis and temperature measured. DGT units were removed from the solution and rinsed with MQ water. DGT devices were then dismantled, and the Fe-oxide gel added to a sterile sample tube with 10ml of 0.25M H_2SO_4 solution. The solution was then shaken on a side-to-side shaker at 300rpm for two hours prior to analysis. The P concentration of the elution solution and the, DGT measured concentration (C_{DGT}) are then measured using the following principle.

The Fe oxide gels were eluted in a 10 ml solution of 0.25M H_2SO_4, and placed on a side-to-side shaker at 300 RPM for 2 hours. The P concentration in the solution was then measured using the molybdenum blue batch method (ISO, 8556:1986). The mass of P in the Fe oxide gel (M) can be calculated using Equation 2-1:

$$M = C_e(V_{acid} + V_{gel}) / f_e \qquad \textbf{Equation 2-1}$$

where Ce is the P concentration, measured by the spectrophotometer in the 0.25M H_2SO_4 elution solution (μg l^{-1}), V_{acid} is the volume of H_2SO_4 used for elution (10 ml), V_{gel} is the volume of Fe oxide gel (V_{gel} = 0.25 cm^2), and f_e is the elution factor for each P, typically 1. The time averaged concentration of phosphorus at the interface of the soil and the DGT device can be obtained using Equation 2-2:

$$C_{DGT} = M \times \Delta g / (D \times A \times t) \qquad \textbf{Equation 2-2}$$

where Δg is the thickness of the diffusive gel (0.96 mm) plus the thickness of filter membrane (0.014 cm), D is the diffusion coefficient of P in the gel, t is the deployment time (s) and A is the exposed area of the gel (A = 2.54 cm^2).

The P concentration in the immersion solution (10ml aliquot) underwent the same procedure as the elution solution described above, however no 0.25M H_2SO_4 was added. The P concentration in the solution was then measured using the molybdenum blue batch method (ISO, 8556:1986). In addition three blank DGT gels were eluted to as a means of quality control and three elution solutions were measured without anything added.

The two concentrations were then compared. If the solution concentration and C_{DGT} were within 10% of each other, the gel produced for DGT analysis was deemed acceptable for use. If not tests were conducted again, and if still not within 10% gels were disposed of.

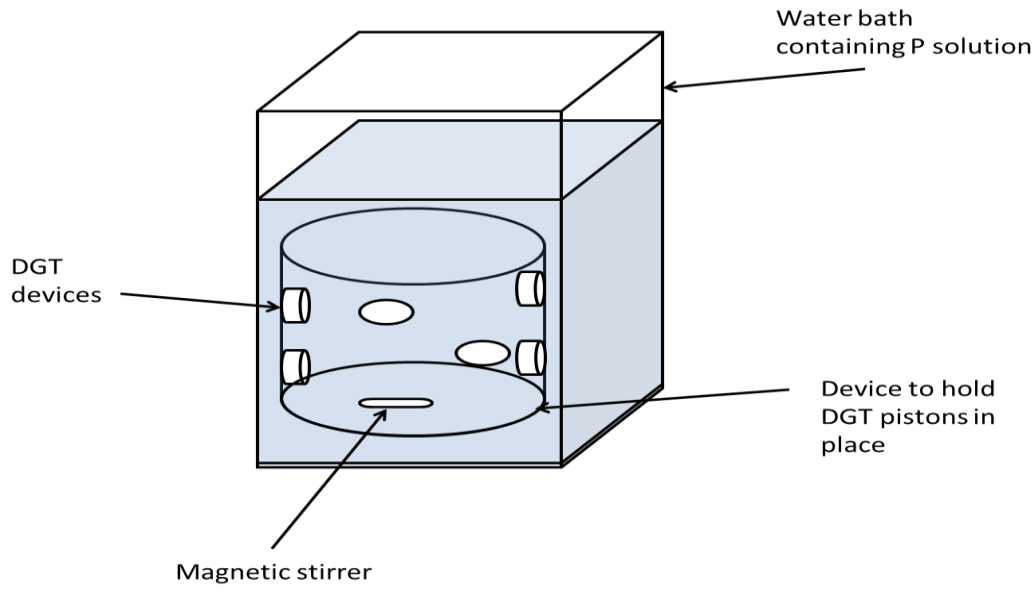


Figure 2-1: Experimental setup for testing DGT gels in water.

2.2.2.2 Deployment in soil

To prepare the soil sample for use with DGT, 30g of air-dried and 2 mm-sieved soil samples were brought to 60% maximum water holding capacity (MWHC) in a petri dish and incubated for 2 days, then raised to 100% MWHC for 24 hours prior to DGT deployment. DGT devices were then placed on the soil twisting gently to complete contact between the filter membrane of the device and the soil. Deployments were carried out for 24 hours at 25±1ºC. DGT devices were then removed from the soil and washed with MQ water to remove soil particles before dissembling (Nolan *et al*, 2005). C_{DGT} was then measured using the procedure described in Section 2.2.1.

The methodology described above represents the standard procedures throughout the thesis. However in order to arrive at this standard methodology, a number of deployment conditions were tested as described below.

2.2.3 Soil analysis

The soils used in this section were collected from Gleadthorpe farm, an ADAS experimental site in Nottinghamshire and has had historical application of FYM, GW, SLRY and ammonium nitrate fertiliser (Control ADAS-QC) for four years, (Bhogal *et al*, 2011). Full details of the experimental soils are provided in **Chapter 3**. This soil had been under arable production and was categorised as loamy sand Table 2-1. Each soil was collected from the top 30 cm of each field brought to the lab, air dried and ground to pass through a 2mm sieve. Standard soil characteristics were determined in the laboratory prior to setting up the experiment. Soil texture was measured using the pipette method (1974; BS 1377 Part 2.0, 1990). The soils maximum water holding capacity was determined in the laboratory based on BS 7755 Section 5.5, (1999). This was carried out in order to work out how much water was required to maintain the soil at appropriate water content for each experiment. In all experiments within this chapter, unless otherwise stated four replicates were used for each experiment.

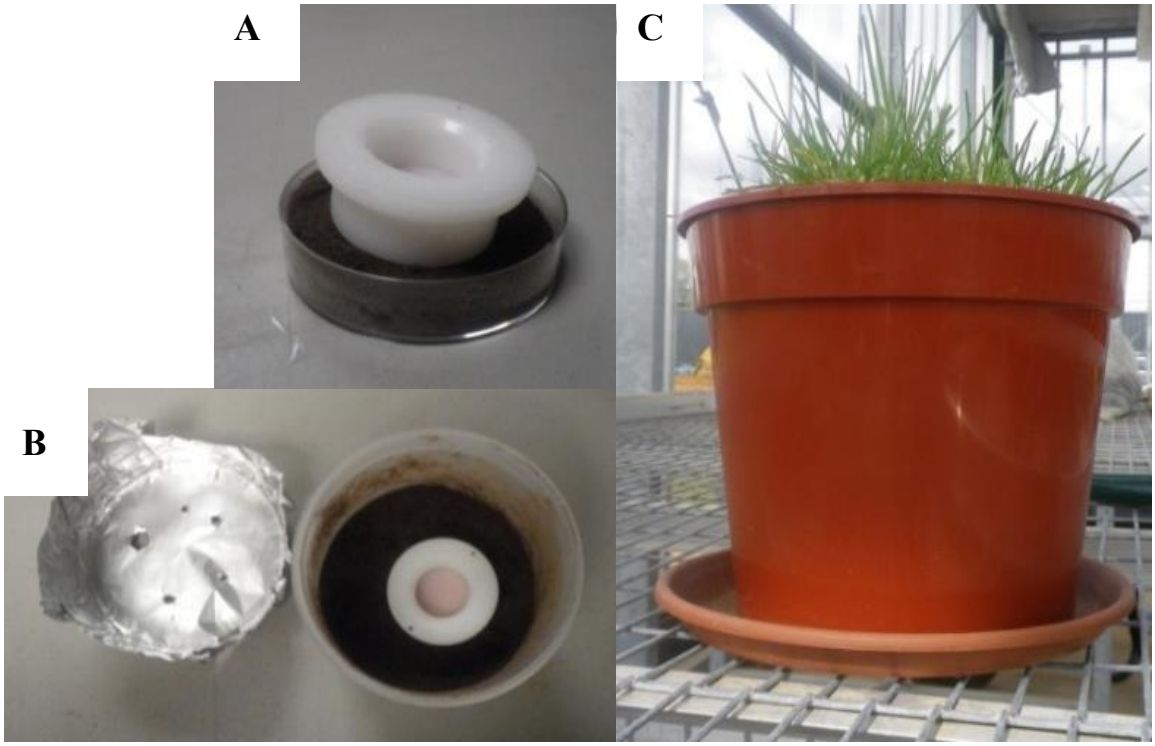


Figure 2-2: Photographs of deployment mediums used a= petri dish b= incubation pot c= glasshouse pot.

Table 2-1: Field capacity, textural analysis and bulk density of the soil used for the incubation study. Data are means (*n*=*3*). Brackets represent ± standard error.

Determination	Treatments historically applied			
	Control ADAS-QC	SLRY	FYM	GW
Field Capacity (%, ww^{-1})	29.6(0.3)	27(0.4)	29.2(0.4)	28.3(0.3)
Bulk density (g cm^{-2})	1.29(0.01)	1.21(0.01)	1.26(0.01)	1.28(0.01)
Textural analysis (%)				
Sand	88.3	88.3	88.3	88.3
Silt	7.8	7.8	7.8	7.8
Clay	4	4	4	4

Table 2-2: Soil analysis conducted prior to experiment. Data are means (*n=3*). Brackets represent ± standard error.

Determination	Control ADAS-QC (Plots)	GW (Plots)	SLRY (Plots)	FYM (Plots)	Method
Total N (%)	0.82(0.03)	0.9(0.09)	0.84(0.05)	0.79 (0.06)	BS EN 13654-2 (2001)
Total C (%)	11.7(0.6)	13.1(0.6)	11.3(0.7)	12.2(0.8)	BS 7755 Section 3.8 (1995)
C:N	14.2(0.2)	14.6(1.4)	13.4(0.1)	15.5(0.8)	
C:P	25.78	29.04	21.04	24.49	
Total OC (%)	10.7(0.8)	12.8(0.8)	10.7(0.4)	11.3(0.1)	BS 7755 Section 3.8 (1995)
Organic matter (%)	18.4 (0.8)	22.1 (0.8)	18.5 (0.4)	19.5 (0.1)	MAFF (1986) Method No.: 56
Extractable P (mg kg^{-1})	39(0.1)	70.1(0.4)	62.8(0.2)	61.9(0.3)	Olsen *et al*, (1954); BS 7755 Section 3.6
Available N (mg kg^{-1})	6.2(0.3)	7(0.5)	6(0.9)	8.3(0.6)	MAFF (1986) Method No.: 53
Total P (%)	0.45(4)	0.45(11)	0.54(4)	0.5(3)	BS 7755-3.13 (1998)
pH	6.3(0.03)	6.5(0.08)	6.6(0.08)	6.6(0.05)	MAFF (1986) Method No.: 32
Oxalate Fe (%)	0.14(0.01)	0.26(0.015)	0.21(0.015)	0.17(0.012)	Carter (1992) Method 23.5
Oxalate Al (%)	0.06(0.009)	0.07(0.004)	0.07(0.002)	0.07(0.002)	Carter (1992) Method 23.5

2.3 Gel Production

Initial attempts to produce gels for DGT use resulted in production of gels which were unsuitable for DGT use. Several months were spent investigating the reasons for the failed gel production and attempting to improve methods of gel production. Table 2-3 outlines the problems encountered when attempting to produce gels, and the solutions for overcoming these problems.

Table 2-3: A guide for overcoming problems encountered, during early attempts to produce gels for DGT use.

Problem	Solution
1. Fe oxide slurry not being mixed well enough with DGT gel solution – slurry pieces got stuck in between glass plates creating an area where slurry cannot move in between plates which created an uneven distribution of slurry within the gel	1. Ensure the gel and Fe oxide slurry are thoroughly mixed before adding the ammonium persulphate. This can be achieved my stirring with a spatula vigorously for 20 seconds.
2. Glass plates scratched – This can cause an accumulation of Fe oxide particles at the scratch, and air pockets, which has an effect on gel setting	2. Replace scratched glasses with new plates, whenever scratches appear.
3. Leaving for too long in the oven. This can cause the gel to dry out, making it stick to glass plates, and when attempting to remove can be broken	3. Gel solution should be placed in the oven for 1 hour.

Table 2-3 highlights that the initial production of DGT gels was not a straightforward process and required a degree of trial and error in order to produce gels which could be used on DGT devices tested for quality assurance tests (Section 2.4). When gels were produced in a form which could be prepared and inserted into DGT devices, the method development process was taken to the next level where gel testing could begin.

2.4 Gel testing in water

Initial tests of DGT gels in water tests confirmed that gels produced were unsuitable for deployment. Results of these attempts are documented in Table 2-4. The *R* value represents the ratio of mean C_{DGT} (μg l^{-1}) to the mean water concentration C_{Soln} (μg l^{-1}) (Equation 2-3).

$$R = C_{DGT}/C_{Soln} \quad \textbf{Equation 2-3}$$

This gives an indication of the accuracy of the DGT gels. An *R* value within 10% of 1 suggests that the DGT gel is accurate in measuring the solution P concentration. Table 2-4 shows that the first 13 tests resulted in gels being discarded as they were not suitable for DGT use. A trial and error procedure was employed to investigate the potential mechanisms for the failures; these correspond to letters in Table 2-5 explaining the reasons for failure. One by one, potential mechanisms for failure were removed, and eventually gels were successfully produced in consistent and reliable manner. Table 2-6 and Table 2-7 summarises all subsequent quality assurance tests, which are mainly successful, and used in soil deployments.

Table 2-4: Attempts to produce DGT gels which failed and reasons for the failure)

Test Number	Mean Water concentration (μg l^{-1})	Mean C_{DGT} (μg l^{-1})	Mean R	Used (U) or Destroyed (D)	Potential reason for failure
1	5950	3890	0.65	D	b,c,d
2	4600	22700	4.93	D	a,b,c,d
3	3840	1830	0.47	D	b,c,d
4	1460	23500	16.1	D	c,d,
5	1640	2220	1.35	D	c,d,
6	1530	910	0.59	D	d
7	1620	830	0.51	D	d
8	1490	1520	1.02	U	c,d,
9	1550	350	0.22	D	d,e
10	1480	800	0.54	D	d
12	1580	863	0.54	D	d
13	1600	913	0.57	D	d

Table 2-5: Corresponding reasons for failures

Corresponding failure reason	Potential reasons for gel not working
a	When making Fe oxide slurry, titrating too much NaOH, resulting in pH increasing above 7
b	Contamination of glass plates
c	Contamination of beakers to measure P in
d	Using the wrong gel thickness in the calculation
e	Reagents going out of date

Table 2-6: Details of DGT gels which were produced successfully in mean water concentration of 200 µg P l^{-1}

Test Number	Mean C_{DGT}	Mean R	Used (U) or Destroyed (D)
1	210	0.98	U
3	230	1.01	U
4	300	1.03	U
5	1200	0.80	D
6	230	0.96	U

Table 2-7: Details of DGT gels which were produced successfully; 1500 µg P l^{-1}

Test Number	Mean C_{DGT}	Mean R	Used(U) or Destroyed (D)
1	1499	0.98	U
3	1533	1.01	U
4	1546	1.03	U
5	1200	0.80	D
6	1486	0.96	U
7	1496	0.99	U
8	1521	0.99	U
9	1506	0.96	U
10	1539	1.00	U
11	1585	0.98	U
12	1563	0.98	U
13	1586	0.99	U
14	1575	0.99	U
15	1627	1.00	U

Table 2-6 and Table 2-7 show a natural progression from unsuccessful gel production, where gels had to be discarded, to successful production, where gels could be kept and used in further quality assurance tests on soils (Section 2.5). The progression was made

possible by identifying potential mechanisms responsible for failure and removing these (Table 2-5).

2.5 Deployment on soil

It has been established by previous authors (Degryse *et al*, 2009: Zhang *et al*, 1998; Menzies *et al*, 2005) that the Fe oxide gel is not an infinite sink for P. The capacity of the gel has been determined experimentally as ~7μg P (Zhang *et al*, 1998) in an experiment deploying DGT in water with different P contents. This is representative of DGT deployment with Fe oxide gel thickness (0.4mm and diffusive gel thickness 0.8mm). This was confirmed in studies by Menzies *et al*, (2005), who furthermore determined that during deployment in heavily fertilised soil, there was a non-linear response to P concentration over 48 hour deployments. From this information it was established that the capacity of the Fe oxide gels used in these experiments was ~2.5 μg P cm^{-2}. As the soils used in this experiment had a history of P additions, a decision was made to produce a thicker Fe oxide gel, containing more Fe oxide slurry, in order to increase the capacity for P uptake. Following manufacture of gel with a higher Fe oxide content, it was necessary to conduct tests to assess the performance of DGT on soils which would be used in this experiment, in order to establish optimum deployment conditions.

After establishment of a consistent and reliable method for gel production, with tests on DGT gel performance in water, tests progressed to deployment in soil to establish optimum deployment conditions. Also some further tests (Section 5.1 - 5.4) were required for this project which was not measured in these other studies. Therefore this section carries out a range of tests for DGT performance in soils in order to establish best deployment conditions for DGT use on the soils in this project.

2.5.1 Time

Measurement of the effect of time on C_{DGT} flux was measured in order to establish the optimum deployment time. Deployments were carried out for 12, 24, and 48 hours (Figure 2-3) on the four soils. It was suggested by authors that saturation of the DGT gel might occur at high solution P concentrations (Zhang *et al*, 1998; Menzies *et al*, 2005). As deployment for longer timescales results in a greater P accumulation on the Fe oxide

gel, tests had to be carried out to ensure saturation of the Fe oxide gel did not occur in soils tested.

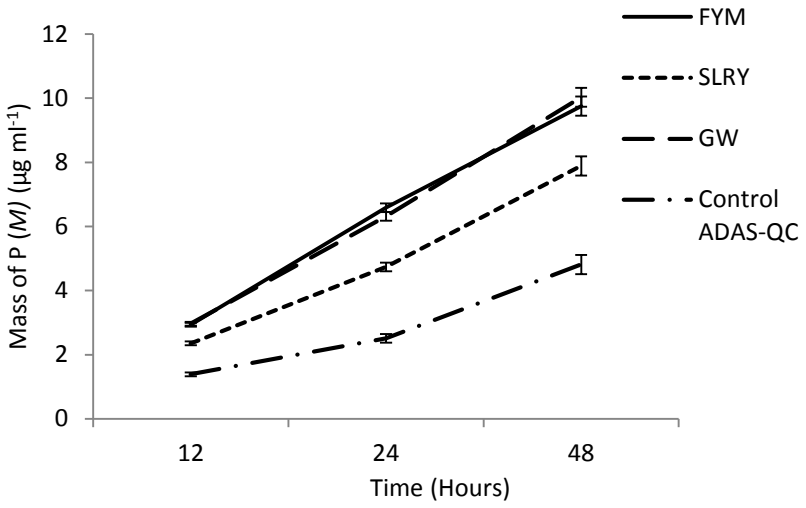


Figure 2-3: Increasing mass of P on the Fe oxide gel (*M*) with increasing time from 12 to 48hrs for four soils. Data are means (*n=4*). Error bars represent ± standard error. There was a significant interaction between each historically treated soil and *M* with time ($p < 0.001$ (Repeated measures ANOVA)).

The effect of time on P accumulation by DGT is displayed in (Figure 2-3). There is an increase in the mass of P accumulated on the resin gel with time for all soils ($p<0.001$). This suggests that DGT saturation of the Fe oxide gel does not occur for at least 48 hours on the soils which will be used in this experiment, and the gel can measure at least 10µg P before saturation. Therefore the DGT gel is expected to effectively act as a zero sink when deployed on these soils. In order to ensure validity of results throughout the study, DGT will be deployed for a maximum of 24 hours, in line with standard procedures (Menzies *et al*, 2005) used in previous studies. If *M* values above 10µg P are recorded, tests will be re-run at a shorter duration to avoid possible saturation of the gel.

2.5.2 Soil moisture content

Measurement of the effects of soil moisture content on DGT flux in the four soils was measured in order to establish the optimum soil moisture content to use for DGT deployment. For the purposes of this thesis it was important to understand the effects of DGT measurements on soil up to 110% field capacity as experiments may have to be conducted under conditions where the soil is above field capacity.

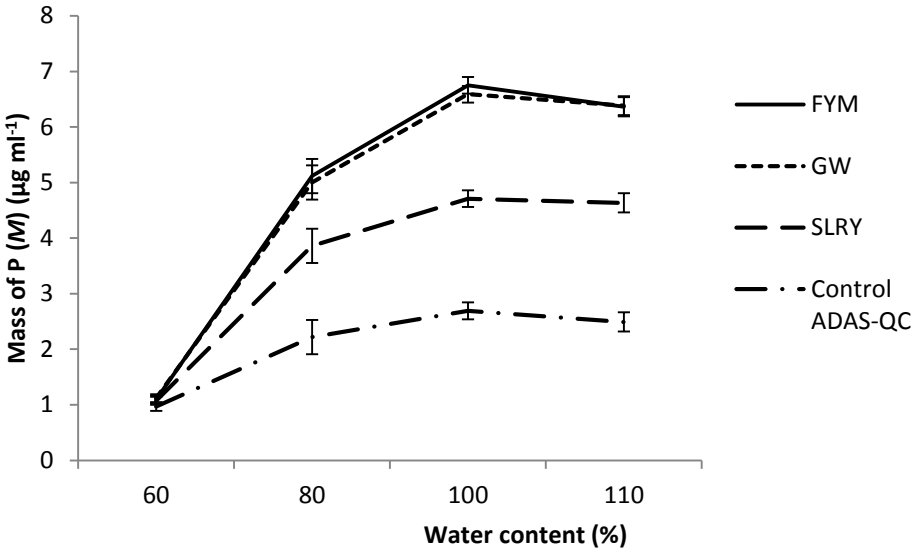


Figure 2-4: Change in mass of P on the Fe oxide gel (*M*) for deployments at increasing soil water contents from 60 to 110% field capacity for the four soils. Data are means (*n=4*). Error bars represent ± standard error. There was a significant interaction between each historically treated soil and *M* with soil water content ($p < 0.001$ (Repeated measures ANOVA)).

The mass of P accumulated by the DGT device increases with increasing soil water content up to 100% MWHC the flux levels from 100 to 110% MWHC (Figure 2-4).

Previous studies have shown that resin sinks accumulate increasing amounts of nutrients, with increasing soil moisture content (Menzies *et al*, 2004; Hooda *et al*, 1999). Hooda *et al*, (1999) reported a similar trend of increasing DGT flux with increasing soil water content to that found in this study, for metal flux. Menzies *et al*, (2004) also found an increase up to 100% MWHC for P; however no measurement above this was measured. The increase in P flux with increasing water content was attributed firstly to decreasing the tortuosity of the diffusion pathway (Hooda *et al*, 2009). In addition at lower water contents, pockets of air form in the soil which reduces the effective surface area of the DGT membrane (Hooda *et al*, 1999; Menzies *et al*, 2004). Therefore deployment at increasing water contents up to 100% MWHC enhances C_{DGT} P flux. However C_{DGT} P flux decreased slightly at deployments above 100% MWHC. Hooda *et al*, (1999) experienced a similar pattern for heavy metals and attributed the decline to dilution of soil solution metal concentrations due to the

increased water concentration. Dilution of P in the soil solution with deployments above 100% MWHC is responsible for the reduced P flux at 110% MWHC in this experiment.

2.5.3 Temperature

The effects of temperature on *M* were measured in order to choose optimum conditions for deployment throughout the thesis. Previous experiments have been conducted to establish the diffusion coefficient of P at different temperatures (Zhang *et al*, 1998). This is usually used to work out the diffusion coefficient and is input into Equation 2-2. However to the knowledge of the author no experiment has been conducted to date measuring the effects of temperature during deployment. This is important to know as planned experiments may take place at different temperatures, therefore its effect on *M* is imperative to understand.

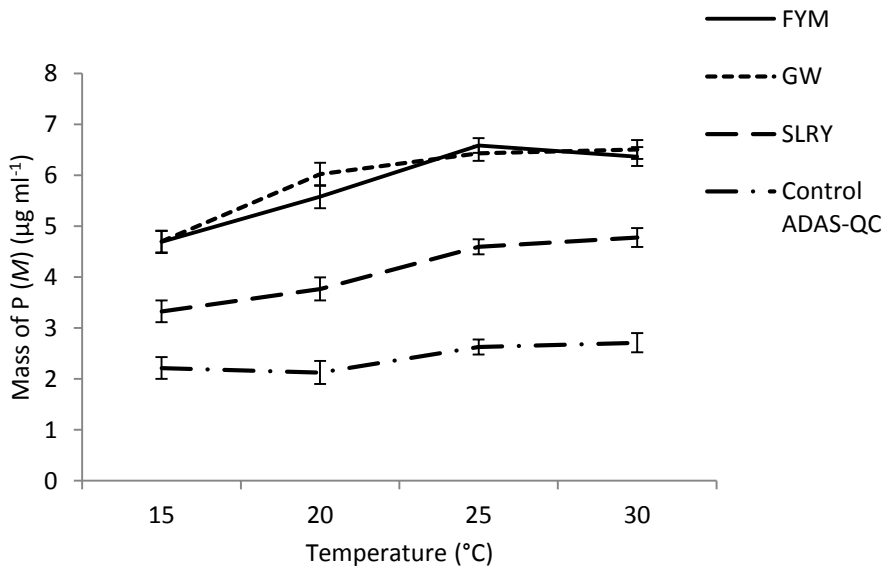


Figure 2-5: Change in mass of P on the Fe oxide gel (*M*) for deployments at increasing air temperature from 15 to 30 °C for the four soils. Data are means (*n=4*). Error bars represent ± standard error. There was a significant interaction between each historically treated soil and *M* with air temperature (p < 0.005 (Repeated measures ANOVA)).

The mass of P accumulated *M* by the DGT device shows an increase from 15 to 25°C, however no significant difference (p<0.005) between 25 and 30°C.

Results confirm that deployment at different temperatures affect the P flux to the DGT gel (Figure 2-5). However the equation to determine C_{DGT} contains a parameter which

allows the output to change with changing temperature based on the diffusion coefficient (Zhang *et al*, 1998). This has implications for the pot experiments (**Chapters 5 and 6**), as the temperature in the glasshouse cannot be maintained at a controlled temperature, methods to overcome this are explained in Section 2.6.1.

2.5.4 Natural variability of the technique

An experiment was carried out to determine the natural variability of the DGT technique in the experimental soils. Ten replicates of the experiment on the same soil sample were determined as sufficiently large sample size for rigorous statistical analysis. Previous work has typically used 2-4 replicates, when measuring the variability of DGT on soils. Results were also compared to Olsen P measurements at ten replicates for experimental soils.

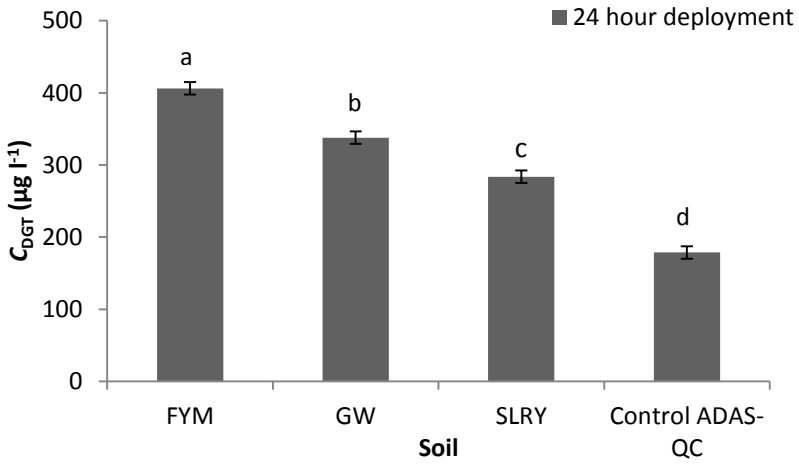


Figure 2-6: Natural variability of the DGT technique for each historically treated field soil. Data are means (*n=10*). Error bars represent ± standard error. There was a significant difference between each historically treated soil ($p < 0.001$ (One-way ANOVA)). Significant differences between soils are denoted by lower case letters (Fisher LSD).

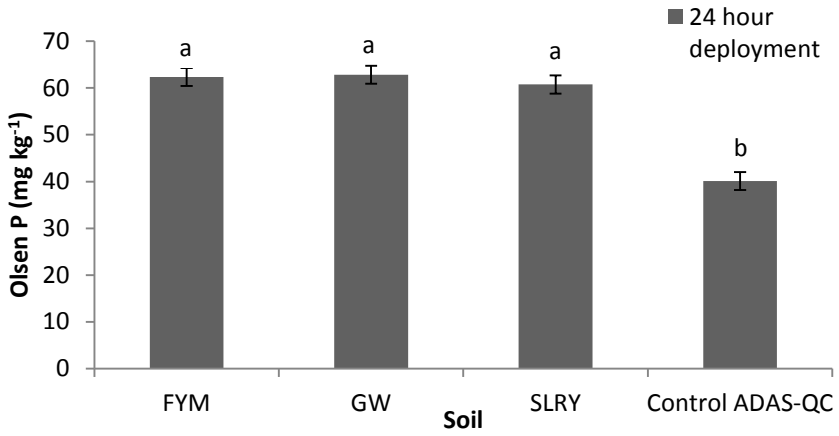


Figure 2-7: Natural variability of the Olsen P technique for each historically treated field soil. Data are means (*n=10*). Error bars represent ± standard error. There was a significant difference between historically treated soils and Control ADAS-QC ($p < 0.001$ (One-way ANOVA)). Significant differences between soils are denoted by lower case letters (Fisher LSD).

The mass of P accumulated by DGT devices show a low standard error (0.013) (Figure 2-6) when deployed on 10 reps of the same sample. The standard error of the Olsen P technique is also low (1.9) (Figure 2-7). This instils confidence that the DGT technique is reproducible on the soils being analysed. Furthermore it confirms that the deployment conditions which were chosen as optimum, as a result of previous tests, are suitable and appropriate for this project. Results are comparable to Olsen P tests. As this has undergone rigorous testing over the years (Horta *et al*, 2007) it can be expected that natural variability of both techniques is low and unlikely to have a significant effect on measured results. Furthermore this information provides evidence to suggest that C_{DGT} measurements are detecting differences in P between soils, which Olsen P is not.

2.5.5 Volume of elution solution

It must be established that throughout this chapter, DGT gels were eluted in 5ml H_2SO_4. However throughout the subsequent chapters, for practical reasons it was sometimes necessary to elute the gel in 10ml H_2SO_4. Where this was the case it is clearly highlighted in the methodology of the individual chapter. Equation 2-2 used to calculate C_{DGT}, allows for different volumes of elution solution. Under conditions where the soil P concentration is low (**Chapters 4, and 6**), it is preferable to use 10ml H_2SO_4, as this

concentrates the total P in the solution, making analysis easier. However when soil P concentration is high, and has received further addition of P such as soils from Gleadthorpe experiments (**Chapters 3 and 5**) 10ml H_2SO_4 is used, in case an aliquot of the elution solution is required for dilution and additional analysis.

2.6 In situ pot deployments

The methodology (Section 2.2) describes deployment conditions in petri dishes. DGT was also deployed directly in incubation pots, and in situ in glasshouse pots (Figure 2-2). Deployments in incubation pots require a procedure similar to those in petri dishes. However 400g of dried and sieved (<2mm) soil, was used instead of 30g. Devices were then deployed as mentioned (Section 2.2.2).

It has been established by previous authors that the practice of drying and re-wetting soil, brings about changes in P dynamics, as a result of soil biological and chemical changes (Soinne *et al,* 2010). Furthermore Nowak *et al,* (2004) found results of heavy metal analysis differed between in situ and homogenised samples, where pools existed in the field, which didn't appear in disturbed and homogenised samples. As it was the intention of this project to carry out experiments across a range of scales, it was important to establish how deployment at different scales influenced results of DGT analysis on the same soil. It was also important to establish a consistent and reliable method for deployment of DGT in situ in glasshouse pots (Full details of pot setup detailed in **Chapters 5 and 6**). The experiments were split into sections based on the order they were conducted. Table 2-8 provides an overview of each trial conducted to establish the best method of deploying DGT in situ, and the outcomes, which led to an improved method of deployment.

Table 2-8: Experiment comparing in situ deployments with deployments on dried and homogenised soils, which have been re-wet in petri dishes.

Attempt	Name	Outcome
1	Trial-1	• Issues with temperature control • Issues with soil drying • Significant difference between P measurements between the two methods.
2	Trial-2	• Reduced difference between the two methods, however still a significant difference.
3	Trial-3	• Destructive • Significant difference between the two methods.

2.6.1 Trial 1

Trial 1 was designed with the initial objective of establishing the best method of deploying DGT devices in situ. Results from this experiment were used to design Trial 2 which is a more accurate way of deploying DGT devices in situ. Table 2-9 outlines the experimental conditions for Trial 1, comparing conditions for in situ analysis and petri dish analysis (disturbed soil). Soil was maintained in pots between 80 and 100% field capacity at all times, 24 hours prior to DGT deployment the pot was brought to field capacity before the DGT device was deployed on the surface of the pot as described above (Section 2.2.2).

Table 2-9: Deployment conditions used in Trial 1

Variable	In situ	Petri dish (Disturbed)
Air Temperature (°C)	16 (Mean*) Max =20 Min=10	25 (Constant)
Soil water content	(Not measured but visually reduced)	Field capacity
Time	24 hours	24 hours
Depth of soil measured	<1cm (Pot surface)	15cm (with auger)

*Mean of 4 samples throughout the experiment

There is a significant difference between deployments made in situ and deployments made in petri dishes ($p<0.001$). Deployments in petri dishes are significantly larger than those measured in situ ($p<0.001$) Figure 2-8.

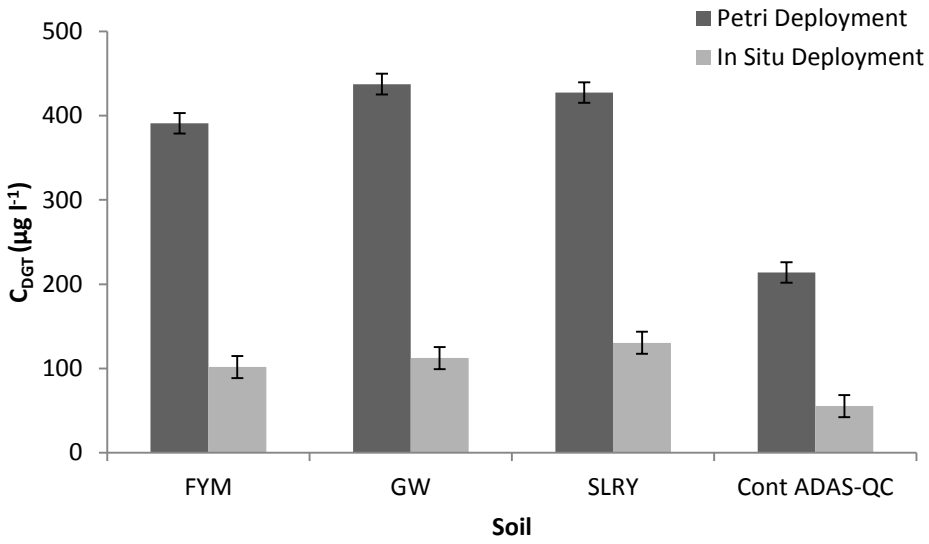


Figure 2-8: Deployments in situ and deployments in petri dishes for Trial 1. Data are means (*n=4*). Error bars represent ± standard error. There was a significant difference between in situ and petri dish deployments for each soil ($p < 0.001$).

It is believed that petri dish deployments took place under optimum conditions for recording DGT flux. Therefore it was expected that petri dish deployments would give a greater P flux than in situ. However the magnitude of difference between the two deployment methods suggests that there was an issue with the accuracy of deployments in situ. A number of reasons are proposed for the differences in P flux between the two deployment methods. Firstly, temperature could not be controlled in the pot experiment, neither was it accurately measured; a mean was recorded from four readings of a thermometer in the glasshouse at different points throughout deployment. Furthermore it is expected that there is a difference between the air temperature which was measured (16°C), and the soil temperature which was not measured. Accurate soil temperature measurement is required to work out the diffusion coefficient of P in the soil during DGT deployment. This in turn is input into the C_{DGT} equation in order to work out an accurate P flux (Equation 2-2). The soil moisture content was neither accurately recorded prior to deployment nor during deployment. Visual inspection suggested the soil dried out during deployment, due to the high temperatures. Figure 2-4 showed how soil moisture affects C_{DGT} P flux.

2.6.2 Trial 2

Trial 1 did not allow for soil moisture content and temperature variations before and during deployment, which affected the difference in P flux between the two deployment methods. This section outlines the new deployment conditions employed to overcome the methodological limitations of Trial 1. A plastic bag was used to reduce water loss from the soil. This had small holes pierced to allow air flow. A soil moisture probe (ΔT Soil moisture kit, CWI Technical Ltd) was used to record the soil moisture content and temperature probe used to record soil temperature, and therefore the diffusion coefficient of P in the soil.

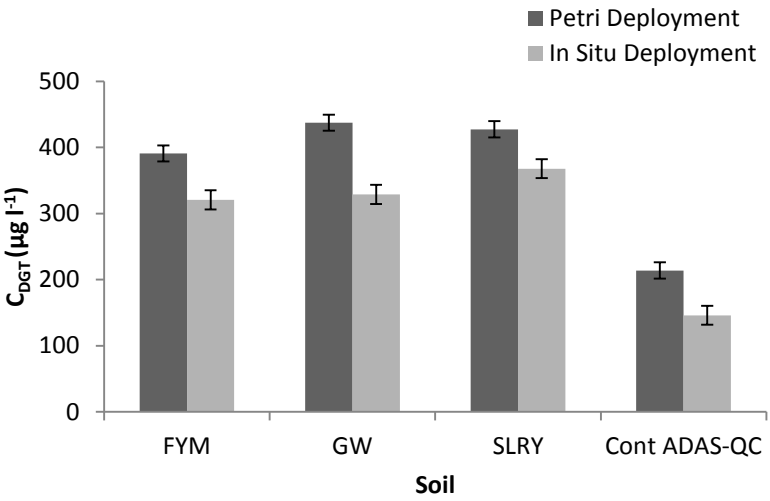


Figure 2-9: Deployments in situ and deployments in petri dishes for Trial 2. Data are means (*n=4*). Error bars represent ± standard error. There was a significant difference between in situ and petri dish deployments for each soil (p < 0.001).

Table 2-10: Details of deployment conditions used in Trial 2

Variable	In situ	Petri dish
Temperature (°C)	14 (Mean of 4 readings throughout experiment) *temperature of soil	25 (Constant)
Soil water content	Field capacity	Field capacity
Time	24 hours	24 hours
Depth of soil measured	<1cm (pot surface)	15cm (with auger)

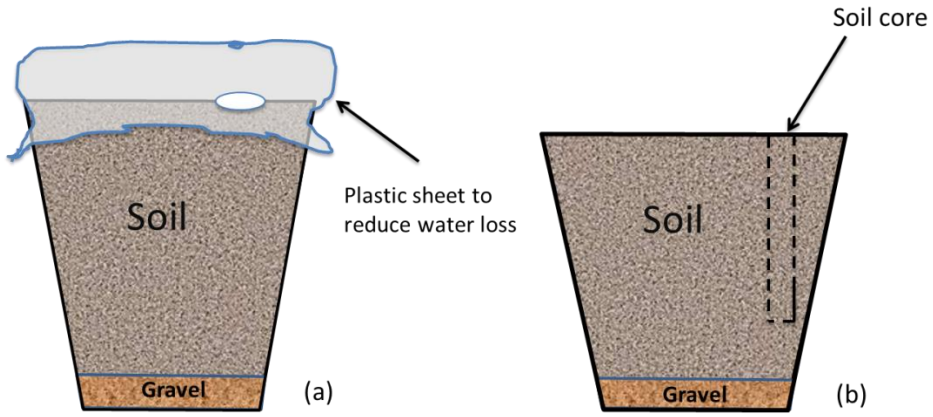


Figure 2-10: (a) methods deployed for water loss control (b) area of soil removed by auger.

Figure 2-9 shows a significant difference ($p<0.001$) between deployments in situ and deployments in petri dishes. Deployments in petri dishes are greater than in situ ($p<0.001$).

The mass of P accumulated by the DGT device shows a similar pattern for in situ and petri dish deployments, however deployments in the petri dish are significantly greater than those measured in situ. Moisture probe readings confirmed soil remained at field capacity throughout deployment. This is a direct result of the addition of a plastic cover to the pot. As a result values from tests in petri dish and in situ are closer compared to Trial 1. This method of deployment is an improvement on Trial 1, and is considered within the context of this project to be the best way of deploying the DGT device in situ on the surface of the pot. However, limitations remain with making a direct comparison of deployment in situ and homogenised samples.

The two deployment methods are measuring P in different zones (Figure 2-10). Koenig *et al*, (2008) indicate that the soil solution P concentration changes with depth in the soil profile. As the P applied remains close to the site of application, shallow sampling can result in higher soil test P values. If the P being measured in situ is being taken from a different zone from homogenised soils, then a direct comparison between the two deployment methods will be inaccurate. However, as the soils sampled in this test were measured 1 week after water was added to the pots, it was expected that there was insufficient time for differences with depth to be established. Furthermore there was no

addition of treatments to these soils for the purpose of these method development experiments, making differences with depth even less likely.

Despite methodological developments to improve water retention and temperature measurement, no allowance could be made for changes in soil chemistry brought about by drying and re-wetting the soil. Soinne *et al*, (2010) explained that rewetting of dried soils enhanced the mineralisation of organic matter. Nutrient bursts originate from solubilisation of organic matter and the disruption of aggregates revealing fresh new surfaces and through microorganisms broken down during drying or rewetting. Turner and Haygarth, (2001) found that air drying increased water, and sodium bicarbonate ($NaHCO_3$)–extractable P (Soinne *et al*, 2010). Therefore this will continue to influence results when comparing in situ to homogenised sampling. Trial 2 was sufficient for measuring the C_{DGT} in situ on the soil surface, throughout the experiment; however information needs to be made available on the difference in C_{DGT} P with depth in the range of soil the corer is removing and homogenising.

2.6.3 Method for deployment of DGT in situ with depth

From the information obtained in Trial 2 it was deemed necessary to assess the variation in soil P dynamics with depth following application of treatments to soil. However it was expected that changes with depth would develop over time, therefore it would be impractical to measure this in an initial trial like those above. Therefore it was decided that a method would be developed in this section, however analysis would take place following cessation of pot experiments, and results reported in **Chapters 5 and 6**.

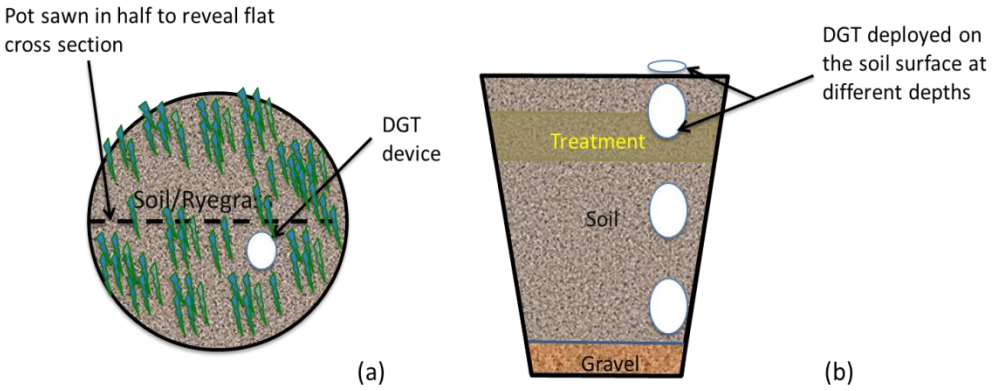


Figure 2-11: The process of deploying DGT devices at different depths after the pot experiments Chapters 5 and 6.

Following cessation of the pot experiments, pots were prepared for in situ analysis as explained in Trial 2. However on this occasion, prior to deployment of the device, pots were sawn exactly in half (Figure 2-11a), and DGT devices were deployed on the soil surface and at 3 depths 0, 5, 10, 15 cm (Figure 2-11b). The pot was then reassembled and held together with an elastic band, and DGT deployments took place for 24 hours, under conditions highlighted in Trial 2 before dismantling the pots and retrieving devices for analysis.

2.7 Conclusions

This chapter acts as a reference for subsequent chapters for deployment of DGT on soil. A range of experiments were conducted to establish optimum deployment conditions. The following conclusions were established.

- DGT gels could be produced in a consistent and reliable manner, after refining the technique of production and establishment of a guide for overcoming issues related to gel production.
- Gel testing in water encountered numerous issues when testing gel performance initially, however issues were resolved when; P contamination of gels, glassware and all lab equipment was removed, when the correct gel thickness was used in the DGT calculation, when care was taken to make sure reagents were not out of date.
- Optimum deployment conditions in soils which have received repeated application of different organic amendments were established by carrying out a range of tests at different times, water contents, temperatures, and with 10 reps on the same soil. All carried out in petri dish deployment.
- Saturation of the Fe-oxide gel was not an issue when DGT was deployed on soils in this experiment for 48 hours, using the detailed methodology. However the optimum deployment time was 24 hours. Furthermore it was determined that if the P content of the gel was >10µg P, following deployment, that the experiment would be re-done at a shorter timescale.
- Optimum conditions were established for DGT deployment in situ in pots by controlling the amount of soil water and accurately recording the soil temperature.

3 Gleadthorpe incubation experiment

3.1 Introduction

Incubation studies have been used extensively to measure P behaviour in soil, following application of a wide range of organic amendments (Mafongoya, 2000; Griffin *et al*, 2003; Gichangi *et al*, 2009; Miller *et al*, 2010). Incubation experiments have the advantage of being convenient to set up and manage (Miller *et al*, 2010). Control over environmental conditions reduces the likelihood of variability as a result of environmental factors. The disadvantage is that optimal conditions are less representative of field conditions. This has been taken into consideration and pot experiments (**Chapters 5 and 6**) have been set up to upscale from incubation experiments with the introduction of plant influences.

The experiment has been designed to understand P availability in soil using DGT and Olsen P, following the application of treatments, (GW, FYM and SLRY and SP) on soils, which have received treatment additions based on crop, N demands, for 5 years.

When considering the influence of treatments on soil characteristics, the history of treatment application is important; however this is often neglected (Griffin *et al*, 2003). This study uses soils, which were taken from an existing field site, described in detail by (Bhogal *et al*, 2011) as part of an ADAS-QC experiment. The site was part of a study designed to develop an improved understanding of the processes and linkages through which organic carbon (OC) additions influence the soil bio-physical and physico-chemical properties. Such practices have previously been shown to cause a build-up of soluble P in soil (Read *et al*, 2007).

The rationale of this study is to quantify P availability in soils used in this study before and after fresh addition of corresponding treatments used in ADAS-QC trials in order to understand how application history influences P availability from fresh treatment addition. In addition, it is important to compare this to P release following SP addition, in order to understand the relative P release from organic amendments (FYM, GW, and SLRY) compared to inorganic P (SP).

Information gained from this experiment will add to knowledge of P availability following incorporation of treatments at the laboratory scale, and add valuable

information about the performance of the DGT technique used on soils receiving application of organic amendments under laboratory conditions. The objectives of the current study are summarised below.

Objectives

1. Determine P availability patterns in soils which have historically received application of organic amendments, with and without addition of further treatments (FYM, GW, SLRY and SP) at agronomic application rates.
2. To investigate how organic amendments perform compared to SP in the aforementioned soils.

Hypotheses

- Historical addition of organic amendments to meet N demands will lead to a build-up of P measured by DGT compared to control soils.
    - The pattern will be influenced by the total mass of C and P added in the organic amendments.
- Treatment application history will be significant in determining P release from fresh treatment additions.
    - Soils which receive SP will show a greater response (C_{DGT}) than those which received addition of organic amendments, due to the slow release of P from organic amendments.
- Olsen P and DGT will show a different trend as they are measuring different P pools

3.2 Methodology

3.2.1 Historical treatment additions

A brief description of experimental sites was provided in **Chapter 1**. Gleadthorpe soils were sampled and removed from existing field trials (ADAS –QC), which had received historical application of organic amendments because they had a well-documented application history. The project entitled organic manure and crop organic carbon returns-effects on soil quality: SOIL QC was a report for Defra project SP0530 (Bhogal *et al*, 2011). The overall objective of the project was to develop an improved

understanding of the processes and linkages through which organic carbon (OC) additions influence soil bio-physical and physio-chemical properties, from seven experimental sites. Each site received a range of organic amendment additions. The study showed that OC additions produce measurable changes in a wide range of soil bio-physical and physico-chemical properties and processes, which are central to maintenance of soil fertility and functioning (Bhogal *et al*, 2009). Findings from the work are important in understanding the influence of organic matter (OM) on soil properties. The soils from Gleadthorpe represent a good opportunity to explore the influence of the documented OM additions on some specific soil properties, which were not in the scope of the aforementioned trial.

The ADAS-QC experiment used soils from a range of locations; however the soils sampled and measured in this experiment were from Gleadthorpe farm, Nottingham, England (Figure 1-7)**.** At this site treatments (FYM, SLRY and GW) with 4 replications of each treatment were added to plots in a randomised block design, with plot sizes measuring a minimum of 5x15m. All treatments were applied at 250 kg N ha^{-1} and balanced with ammonium nitrate, using MANNER (a practical software tool that provides farmers and advisers with an estimate of crop available nitrogen, phosphate and potash from applications of organic manure) model predictions of manure crop available N availability (Chambers *et al,* 1999), to ensure similarity of crop available N supply across the treatments (with the control treatment receiving the economic recommended rate of only ammonium nitrate N (Defra, 2010)). Treatments were applied to soil annually from 2004 to 2008, in order to grow winter wheat and spring oil seed rape on alternate years. Total aboveground biomass production (grain/seed+straw) was measured at each harvest. Each year treatments were fully characterised according to standard analytical techniques. Appendix Table A.2-1 shows mean values from the 4 applications. Converted P application rates were 61, 41, 49, kg P ha^{-1} for FYM, SLRY, GW respectively. A description of each soils characteristics (soil texture, field capacity and bulk density measurement was provided (Table 2-1).

3.2.2 Soil and treatment setup

The treatments (FYM, GW, SLRY and SP) used in this experiment are the same in all glasshouse and incubation studies. The composition of each organic amendment is

reported in Table 3-1. Soil analysis conducted prior to experiment is reported in Table 2-2. The application rates used (Table 3-2) were used based on RB209 fertiliser manual recommendations for the P index of each soil to maintain sufficient extractable P for grass establishment (Defra, 2010). The mass of treatment added to the soil was calculated based on the application rate required, the bulk density of the soil (Table 3-2), and the total P content of each treatment (Table 3-1). To determine the mass of each treatment to add to each pot the following calculation (Equation 3-1) was used.

$$Mass\ of\ treatment\ required(g) = \left(\frac{MS_{pot}}{MS_{ha}}\right) * RA * 1000000 \qquad \textbf{Equation 3-1}$$

Where MS_{pot} = Mass of soil in the pot (kg), MS_{ha}= Mass of soil in a hectare (kg), and RA= Application rate of treatment (t ha^{-1}).

Treatments were applied based on total P following recommendations from the RB209 fertiliser manual (Defra, 2010). However it was expected that other treatment characteristics such as extractable P, C:P ratio and pH would influence P availability in the soils. Soil from the Gleadthorpe experimental site detailed above was weighed into incubation pots. Each pot was labeled and received soil from a historically amended plot (FYM, GW, SLRY, and Control ADAS-QC)). Each historically amended soil then received a fresh batch of its corresponding treatment. Therefore FYM, SLRY and GW were added to their respective soil and SP was added to Control ADAS-QC (Table 3-2). Treatments were applied at two application rates 15 and 25 kg P ha^{-1}, actual volumes of each treatment added to the soil are shown in (Table 3-2). Treatments labeled 0 are soils from each historically amended plot which have not received addition of any further treatment in this experiment and therefore represent an un-amended control for each treatment. The relatively low application rates used reflect the initial P initial index of the soil, and the amount of P which is required to sustain crop growth. Four repetitions per treatment were required for robust statistical analysis, taking into account variability in chemical analysis (Kokkora, 2008; Antille, 2011).

It must be noted that the terms “organic amendment” and “treatment” are used throughout the study. The term treatment is the main term used to describe any material added to the soil in the studies. However it is often necessary to use the term organic

amendment, in order to distinguish this from inorganic materials, particularly when comparing applications of each material.

Table 3-1: Treatment analysis conducted prior to experiment, numbers in brackets indicate standard error, measures made on a dry weight basis for all amendments except SLRY (wet); Data are means *n=4*.

Amendment	GW	FYM	SLRY(Wet)	SLRY(Dry)
pH	8.9(0.02)	8.9(0.01)	7.1 (0.01)	7.3(0.01)
TC (%)	25.7(0.4)	45.9(0.05)	2.5(0.02)	44.4 (0.06)
TN (%)	1.3(0.03)	1.7(0.03)	N/A	2.6 (0.06)
TP (%)	0.22(0.06)	0.21(0.3)	0.053(0.02)	0.33 (0.17)
C:N	11.8(0.02)	27.6(0.4)	N/A	17.3(0.6)
C:P	110.3(1)	276.5(2)	46.9(1)	146 (1.5)
Extractable P (mg kg^{-1})	189.6(2)	448.9(1)	495.9(27)	N/A

Table 3-2: Mass of each treatment added to each incubation pot for the study; *n=4*

	Treatment(g)			
	FYM	**SLRY**	**GW**	**SP**
App rate (kg P ha^{-1})				
0	0	0	0	0
15	1.5	6.2	1.5	0.05
25	2.6	9.9	2.6	0.08

3.2.3 Soil incubation

Incubation pots received 400g of soil, which had been dried and sieved (<2mm). Treatments were added to each pot and thoroughly mixed with the soil. De-ionised water was then added to the soil to bring it to the required water content (Table 2-1). Pots were placed in the incubator in the absence of light and incubated at 25°C. Pots were covered with aluminium foil and holes pierced in the top to allow oxygen circulation, but prevent water loss. A tray of de-ionised water was also maintained at the bottom of the incubator to keep conditions humid and stops the soils drying out (Antille, 2011). Pot weights were monitored weekly for water loss, and any loss was replenished

with de-ionised water. The duration of the experiment was 90 days, (as information required was for short term P dynamics) and sampling carried out on days 1, 5, 10, 30, 60 and 90.

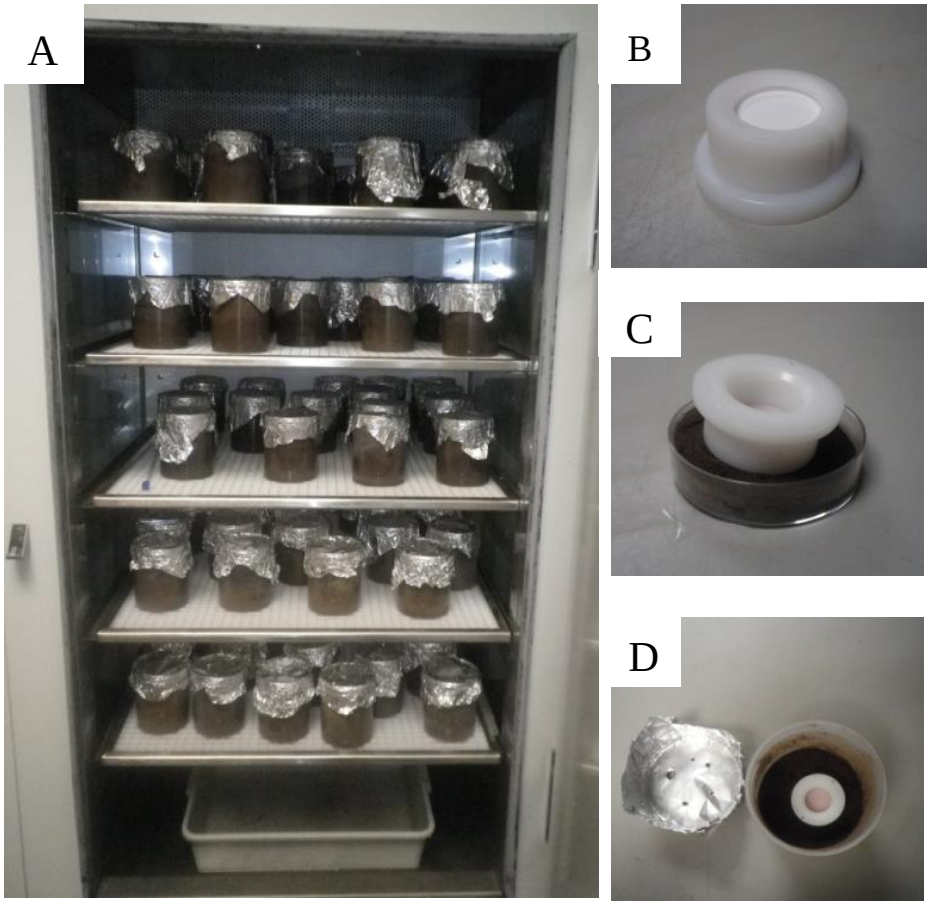


Figure 3-1: Incubation experiment showing (a) The incubator containing the pots, (b) a DGT device (c) a DGT device sampling from a petri dish (d) a DGT device sampling from a pot directly.

3.2.4 Measurement and analysis

DGT devices were deployed in situ in the incubation pots. This involved inserting the DGT device onto the soil surface and gently twisting to allow contact between the soil and the device. Two days prior to sampling pots were brought to 100 % field capacity. Devices were deployed for 24 hours in the incubator at 25°C; the devices were then dismantled and measured as described (Section 2.2.2).

The percentage extractabe P in the soil recovered from each treatment was determined using (Equation 3-2). This is described as percentage phosphorus recovery ratio (% PRR) by Miller *et al,* (2010), and is derived from a similar equation termed % efficiency by Griffin *et al,* (2003). %PRR provides a ratio of the net P available to the amount of P added. The basis of the calculation involves first of all correcting extracted P available from the treatment at each sampling point for the soil before treatment application with (Equation 3-2)

$$P_{corrected} = P_{amended} - P_{control}$$ **Equation 3-2**

The efficiency of an added P source in altering a soil P pool is then determined by (Equation 3-3)

$$\%PRR = P_{Corrected}/P_{added} \times 100$$ **Equation 3-3**

Where $P_{added} = mg\ P\ kg^{-1} soil$

This type of calculation has previously been carried out on soils where extraction techniques were used to derive the amount of available phosphorus in the soil. It has not however been used to quantify the % PRR from the DGT device. Using the C_{DGT} to derive %PRR gives value which represents the percentage of total P in each treatment which is made plant available across the experiment, rather than that which is potentially available as in with extraction techniques such as Olsen P. C_{DGT} values had to be converted from μg l^{-1} to mg kg^{-1} to facilitate the measured total P concentration which was in g kg^{-1}.

3.2.5 Statistical analysis

Statistical analysis was carried out to determine the effects of treatments on C_{DGT} and Olsen P. This was achieved using repeated measures analysis of variance (ANOVA). Post-hoc analysis was conducted using Fisher least significant difference (LSD) Homogeneous groups (0.005) where there is no significant difference; the same lower case letter is used. Error bars on each graph represent ± standard error. All statistical analysis was carried out using STATISTICA 11 software. Residuals were all normally distributed. Results of all ANOVA analysis are displayed in Appendix Table A.1-1 to Table A.1-4.

3.3 Results

3.3.1 Historical treatment additions

To evaluate the effects of historical treatment practices conducted in ADAS-QC experiments, mean values for each soil sampled were calculated and summarised for C_{DGT} and Olsen P in Figure 3-2 and Figure 3-3 respectively. Figure 3-2 shows soils sampled from ADAS-QC experiments after four annual applications of the described treatment, for C_{DGT} measurements. There is a significant difference ($p<0.001$) between all soils, with the pattern following GW≥FYM>SLRY>CONT.

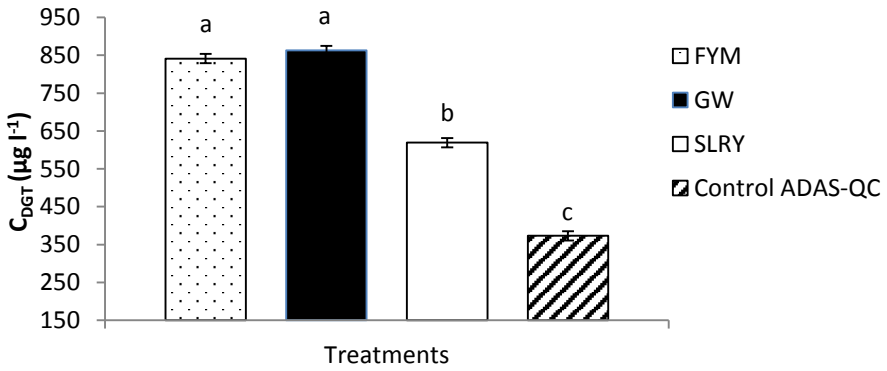


Figure 3-2: Results of C_{DGT} measurements on soils, which had received application of treatments from 2004-2008. Data are means (*n=4*). Error bars represent ± standard error. Overall there was a significant difference between soils ($p < 0.001$). Significant differences between soils are denoted by lower case letters (Fisher LSD).

Figure 3-3 shows soils sampled from ADAS-QC experiments, for Olsen P measurements. There is a significant difference ($p<0.001$) between all soils, with the pattern following GW> SLRY>FYM> CONT.

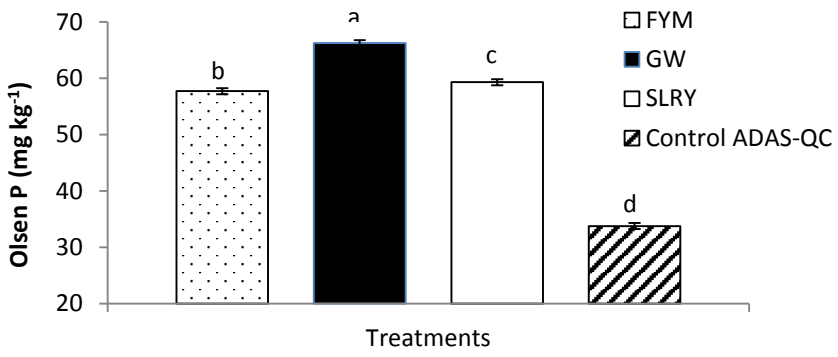


Figure 3-3: Results of Olsen P measurements on soils which had received application of treatments from 2004-2008. Data are means (*n=4*). Error bars represent ± standard error. There was a significant difference between each soil (p < 0.001). Significant differences between soils are denoted by lower case letters (Fisher LSD).

3.3.2 Incubation experiments

To evaluate the effects of the rate of added SP, GW, FYM and SLRY on C_{DGT} mean values for each treatment and rate were calculated and summarised for C_{DGT}, C_{DGT} (% PRR). Figure 3-4 shows soils sampled from incubation experiments, where corresponding treatments were added to ADAS-QC experiments for C_{DGT} measurements. There is a significant difference (p<0.001) between control and treated soils with time. However there was no significant difference between the two rates of application (15 and 25kg P ha^{-1}).

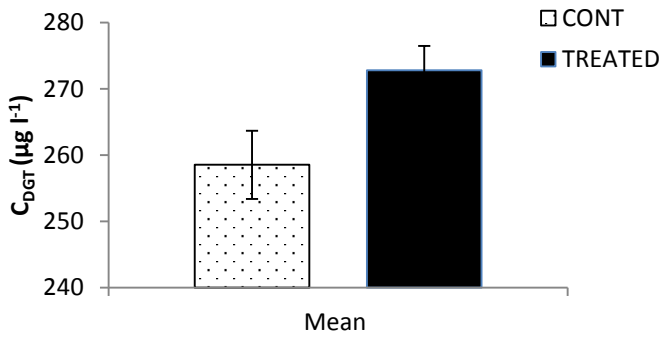


Figure 3-4: C_{DGT} measurements on overall control and treated soils for each sampling date for incubation experiments. Data are means (Control soils *n=4*))(Treated soils *n=8*). Error bars represent ± standard error. There was a significant difference between control and treated soils (p =0.029).

There is a significant difference between the control and treated soils for Olsen P (as a mean of all sampling dates and over time (Figure 3-5)); however there is no significant difference between the individual treated soils (FYM, GW, SLRY, and SP).

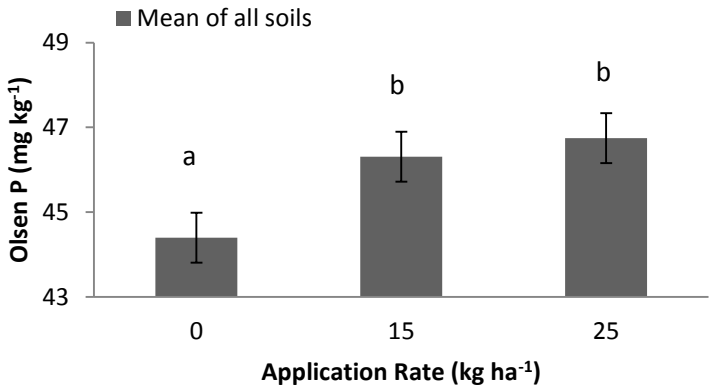


Figure 3-5: Olsen P measurements on overall control and treated soils with increasing application rate as a mean of each sampling date for incubation experiments. Data are means (Control soils *n=4*))(Treated soils *n=8*). Error bars represent ± standard error. There was a significant difference between control and treated soils but no significant difference between the treated soils (p <0.001). Significant differences between soils are denoted by lower case letters (Fisher LSD).

There is a decrease in C_{DGT} with time for both control soils (Figure 3-6a) and treated soils (Figure 3-6b). Although there is a significant difference in C_{DGT} between control and treated soils over time, both show a similar trend.

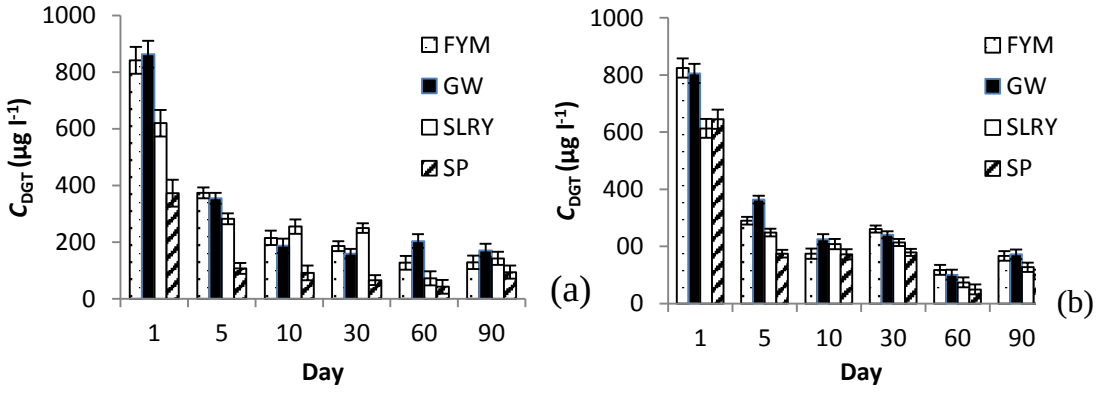


Figure 3-6: C_{DGT} measurements showing (a) control and (b) treated soils over time for each treatment. Data are means *n=4*. Error bars represent ± standard error. There was a significant difference between control and treated soils over time (p<0.001).

There is a significant difference between treated soils and controls (Figure 3-7). However there was no significant difference between the two rates of application (15 and 25kg P ha^{-1}). There is a decrease in Olsen P with time for both control soils (Figure 3-7a) and treated soils (Figure 3-7b).

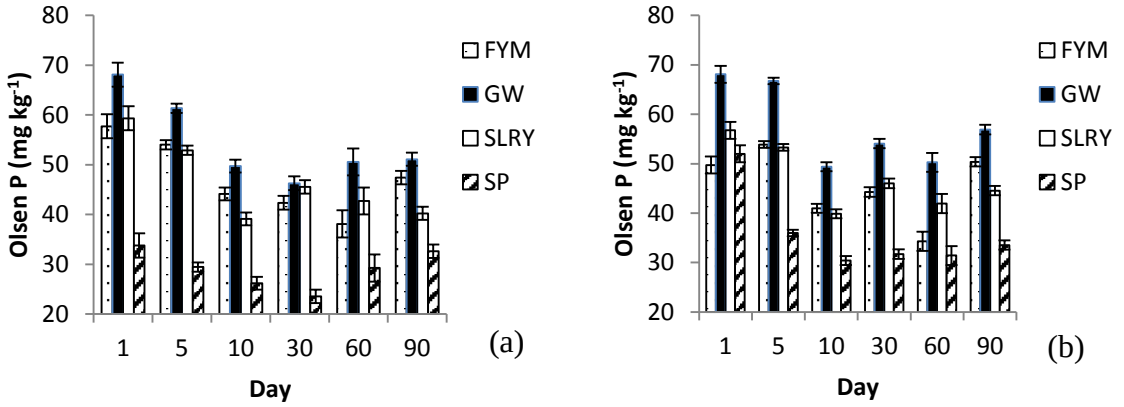


Figure 3-7: Olsen P measurements showing (a) control and (b) treated soils over time for each treatment Data are means *n=4*. Error bars represent ± standard error. There was a significant difference between control and treated soils over time (p<0.001).

Figure 3-8 shows the overall difference (p<0.001) between treatments as a mean of all sampling dates the trend follows (GW>FYM>SLRY>SP).

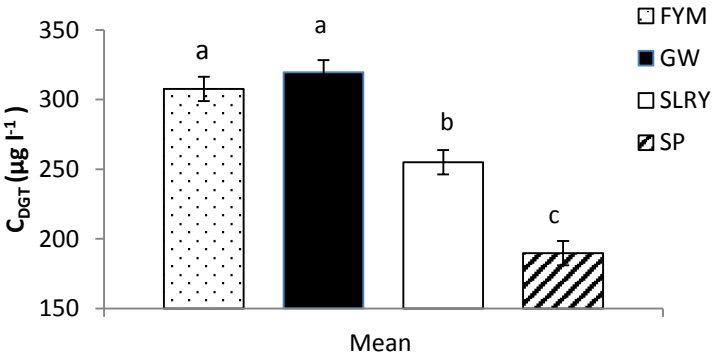


Figure 3-8: C_{DGT} measurements showing mean values of soils from each treatment source for all sampling dates throughout the experiment. Data are means (*n=4*). Error bars represent ± standard error. There was a significant difference between each soil (p < 0.001). Significant differences between soils are denoted by lower case letters (Fisher LSD).

Figure 3-9 shows mean values of all sampling dates for each treatment C_{DGT} %PRR. Results show there is no significant difference between organic amendment (FYM, GW, SLRY) C_{DGT} %PRR, and the negative values suggest no significant P release from each organic amendment. However there is a significant increase for the soil which received SP.

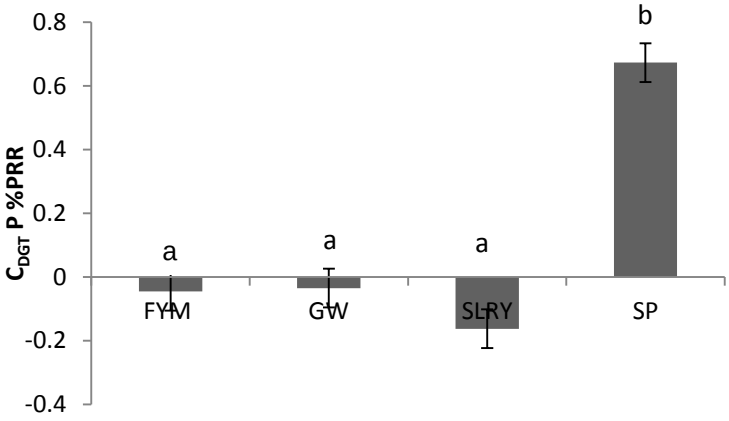


Figure 3-9: C_{DGT} % PRR showing the P recovered from each treatment as a mean of the two treatments (15 and 25 kg P ha^{-1}) and a mean of all sampling dates (*n=4*). Error bars represent ± standard error. There was a significant difference between treatments overall ($p < 0.001$). Significant differences between treatments are denoted by lower case letters (Fisher LSD).

Figure 3-10 shows mean values of all sampling dates for each treatment Olsen P %PRR. Results show there is a significant difference between all treatments with the pattern of P release following SP>GW>SLRY>FYM. Overall all treatments released P, except FYM.

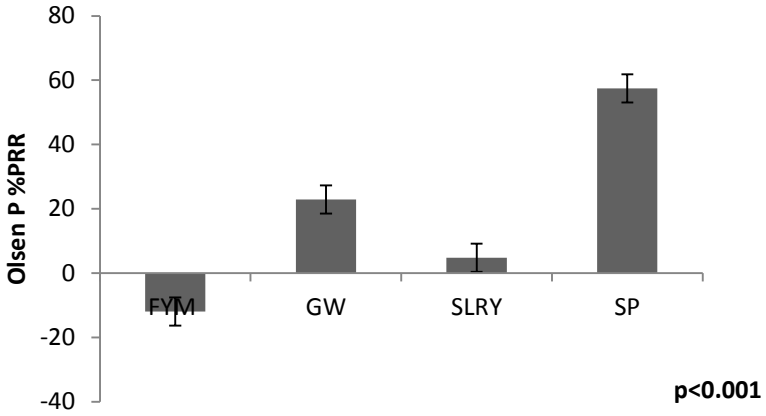


Figure 3-10: Olsen P % PRR showing the P recovered from each treatment as a mean of the two treatments (15 and 25 kg P ha^{-1}) and a mean of all sampling dates (*n=4*). Error bars represent ± standard error. There was a significant difference between treatments overall (p < 0.001).

Table 3-3 and Figure 3-4 show results of regression analysis between treatment characteristics, and mean values, for Olsen P and C_{DGT} %PRR. Analysis of results of %PRR was conducted to identify differences, following treatment application in the incubation experiment, rather than from historical treatments. Table 3-3 shows regression analysis for all treatment sources, and highlights a poor correlation between treatment characteristics measured. Figure 3-4 shows regression analysis for organic amendments only and also shows a poor correlation between treatment characteristics measured.

Table 3-3: Results of linear regression analysis between Olsen P/C_{DGT} and treatment properties for all treatments. Values represent the correlation coefficient displayed as R^2. There was a significant relationship for all tests p<0.05.

	Olsen P	**C_{DGT}**	**$TP_{treatment}$**	**$C:P_{treatment}$**
Olsen P	x	0.48	0.33	0.27
C_{DGT}	0.48	x	0.8	0.18

Table 3-4: Results of regression analysis between Olsen P/C_{DGT} and treatment properties (Excluding SP). Values represent the correlation coefficient displayed as R^2. Values in red indicate no significant relationship for the test p>0.05. Values in black represent a significant relationship for the test p<0.05.

	Olsen P	C_{DGT}	$TP_{treatment}$	$AVP_{treatment}$	$C:P_{treatment}$
Olsen P	x	-0.03	0.27	0.21	0.13
C_{DGT}	-0.03	x	-0.01	0.04	0.04

3.4 Discussion

3.4.1 Historical treatment applications

Historical addition of organic amendments to meet N demands will lead to a build-up of P measured by DGT compared to the control. Treatments were added to meet plant N demands; therefore the application rates of each treatment in (both overall treatment addition and total P addition) were different (Appendix Table A.2-1).

Adding organic amendments based on crop N demand can lead to a build-up of extractable P in soil (Read *et al*, 2007). N:P ratios differ between organic amendments and depend on source material, however it has been established by a number of authors (Read *et al*, 2007; Evers, 2002) that the P content of organic amendments generally provide a greater proportion of the available nutrient required by the plant, than N. This results in a build-up of extractable P characteristic of soils which have received long term amendment application based on N demands.

Read *et al*, (2007) explained that an N:P ratio of broiler litter is lower than the ratio of N and P absorbed from the soil by plant root (Bermudagrass), (2:1 vs 10:1) (Evers, 2002), this causes a build-up in soil P levels substantially greater than those required for optimum yield. Although this is specific to the aforementioned crop and treatment, the trend is characteristic of organic amendment application to agricultural soils.

The target P index for optimum wheat and oilseed rape yield is index 2 (16 to 25 mg P l^{-1} which translates to 13 to 21mg P kg^{-1} based on a bulk density of 1.2) to replace the off take in the yield and maintain the soil at the target index, based on RB209 fertiliser

recommendations (Defra, 2010). These crops were grown alternately in the 4 years of the ADAS –QC trials. Therefore it is evident (Figure 3-3) that for all soils including the control, the soil P status is already sufficiently high to meet crop P demand without addition of supplemental P.

With the previous information considered, it is likely that the greater P status of soils which had received application of organic amendments (FYM, SLRY, GW) compared to ammonium nitrate addition (Control ADAS-QC)) resulted from the applications of P based on N demand. As nutrient availability from inorganic sources is (relatively) well established in terms of uptake efficiency, inorganic inputs based on N demand were likely to have resulted in uptake of the required nutrients for plant needs. However the higher P status of soils which received application of organic amendments is likely to have resulted from the P supply in excess of plant needs being supplied by the organic amendments.

Reasons for the differences between the treatments, particularly the organic amendments are believed to have resulted from a range of factors. These factors are as follows;

- Differences in mass of treatment added to the soil.
- Differences in TP added to the soil from each treatment.
- Differences in C:P of each treatment added to the soil.
- Differences in N:P of treatments added to the soil.

P addition from organic amendments to each soil follows FYM>GW>SLRY. Although the TP added to the soil is expected to influence the pattern of P availability, it is also expected that other characteristics of the organic amendments added would have played a role in determining the P availability when measured for the purpose of this experiment.

The C:P of the treatments added is also expected to have been important in determining P transformations. Appendix Table A.2-1 shows differences in organic C to total P ratio (no information was available for total C:P ratio). The pattern follows SLRY>FYM>GW. The different organic C additions from each treatment are likely to have had different influences on soil biological processes, which determine P

transformations. Addition of C provides a substrate for stimulation of microbial processes, which can result in immobilisation of soil nutrients, reducing availability (Fuentes *et al*, 2006).

The effect of organic matter on overall soil health has been described in detail by (Fuentes *et al*, 2006). Its benefits are described in detail in **Chapter 1**. Amongst these benefits organic matter in the soil can help to improve P availability, by increasing soil chemical, biological and physical health, facilitating increases in cation exchange and pH, improving soil structure, increasing water holding capacity, modifying microbial activity, which have positive effects on P bioavailability (Fuentes *et al*, 2006).

A full explanation of the effects of organic amendment characteristics is described in (Section 3.3.2). The intention of this section is to highlight that there are differences between treatments which were applied to the soils previously, which are the factors most likely to have influenced the soils P characteristics and transformations following the field trials described.

As limited information is available regarding the previous treatment additions to Gleadthorpe soils, robust analysis on the effects of these is not possible. Therefore it is likely that these factors have played a role in determining the P status of these soils to date, however to fully understand how treatment characteristics influence P availability further experiments were designed.

3.4.2 Incubation experiment

3.4.2.1 P release from fresh treatment additions

Treatment application history was significant in determining P release from fresh treatment additions. The mean application rate of treatment additions to soil for the ADAS–QC trials experiments, over 4 years is displayed in Appendix Table A.2-1. It was established (Section 3.4) that management practices in ADAS-QC trials resulted in P application rates being supplied in excess of plant demand. Therefore when designing this incubation study, P application rates was based on RB209 fertiliser manual recommendations, for grass establishment, which were significantly lower than ADAS-QC trials rates (application rates used in this experiment were 59%, 49%, 39% lower than ADAS-QC trials for FYM, GW and SLRY respectively at 25kg P ha^{-1} addition).

It is expected that the higher application rates used in ADAS-QC trials is the main reason there is no significant change in P following treatment addition at rates used in this study. It was previously explained that the P supplied was greater than that required by the plants; however the availability of this P is largely influenced by the C content of the treatment. Damodar Reddy *et al,* (1999) suggested that addition of organic amendments to soil over time causes an increase in the organic P fraction, which is made available slowly over time. The C in the organic amendment provides a substrate for stimulation of microbial processes, which can cause immobilisation of soil nutrients, reducing availability (Fuentes *et al*, 2006) if the C:P is >200. This is likely to result in P release from previous additions still being the major factor influencing P release. Consequently, fresh organic amendment applications at recommended rates (which are much lower than ADAS-QC trials), results in no significant change in C_{DGT} compared to the control soil, (Figure 3-9).

It was important to determine how each treatment influenced available P at different rates of application. Previous experiments have determined that a linear relationship exists between application rate and extractable P (Iglesias Jimenez *et al*, 1993; Indiati and Sharpley, 1997, Laboski and Lamb, 2003). Results suggest that overall; addition of the different treatments result in an increase in treated soils compared to control soils (C_{DGT}) (Figure 3-4). However, between treatments, it is only following SP addition that there is a significant increase in P availability, organic amendment applications result in no significant difference, therefore in Figure 3-9 the increase in the soil which had received SP is masking the fact that there is no change following addition of organic amendments. It is anticipated that there is no response of soil P to fresh applications of organic amendments. Factors responsible are outlined below.

Soils which received SP showed a greater response (C_{DGT}) than those which received addition of organic amendments, due to the slow release of P from organic amendments (Figure 3-9). Soils which received SP addition had no documented organic amendment additions within the past 5 years. Fertilisation consisted of inorganic N addition in the form of ammonium nitrate. It is expected that this is the main reason for the significant release P with increasing application rate. It has been established by previous authors

that inorganic P sources are more soluble and thus release more readily than from organic amendments (Prasad, 2009; Sharpley and Sisak, 1997).

Previous authors have found contradicting results regarding comparison of the contribution of organic amendments to P availability compared to inorganic P sources. Some authors found that organic amendments can supply as much or more P than inorganic sources (Laboski and Lamb, 2003; Sikora and Enkiri 2005; Sneller and Laboski 2009). However, (Gracey, 1984; Griffin *et al*, 2003; Sharpley and Sisak, 1997) found inorganic sources supply more P then organic amendments. However in each of these studies there was no standard for soil type treatment source or application rate, so it is difficult to infer any meaningful findings from these studies.

Measures were taken to determine the effect of treatment characteristics on P release. Table 3-3 and Figure 3-4 show results of regression analysis, conducted to test the effects of treatment properties (C:P, TP, AVP) on overall P release in this experiment (C_{DGT} and Olsen P %PRR). This demonstrates that there is no significant relationship between treatment characteristics tested and P release following application to soils. As there was no significant P release from organic amendments (From C_{DGT}) following addition to soil, it was not possible to accurately determine the effects of these amendments on P release based on treatment chacteristics.

3.4.2.2 Comparison between Olsen P and DGT

Olsen P and DGT show a different trend as they are measuring different P pools. Overall there is a relationship between Olsen P and DGT throughout the experiment (Table 3-3 and Table 3-4) however, the correlation between the two methods is relatively poor (R^2=0.48 between all treatments). This reflects the differences in the way each method measures the P. A comparison between Figure 3-9 and Figure 3-10 shows that each method is showing a different trend. This can be linked to the forms of P each method is measuring, with DGT representing P available by diffusive supply, and Olsen P representing P which is both readily available, as well as a fraction of potentially available P (extractable P).

Analysis of Olsen P is particularly useful for comparing P transformations to studies carried out by previous authors; this is not possible with DGT as there are no previous

similar studies to compare. Previous studies on P mineralisation have suggested that P released from organic amendments can range between 5 to 30% (Lucero *et al*, 1995; Damodar Reddy *et al*, 1999), based on chemical extraction procedures. However Griffin *et al*, (2003) showed all P sources were <5% efficient in altering extractable P pools. This incubation experiment has shown mean P released over the whole experiment from SP as 58% efficiency, whereas GW, SLRY and FYM are 23, 5,-11 respectively (Figure 3-10),suggesting results in this study are comparable to previous in terms of Olsen P.

Treatment characteristics have a significant effect on P release (Griffin *et al*, 2003, Azeez *et al*, 2009, Miller *et al*, 2010). Laboski and Lamb (2003) suggested a critical treatment P content of 0.2-0.3 % above which there is no net immobilisation from organic amendments. Mafongoya *et al*, (2000) and Gichangi *et al*, (2009) confirm this. Application of treatments with a low P content (C:P ratio >300:1) can result in immobilisation, limiting availability. However treatments with a C:P ratio <200:1(Brady and Weil, 2002) result in net P mineralisation.

There is no significant effect of treatment characteristics on P release in this experiment (Table 3-3 and Figure 3-4). It was previously explained that a poor relationship between DGT and treatment characteristics existed because there was little release of available P following treatment application, compared to control soils. However for Olsen P there was a clear effect of organic amendments on P availability (Figure 3-10). Table 3-1 shows that GW and SLRY have a C:P <200, whereas FYM>200 which is expected to have influenced the trend shown in Figure 3-10 where FYM was the only treatment which resulted in an overall immobilisation. However these principals only apply for Olsen P measurements and not DGT, due to the forms of P being measured, as described above.

3.4.3 Soil pH

It is well established that soil pH plays an important role in determining P availability. It is important in controlling P speciation, precipitation–dissolution and adsorption–desorption reactions, this in turn influences P solubility and availability to the plant (Hinsinger, 2001) Mechanisms are described in detail in **Chapter 1**. Waldrip *et al*, (2011) showed that addition of organic amendments could increase pH and in turn P availability. At pH 7.2 there are approximately equal amounts of $H_2PO_4^-$ and HPO_4^{2-}.

Below this $H_2PO_4^-$ is the major form in solution, whereas HPO_4^{2-} is the main form above pH 7.2. fluenced by treatment addition.

Table 3-5 shows an overall increase in soil pH over the duration of the experiment for all soils which received organic amendment application (FYM, SLRY, and GW). However the soil which received SP showed an overall decrease over the period studied. It is unlikely that the changes had a significant influence on P availability, as P availability was not influenced by treatment addition.

Table 3-5: Soil pH at the start and end of the experiment for each treatment (*n=8*). STD error represents ± standard error. There was a significant difference between treatments overall st the start and end of the experiment (p < 0.001) (One way ANOVA).

Treatment	Start	End
SP	6.31	6.17
FYM	6.62	7.39
SLRY	6.61	7.3
GW	6.5	7.28
STD error	0.004	0.042

3.5 Conclusions

The work in this chapter refers to analysis carried out in two stages. Firstly analysis of soils sampled from ADAS –QC studies (Bhogal *et al*, 2011). These soils were then used in an incubation experiment to determine P availability following release from fresh treatment application. The conclusion will therefore consider each stage separately.

Historic treatment applications from ADAS –QC studies.

- Historical treatment additions were based on crop N requirement; this resulted in P supply in excess of plant demand. This resulted in a build-up of P measured by C_{DGT} in historically treated soils compared to control ADAS-QC soils by 126, 131 and 66% for FYM, GW and SLRY respectively.
- The principal mechanisms responsible for the trend, determined by investigation of the data available suggest

a. Differences in the total mass of P added in to soil differed between amendments, which influences the quantity of P added to the soil.
b. Differences in the total mass of C added to the soil differed between amendments, which primarily influences P mineralisation-immobilisation patterns, which have an important role in determining P availability.

Incubation experiment

- Following fresh addition of treatments in the incubation studies, there was a significant increase in C_{DGT} with increasing application rate following addition of SP by 71% overall, however there was no significant change in C_{DGT} measurements compared to control soils following addition of organic amendments.
- It is expected that the previous fertilisation regime for ADAS –QC studies, where P application rates were significantly higher than those used in this experiment (59%, 49%, 39%) for FYM, GW and SLRY respectively at 25 kg P ha^{-1} addition, meant that fresh additions in the incubation experiment were insufficient to significantly alter soil available P. This is because P release from organic amendments occurs slowly over time.
- The increase in C_{DGT} following SP addition occurred as the soil it was added to had no history of organic amendment addition, and solubility of SP is greater than organic amendments, thus a faster P release is the result.
- Olsen P and DGT showed a different trend as they were measuring different P pools. DGT represents P available by diffusive supply, and Olsen P represents P which is both readily available, as well as a fraction of potentially available (extractable P).

4 Kincraigie incubation experiments

4.1 Introduction

To fully understand the influence of treatments (GW, FYM, SLRY and SP) on soil P availability, following application, it is useful to eliminate the residual effect of previous additions. Therefore, soil with no prior history of organic amendment application, was used to investigate this influence. This experiment used soils, which were deficient in P (P index 0). Treatments were applied at rates recommended by the RB209 fertiliser manual (Defra, 2010) for the relevant P index, for grass establishment.

The rationale of this study was to identify differences in P availability, from treatments at two agronomically relevant application rates. It was expected that microbial mineralisation-immobilisation of P would be important in determining P transformations. Therefore the contribution of each treatment to soil microbial biomass P (MBP) was also investigated. The objectives are outlined below.

Objectives

1. To determine P availability patterns in soils deficient in plant available P following addition of the aforementioned treatments, at agronomic application rates.

2. To investigate how organic amendments perform compared to SP in the aforementioned soils.

Hypotheses

- Following lysis of microbial biomass P, there will be a significant increase in C_{DGT} at the subsequent sampling date. Olsen P will not detect this increase.
- SP will be responsible for greater P release than organic amendments.
- The treatment characteristics $C{:}P_{treatment}$ and $TP_{treatment}$ play a significant role in determining P release from each treatment.

4.2 Methodology

4.2.1 Soil and treatment setup

The treatments (FYM, GW, SLRY and SP) used in this experiment are the same in all glasshouse and incubation studies. The composition of organic amendments is reported in Table 3-1. The volume of each treatment added to the soil was calculated based on the application rate required, the bulk density of the soil (Table 4-1), and the total P content of each treatment. To determine the volume of each treatment to add to each pot the calculation (Equation 3-1).

Soil from Kincraigie farm, Strathmiglo, Fife, Scotland (Figure 1-7) was weighed into incubation pots and labeled accordingly. Treatments were applied at two application rates 80 and 120 kg P ha^{-1}, actual volumes of each treatment added to the soil are shown in Table 4-2, and there was an un-amended control which received no treatment addition. The application rates used reflect the initial P index of the soil, and the amount of P which is required to sustain ryegrass growth. Four replications per treatment were required for robust statistical analysis, taking into account variability in chemical analysis (Kokkora, 2008; Antille, 2011).

Table 4-1: Soil textural analysis, bulk density and field capacity at the start of the experiment

Determination	**SOIL**
	Kincraigie
Field Capacity (%, w w^{-1})	51.6 (2.3)
Bulk density (g cm^{-3})	1.12 (0.03)
Textural analysis	
Sand	42.7
Silt	38.5
Clay	18.9

4.2.2 Soil incubation

Incubation pots contained 400g of soil, which had been dried and sieved (<2mm). Treatments were added to each pot and thoroughly mixed with the soil. De-ionised water was then added to the soil to bring it to the required water content (60% filed capacity) (Table 4-1). Pots were placed in the incubator in the absence of light and

incubated at 25°C. Pots were covered with aluminium foil and holes pierced in the top to allow oxygen circulation, but prevent water loss. A tray of de-ionised water was also maintained at the bottom of the incubator to keep conditions humid and stops the soils drying out (Antille, 2011). Pot weights were monitored weekly for water loss, and any loss was replenished with de-ionised water. The duration of the experiment was 90 days, (as information required was for short term P dynamics) and sampling carried out on days 0, 1, 7, 14, 30, 60 and 90.

Table 4-2: Mass of each treatment added to each incubation pot for each incubation experiment for the study of P availability following addition of organic amendments; n=4.

	Treatment(g)			
	FYM	**SLRY**	**GW**	**SP**
App rate (kg P ha^{-1})				
0	0	0	0	0
80	8.9	29.8	8.9	0.28
120	13.3	44.4	13.3	0.42

4.2.3 Measurement and analysis

On each sampling date, 35 g of soil was removed from each pot and subsequently analysed for Olsen P, and MBP, by methods outlined in Table 4-3. C_{DGT} analysis differed from (**Chapter 3**), in that sampling was not carried out in situ in the pot, instead standard protocol (Section 2.2.2.2) was used where soil was dried, ground, added to a petri dish and brought to 100% field capacity before deployment. The decision was made to use this method of analysis based on the practicalities of measuring soil MBP. As explained above pots were filled to a water content of 60% field capacity, for optimum soil microbial biomass phosphorus (MBP) measurement, however it was determined, that DGT measurements should be carried out at field capacity. It was therefore impractical to deploy DGT devices in situ in pots.

The percentage extractable P in the soil recovered from each treatment was determined using (Equation 3-2). This is described as percentage phosphorus recovery ratio (% PRR) by Miller *et al*, (2010), and is derived from a similar equation termed % efficiency by (Griffin *et al*, 2003).

4.2.4 Statistical analysis

Statistical analysis was carried out to determine the effects of treatments on C_{DGT}, Olsen P and MBP. This was achieved using repeated measures analysis of variance (ANOVA). Post-hoc analysis was conducted using Fisher least significant difference (LSD) Homogeneous groups (0.005) where there is no significant difference; the same lower case letter is used. Error bars on each graph represent ± standard error. All statistical analysis was carried out using STATISTICA 11 software. Residuals were all normally distributed. Full results of ANOVA analysis are displayed in Appendix Table B.1-1to Table B.1-5.

Table 4-3: General soil characteristics before the experiment, SE indicates ± standard error; Data are means *n=3*.

Determination	Kincraigie	SE	Method
Texture	Loam	-	
Total N (%)	0.52	0.01	BS EN 13654-2 (2001)
Total C (%)	5.10	0.06	BS 7755 Section 3.8 (1995)
C:N	9.85	0.003	
C:P	33.6	0.013	
Total OC (%)	4.7	0.05	BS 7755 Section 3.8 (1995)
Organic matter(%)	8.01	0.08	MAFF (1986) Method No.: 56
Extractable P (mg kg^{-1})	7	0.10	Olsen *et al*, (1954); BS 7755 Section 3.6
P Index	0	-	
Microbial Biomass P (mg kg^{-1})	0.8	0.66	Brookes *et al*,(1982)
Extractable K (mg kg^{-1})*	19.1	-	MAFF (1986) Method No.: 63
K Index	0	-	
Available N (mg kg^{-1})	2.6	0.01	MAFF (1986) Method No.: 53
Total P (%)	0.15	0.01	US EPA Method No.: 3051; BS 7755-3.13 (1998)
pH	6.57	0.01	MAFF (1986) Method No.: 32
Oxalate Fe (g kg^{-1})	6.2	0.3	Carter (1992) Method 23.5
Oxalate Al (g kg^{-1})	9.4	0.5	Carter (1992) Method 23.5
Mg (mg l^{-1})*	124.1	-	MAFF (1986) Method No.: 40
Ca (mg l^{-1})*	2175	-	MAFF (1986) Method No.: 40

*Measurements conducted prior to this study and displayed in Figure B.2-1

4.3 Results

4.3.1 C_{DGT}

To evaluate the effects of the rate of added treatments on C_{DGT} values, mean values for each rate were calculated and summarised in Figure 4-1, using repeated measures ANOVA. Results for all treatments suggest C_{DGT} decreases until day 30, then increases between day 30 and 60, then decreases again between day 60 and 90.

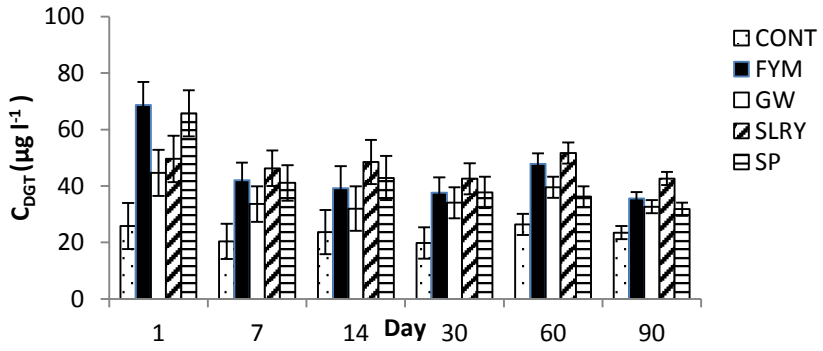


Figure 4-1: Mean C_{DGT} measurements as a mean of both application rates (80 and 120 kg P ha^{-1}) for each soil treatment. Control *n=4* and treated *n=8*. Error bars represent ± standard error. There was a significant difference between time and soil treatments p=0.008.

There was a significant difference between treatments as a mean of all sampling dates (p=0.0019) (Figure 4-2). Overall C_{DGT} from treatment addition followed the trend SLRY≥FYM≥SP>GW. All treatments resulted in a higher C_{DGT} than the control.

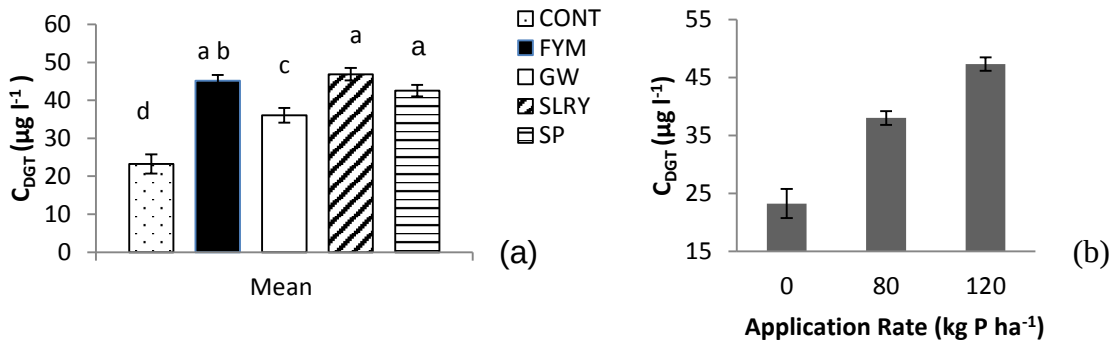


Figure 4-2: (a) C_{DGT} of treatments as a mean of all sampling dates and application rates. Control *n=4* and treated *n=8*. There was a significant difference between soil treatments

p=0.002. (b) Increasing C_{DGT} with increasing application for control and treated soils with increasing application rate from 0 to 120 kg P ha^{-1}as a mean of each sampling date for incubation experiments (p<0.001). Data are means *n=4*. Error bars represent ± standard error. Significant differences between treatments are denoted by lower case letters (Fisher LSD).

There is a significant different between control and treated soils, and a significant difference between the two rates of application (p<0.001) (Figure 4-2(b)). Results show an increase in C_{DGT} with increasing application of treatments. These results are displayed as a mean of all treatments. This is because there was no significant difference between treatments with increasing application rate.

4.3.2 Olsen P

To evaluate the effects of the rate of added SP, GW, FYM and SLRY on Olsen P values, mean values for each rate were calculated and summarised in Figure 4-3. Analysis showed there was a significant difference (p<0.001) between time and soil treatment for all analysis.

There is no significant difference between treatments as a mean of both application rates for Olsen P over time (p=0.18) (Figure 4-3). However there is a significant difference between treatments as a mean of all sampling dates (p=0.003) (Figure 4-4(a)). Results for all treatments suggest that Olsen P decreases until day 14, and then stays relatively constant until day 90 for all treatments.

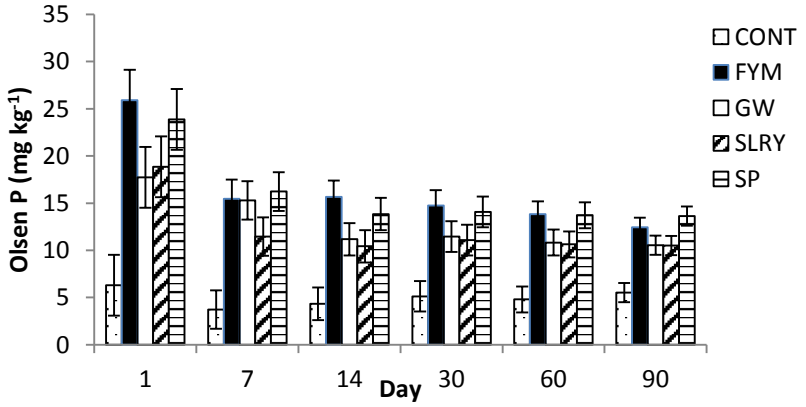


Figure 4-3: Mean Olsen P measurements as a mean of both application rates (80 and 120 kg P ha^{-1}) for each soil treatment. Control *n=4* and treated *n=8*. Error bars represent ±

standard error. There was a no significant difference between time and soil treatments p=0.179.

There was a significant difference between all treatments as a mean of all sampling dates ($p<0.001$) (Figure 4-4(a)). Overall Olsen P from treatment addition followed the trend FYM≥SP>GW≥SLRY. All treatments resulted in a higher C_{DGT} than the control.

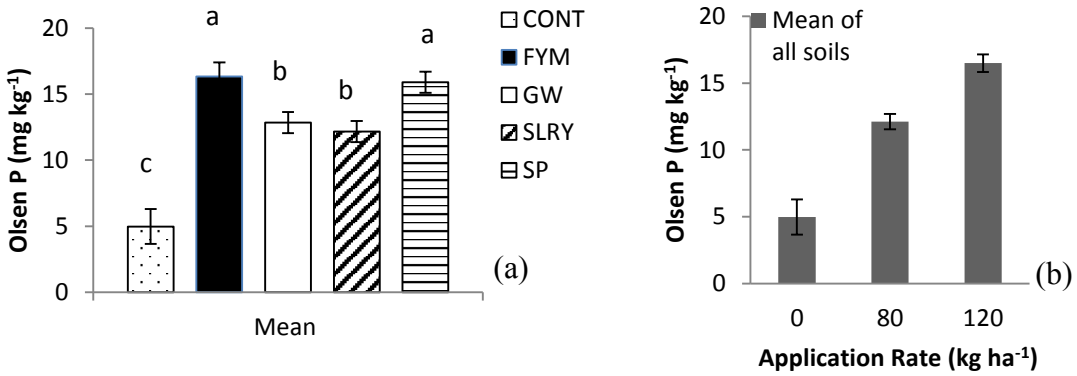


Figure 4-4: (a) Treatment mean of all sampling dates and application rates for Olsen P. Control *n=4* and treated *n=8*. There was a significant difference between soil treatments overall p=0.003 (b) Increasing Olsen P with for control and treated soils with increasing application rate from 0 to 120 kg P ha^{-1}as a mean of each sampling date. Data are means *n=4*. There was a significant difference between control and treated soils and between both rates p <0.001. Error bars represent ± standard error. Significant differences between treatments are denoted by lower case letters (Fisher LSD).

There is a significant different between control and treated soils, and a significant difference between the two rates of application ($p<0.001$) (Figure 4-4(b)). Results show an increase in Olsen P with increasing application of treatments. These results are displayed as a mean of all treatments. This is because there was no significant difference between treatments with increasing application rate (Appendix Table B.1-2).

Mean values of Olsen P (%PRR) are displayed in Figure 4-5. Results show significant differences between all treatments. Treatment efficiency follows the pattern (FYM≥SP>GW≥ SLRY). It must be noted that % PRR values are displayed only for Olsen P and not C_{DGT}. % PRR for C_{DGT} would not add any additional information to that displayed in Figure 4-1and Figure 4-2. %PRR was conducted for Olsen P in order to compare with similar studies conducted by pervious authors.

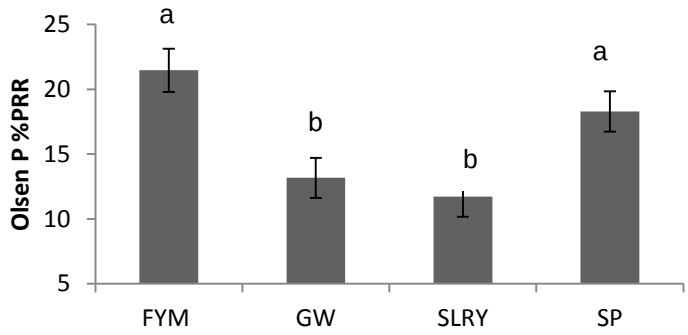


Figure 4-5: Olsen P % PRR showing the P recovered from each treatment as a mean of the two treatments (80 and 120 kg P ha^{-1}) and a mean of all sampling dates (*n=4*). Error bars represent ± standard error. There was a significant difference between treatments overall (p < 0.006). Significant differences between treatments are denoted by lower case letters (Fisher LSD).

4.3.3 Microbial biomass P (MBP)

There is a significant difference between treatments for microbial biomass phosphorus (MBP) over time (p<0.001) (Figure 4-6) and as a mean of all sampling dates (p<0.001) (Figure 4-7). However there was no significant difference between treatments at different application rates over time. Results for all treatments suggest that MBP increases steadily until day 30, and then decreases until day 90 for all treatments.

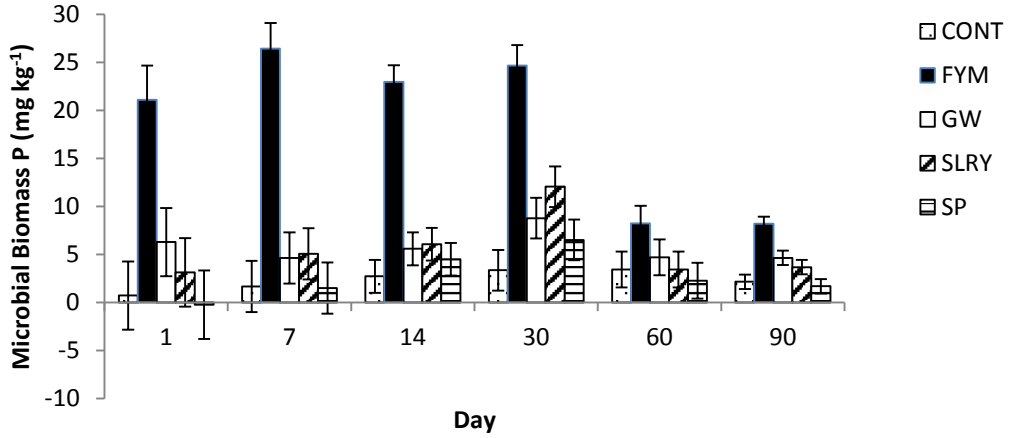


Figure 4-6: Results of mean MBP P measurements for both application rates (80 and 120 kg P ha^{-1}) for each soil treatment. Control *n=4* Treated n=8. Error bars represent ± standard error. There was a significant difference between treatments overall p <0.001.

There was a significant difference between all treatments as a mean of all sampling dates (Figure 4-7) ($p<0.001$). Overall MBP production from treatment addition followed the trend FYM>SLRY≥GW≥SP. All treatments resulted in a higher MBP than the control.

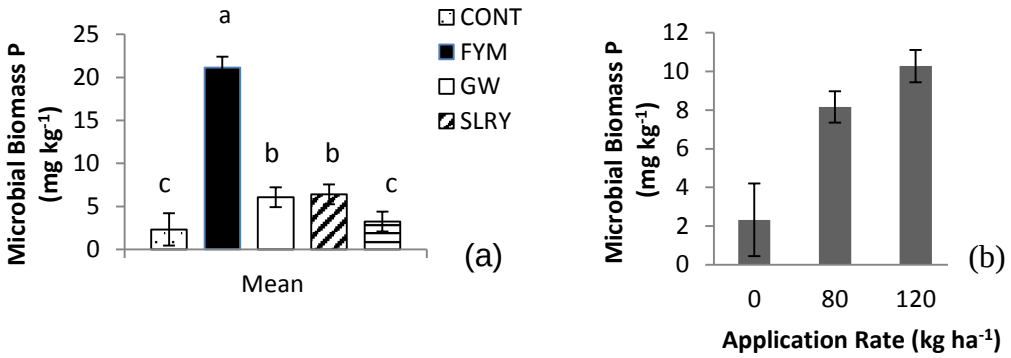


Figure 4-7: (a) Overall MBP of treatments as a mean of all sampling dates and application rates. Control *n=4* and treated *n=8*. Error bars represent ± standard error. There was a significant difference between soil treatments overall p=0.001. (b) Increasing MBP with increasing application rate as a mean of all treatments. *n=4*. There was a significant difference between application rates overall p=0.001. Significant differences between treatments are denoted by lower case letters (Fisher LSD).

There is a significant difference between control and treated soils, and a significant difference between the two rates of application ($p<0.001$) (Figure 4-7b). Results show an increase in MBP with increasing application of treatments. These results are displayed as a mean of all treatments. This is because there was no overall significant difference between treatments with increasing application rate.

4.3.4 Relationship between MBP and OlsenP/C_{DGT}

Results for C_{DGT} (%PRR) for all treatments were significantly different over time ($p<0.001$) (Table 4-4). However there was no significant difference between the two application rates over time. Results show a decrease for each treatment from day 1 to day 30, followed by an increase from day 30 to day 60, and a subsequent decrease from day 60 to 90, for all treatments except SP (therefore only for organic amendment applications).

Table 4-4: C_{DGT} (%PRR) for as a mean of both application rates (80 and 120 kg P ha^{-1}) for all treatments for each sampling date for 90 days of the experiment. SE indicates ± standard error; Data are means *n=8*.

Soil treatment	FYM	GW	SLRY	SP	SE
Time (Days)					
1	0.056	0.030	0.034	0.054	0.007
7	0.034	0.023	0.035	0.031	0.006
14	0.021	0.014	0.036	0.031	0.007
30	0.025	0.017	0.031	0.026	0.005
60	**0.030**	**0.022**	**0.035**	**0.013**	**0.003**
90	0.017	0.006	0.026	0.012	0.002

Results for Olsen P (%PRR) for all treatments were significantly different over time ($p<0.001$) (Table 4-5). However there was no significant difference between the two application rates over time. Results show a decrease for each treatment from day 1 to day 90.

Table 4-5: Olsen P (%PRR) for as a mean of both application rates (80 and 120 kg P ha^{-1}) for all treatments for each sampling date for 90 days of the experiment. SE indicates ± standard error; Data are means *n=8*.

Soil treatment	FYM	GW	SLRY	SP	SE
Time (Days)					
1	43.6	19.0	20.8	29.2	4.3
7	28.9	19.3	12.6	21.0	3.2
14	23.6	11.4	10.2	15.9	2.4
30	12.0	10.7	9.6	15.0	1.9
60	**10.8**	**10.1**	**9.3**	**15.0**	**1.5**
90	9.9	8.5	7.8	13.7	1.2

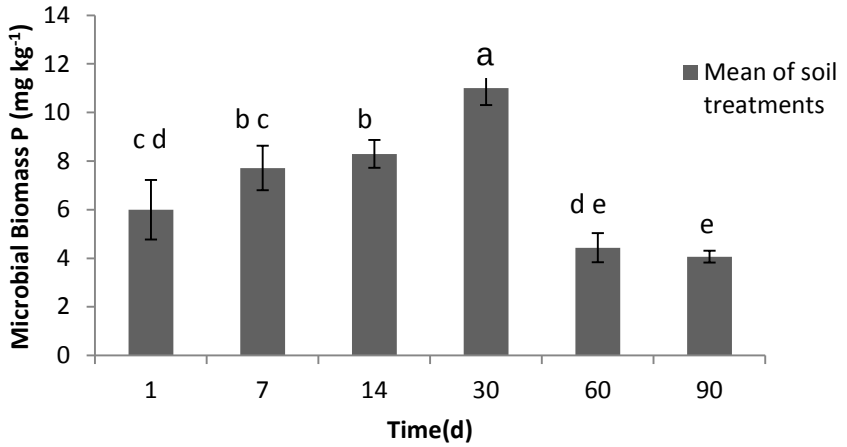


Figure 4-8: The overall trend of soil MBP production with time as a mean of all soils. Error bars represent ± standard error. There was a significant difference in overall MBP with time p=0.001. Significant differences in MBP with time are denoted by lower case letters (Fisher LSD).

Regression analysis highlighted in Table 4-6, suggests there is no significant relationship ($p>0.05$) between C_{DGT} and treatment characteristics, or no significant relationship with MBP. There is a relationship with Olsen P, however this is a poor correlation ($R^2=0.2$).

Table 4-6: Regression analysis between treatment characteristics, Olsen P/C_{DGT} and soil microbial biomass P (MBP). Values represent the correlation coefficient displayed as R^2. Values in red indicate no significant relationship for the test p>0.05. Values in black represent a significant relationship for the test p<0.05.

R^2	Olsen P	C_{DGT}	$TP_{treatment}$	$C:P_{treatment}$
Olsen P	x	0.20	0.07	-0.02
C_{DGT}	0.20	x	-0.04	-0.03
MBP	0.01	0.02	0.17	0.76

4.4 Discussion

4.4.1 C_{DGT}

There is a poor relationship between C_{DGT} and treatment characteristics (Table 4-6). As C_{DGT} has been proven to be an accurate indicator of plant available P (Mason *et al*, 2008, Menzies *et al*, 2005, Mcbeath *et al*, 2007), it was in the interest of this work to establish how treatment characteristics influence C_{DGT} in order to gain an understanding of how treatment characteristics can provide P in a form which is readily available to the plant. Therefore treatment C:P ratio ($C:P_{treatment}$) and TP content ($TP_{treatment}$) were used as a predictor of C_{DGT}. Other authors have found that these characteristics cannot be used as an accurate predictor of P availability. Nwoke *et al*, (2004) highlighted that previous attempts to elucidate the relationship between treatment characteristics and soil P availability have led to inconsistent results. These were attributed to (1) differences in soil sorption capacity (Singh and Jones, (1976) and (2) Modification of the availability of native soil P (Struthers and Sieling, (1950). This suggests that over the short term it is difficult to obtain accurate information on P released using treatment characteristics. It is expected that P release is influenced by a more complex relationship between soils physical, chemical and biological property changes. It is evident that the characteristics of the treatments differ significantly from each other in more than just their P contents.

In addition to measured differences between the treatments in this study (Table 2-1), information from Fuentes *et al*, (2006), suggests that properties of manures, compost and inorganic P differ in many respects, which will result in different P transformations following application to soil. Laboski and Lamb (2003) found that manures contained high molecular weight compounds, probably DNA, polyphosphates and inositol phosphate, which can be adsorbed onto the soil surface, can contribute to the release of inorganic P bound to the soil surface. As a result, manures were always significantly more available than fertiliser in incubation studies conducted between one and nine months. In addition during microbial degradation organic acids are produced, which can compete with P for adsorption sites on soil surfaces, causing P from the manure to be more available. Scherer and Sharma (2002) indicated the positive effect of FYM on extractable P is probably due to these organic anions acting as chelating agents for Fe and Al blocking potential P adsorption sites. It is conceivable that this interaction

between organic compounds and soil surfaces has resulted in the trends in this experiment where P from FYM and SLRY is greater than SP and GW for C_{DGT} measurements (Figure 4-2).

When analysing the total P content of composts, Park *et al,* (2004) explained that neither the quantities, nor the P species which are available are entirely clear. Following P application to managed soils, an accumulation of P species which are unavailable for plant uptake, can occur. Frossard *et al,* (2002) found that only 30 to 50% of the available P in compost is readily plant available, and a large portion is in the form of condensed calcium phosphates. The act of composting leads to the formation of a more stable product over time. A more mature product leads to a reduction in easily degradable organic compounds such as organic acids (Smith and Hughes, 2004) reduction in microbial biomass, and thus enzymatic activity. These factors may therefore be important mechanisms determining the C_{DGT} of GW (lower than FYM and SLRY (Figure 4-2)), which underwent composting until a stable product was formed. However a caveat of this statement is that although GW has undergone more stabilisation than SLRY and FYM, the materials which were used to create the product were vastly different, creating a further avenue for P availability differences between treatment sources.

SP was no more efficient at releasing P to soil than all other treatments. It was established in **Chapter 3** that SP was significantly more efficient at releasing P than organic amendments; however it was expected that this was influenced more by the treatment application history than the treatment characteristics. In this experiment a different trend is identified. SP is more efficient at releasing P than GW, but not FYM or GW (Figure 4-2). Reasons for the differences between treatments have already been established in the previous paragraphs. Information above helps identify potential mechanisms responsible for differences in P availability following treatment application.

It has previously been established that increasing P availability with increasing treatment addition provides a model for which the efficiency of the treatment can be judged (Sikora and Enkiri 2005). Therefore treatments were applied with increasing application rates, in order to establish the efficiency of each treatment. Figure 4-2b

shows a significant increase in P availability with increasing application rate for all treatments ($p<0.001$) however there was no significant difference between each treatment overall (Appendix Table B.1-1). This suggests that over the timeframe of this experiment each treatment applied resulted in similar levels of efficiency of P release with increasing application rate.

4.4.2 Olsen P

Results of Olsen P measurements showed a similar overall trend to $C_{DGT,}$ where following treatment addition there was an immediate release of P, at day 1, then the concentration decreased over time for all treatments. It is likely that similar mechanisms are responsible for overall general trends, therefore to avoid repetition reference will be made to the previous section (Section 3.2) for factors which influence P availability over time. In addition, like DGT, Olsen P shows a poor relationship with treatment characteristics (Table 4-6), it is expected that the mechanisms described for DGT are also relevant for Olsen P. Although general mechanisms influencing P availability over time are thought to be highlighted from month methods, an important factor to consider is that although there is a relationship between the two techniques ($p<0.005$), the correlation is not strong ($R^2=0.2$), therefore the influence of these mechanisms on P release is measured to a different extent by the two methods.

Previous studies on P mineralisation have suggested that P released from organic amendments can range between 5 to 30% (Lucero *et al*, 1995; Damodar Reddy et al, 1999), based on chemical extraction procedures. However a study by Griffin *et al*, (2003) showed all P sources were < 5% efficient in altering extractable P pools. This experiment has shown mean P released over the whole experiment from SP as 18% efficiency, whereas GW, SLRY and FYM are 13, 11, 21 respectively (Figure 4-5). This is in line with estimates from previous studies. This suggests that from analysis of soil P by conventional methods, adding organic amendments based on their TP content at the same rate as SP, it is possible to obtain soil P values at levels greater than(FYM) or less than (GW and SLRY), inorganic fertilisation (SP). In addition it shows that all treatments could alter available P pools by >5%.

4.4.3 MBP

The addition of organic amendments significantly influenced MBP production. Following treatment incorporation, MBP increased progressively for all treatments until day 30 and decreased and remained constant from day 60 to day 90. It is expected that the trend of increase until day 30 represented immobilisation of P by the soil microbial biomass. The decline from day 30 is thought to represent lysis of microbial P cells, distributing it from the soil microbial biomass into available and extractable P forms.

It has been established that microorganisms constitute a large pool of P in the soil and mediate several key processes in the biogeochemical P cycle. Microbial uptake of P and its subsequent release and redistribution significantly affect P availability to plants, especially following addition of organic amendments (Oberson and Joner, 2005). Zhang *et al,* (2005) explained that soil microbial biomass is related to several factors, such as organic C and N limitation, residue and nutrient management, differences in plant species, soil texture, soil moisture and temperature. In this study, temperature was kept constant, and water regime was adjusted regularly based on optimal conditions, the soil texture was constant and there was no plant effect. Control over environmental conditions helped to isolate limiting factors based on the treatments applied. However treatment addition to soil had an effect on its physical, chemical and biological properties. Differences in C, N and P input in association with each treatment are likely to have effected soil microbial biomass production (Table 4-6).

4.4.4 Relationship between MBP and Olsen P/C_{DGT}

Following lysis of microbial P cells, there was a significant increase in C_{DGT} at the subsequent sampling date. Olsen P does not detect this increase. As the soil microbial biomass P decreases significantly between day 30 and 60 (Figure 4-6) there is an increase in the C_{DGT} flux (Table 4-4) this shows that following lysis P is redistributed back to the soil solution and measured by an increase in C_{DGT} flux. As C_{DGT} then decreases again between day 60 and 90, the P is being redistributed from soil solution to less available P forms.

Unlike the behaviour found in measurements of $C_{DGT,}$ there is no significant change in Olsen P measurements after day 7 (Table 4-5). This suggests that measurements of

Olsen P do not reflect significant changes to P availability in response to microbial immobilisation in the first 30 days and subsequent lysis thereafter.

Previous authors have determined that the relationships between different soil available P pools and microbial biomass P following application of treatments can be explained by the relationship between different methods of extraction (Sequential extraction). Gichangi *et al*, (2009), carried out an experiment to investigate changes to resin P, $NaHCO_3$ (Olsen P), MBP and HCl, representing P forms in terms of availability respectively. A similar pattern for MBP to that in this experiment was identified, where available P (resin P) decreased with time and was resupplied to MBP representing a gradual immobilisation before mineralisation. However results in Gichangi *et al*, (2009) show a different trend to this study, as mineralisation coincided with an increase in $NaHCO_3$ (Olsen P), thus it was suggested that this was evidence to support the theory of mineralised P being transferred to extractable P pools.

The significance of the distribution from soil solution P to MBP is profound, as this P which is immobilised by the soil microbial biomass, is unavailable for fixation to soil colloids, and released later. This information is useful, as it provides evidence to support the theory that use of organic amendments can be used to regulate the quantity of P released over time. Release of P at a time when plants require it most, can help significantly improve P use efficiency (Eghball and Power, 1999; Syers *et al*, 2008). However conditions in this study do not resemble variability experienced in the field. Oberson and Joner, (2005) describe the importance of temporal fluctuations in determining MBP concentrations under field conditions.

4.4.5 pH

There is a significant change in soil pH from the start to the end of the experiment for all treatments including the control ($p<0.001$). Table 4-7 shows a similar trend of decrease in pH following application of treatments; however GW application results in a smaller decrease in pH than other treatments. Results show little change in soil pH in this experiment compared to the control; however the pH of the soil treated with GW increases compared to the control. It is therefore expected that changes in pH have less influence on overall P availability in this experiment than others.

Table 4-7: pH values of each treated soil at the start and end of the 90 day experiment. SE indicates ± standard error; Data are means *n=8*.

Treatment	Start	End
GW	6.63	6.1
SLRY	6.63	6.15
FYM	6.63	6.27
SP	6.63	6.1
Std error	**<0.01**	**0.02**

4.5 Conclusions

- SP was no more efficient at releasing P to soil than all other treatments. SP addition increased C_{DGT} over control soils by 83% overall, compared to 94, 59 and 99% for FYM, GW and SLRY respectively. Soils showed an increase in C_{DGT} with increasing application rate of each treatment.
- There is a poor relationship between C_{DGT} and treatment characteristics for $C{:}P_{treatment}$ and $TP_{treatment}$. It is expected that the biological, chemical and physical characteristics of the treatments differed so much that a complex range of interactions was responsible for changes to soil P; therefore changes could not be explained by a single characteristic.
- Following lysis of microbial biomass P, there was a significant increase in C_{DGT} at the subsequent sampling date. This signifies a transfer of P from the soil microbial biomass to the soil solution, causing an increase in P available by diffusive supply. Olsen P does not detect this increase. This suggests that the DGT technique shows promise in measuring the P availability following mineralisation.
- Treatments had little effect on soil pH, compared to the control except for GW. Therefore there appeared to be no influence of pH change on P availability.

5 Gleadthorpe pot experiments

5.1 Introduction

Glasshouse pot studies have been used extensively to assess P behaviour in soil and its transfer to plants, following application of a wide range of treaments (Read *et al*, 2007; Yilvano *et al*, 2008; Waldrip *et al*, 2011). Pot experiments were set up as a natural progression to the incubation studies, with the advantage of being able to assess plant response to treatment addition, under controlled conditions. The experiment was designed to understand changes in P availability, in soils, which have had a history of repeated application of organic amendments and the transfer of P from soil to plant in two different scenarios.

1. On soil which has had no additional application of organic amendments over a 6-month period.

2. By adding the corresponding organic amendment to the historically-amended soil over a 12-month period.

The main aim of the chapter is to understand how repeated application of different organic amendments influence C_{DGT}, Soil Solution P, Olsen P, and the subsequent influence on plant characteristics (dry matter yield (DMY) and total phosphorus uptake (TP_{uptake})) compared to soil which has only received inorganic fertilisation. The objectives are summarised below.

1. Determine P availability patterns in soils which have historically received application of organic amendment, and the subsequent impact on ryegrass yield and P uptake, with and without addition of further treatments (FYM, GW, SLRY and SP) at agronomic application rates.
2. To investigate how organic amendments perform compared to SP in the aforementioned soils.

Hypotheses

- Historical addition of organic amendments to meet N demands will lead to a build-up of P measured by DGT compared to control soils.
- DGT will show a better relationship with DMY and TP_{uptake} than other methods of P analysis.
- Treatment application history will be significant in determining P release from fresh treatment additions.
    - Soils which receive SP will show a greater response (C_{DGT}) than those which received addition of organic amendments, due to the slow release of P from organic amendments.
- Historical treatment additions are more important at determining yield and P uptake than fresh additions.

5.2 Methodology

5.2.1 Experiment details

Experiments were conducted in the Cranfield University glasshouse facility, (Appendix Figure C.3-1). Field capacity, textural analysis and bulk density of the soil used are displayed in Table 2-1. Soil analysis is displayed in Table 2-2 and was described in detail in Section 3.2.1. In addition, soil solution P was measured. Measurement was based on the centrifugation method described by Zhang *et al*, (2006) where immediately following DGT deployment, soil was centrifuged at 13000 rpm for 5 minutes and filtered through a 0.45µm pore filter syringe, and the soil solution was then measured using a spectrophotometer by the same method as DGT. Pots were of 10 litre capacity and were filled with 8kg of air-dried soil which was sieved to <2mm. The base of the soil was also filled with >25mm gravel, which stopped soil loss, but allowed free drainage. When filling the pots with soil, urea was applied at 5cm below the ryegrass seeds in order to restrict direct contact during early stages of germination, as urea causes damage to seeds and developing roots. Tap water was added to soils gradually to avoid leaching of N. Ryegrass seeds were spread evenly across the soil surface at a rate of 4g m^{-2}. Pot diagrams are displayed in Figure 5-1; photographs of experimental setup are displayed in Figure 5-2.

The experiment comprised one soil type, categorised by four different soil treatments it had historically received, FYM, SLRY, GW and a control (control ADAS-QC).

The pot experiment consisted of 2 stages; Table 5-1 provides a summary of the experiment. Stage 1 used the aforementioned soils without additional P addition, but with an application of urea at a rate of 150 kg N ha^{-1}, to provide sufficient N for crop growth, and so that it was not a limiting factor. This stage was conducted for six months from October 2011 to April 2011. A randomised block design was used with 4 replicates of each treatment. Stage 2 used the aforementioned soils, which had been established for 6 months. Corresponding organic amendments (Table 5-2) were added to each soil and superphosphate (SP) added to control ADAS –QC at two application rates, 15 and 25 kg P ha^{-1}. It is important to establish here that treatments refers to the application of (SP, GW, FYM, SLRY), and organic amendments refers to (GW, FYM, SLRY). This is for the purpose of distinguishing superphosphate (SP) and organic amendments. A blanket application of N was applied like Stage 1 at 150 kg N ha^{-1}. All materials were added to the surface of the soil immediately following the final harvest of Stage 1. Treatments were applied in April 2011 and grown until April 2012, in which time 4 harvests were taken. Weeds were removed manually from pots; all treatments were added to the soil manually.

When reporting change in C_{DGT} over time between treatments, Equation 5-1 is used to calculate % change.

$$\% \text{ change} = \frac{x - y}{x} * 100 \qquad \textbf{Equation 5-1}$$

Where x is the C_{DGT} concentration of the untreated soil at day 0 and y is the C_{DGT} concentration of the treated soil at the sampling day.

5.2.2 Soil and crop measurement and analysis

Soil was analysed prior to the experiment following collection from the field. Soil sampling was conducted with an auger 15cm length * 1.5 cm diameter. Three sub samples were taken from the pot on each sampling date, and holes re-filled with soil from the initial batch. Ryegrass was cut 2cm above the soil surface (Antille 2011, Cordovil *et al*, 2007)). Ryegrass was oven dried at 60°C for 48hours (MAFF, 1986; Method No.: 1), this was then reported as DMY (kg ha^{-1}). Determination of total P in

plant material (TP_{plant}) (mg kg^{-1}) was required for the estimation of total P uptake (TP_{uptake}) (kg ha^{-1}) (Equation 5-2).

$$TP_{Uptake} = TP_{Plant} * \text{DMY}$$ **Equation 5-2**

Table 5-1: Representing overall experimental setup, highlighting timescales for each stage and when treatments were added and samples taken.

Year	2010			2011												2012			
Month	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4
Experimental details	Stage 1						Stage 2												
Added N	150 kg N ha^{-1} ~~~~~~~~~~~~~~						150 kg N ha^{-1} ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~												
Added treatment	~~~~~No additional treatments~~~~						Treatment addition ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~												
Ryegrass cuts/Soil sampled	x			x		x				x		x		x				x	

Table 5-2: Details of which treatment was added to the corresponding soil taken from ADAS-QC plots, for the incubation experiment.

	Treatment(g)			
	FYM	**SLRY**	**GW**	**SP**
App rate (kg P ha^{-1})				
0	0	0	0	0
15	18.6	80	18.6	0.53
25	32	120	32	0.88

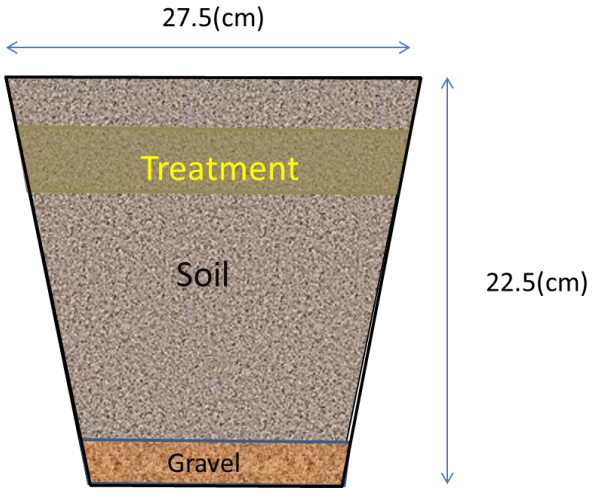


Figure 5-1: Schematic diagram showing the profile of the pots used in the experiment.

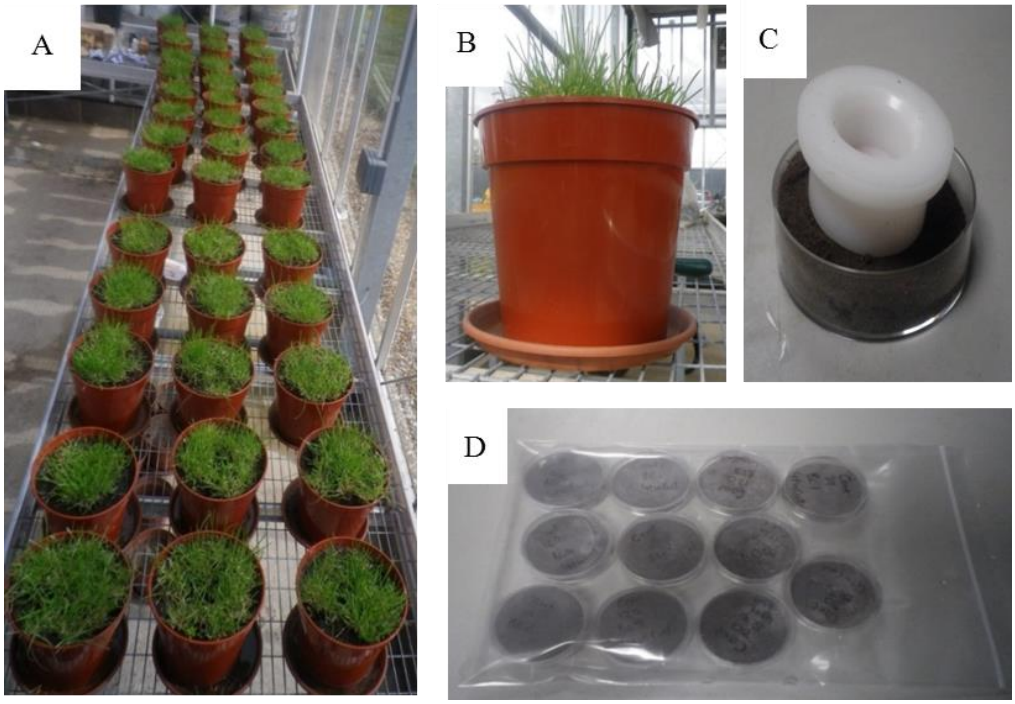


Figure 5-2: Experimental photographs displaying (A) the pot experiment set up (B) A diagram with established ryegrass (C) A DGT device in contact with the soil in a petri dish (D) A plastic bag containing Petri dishes of soils in preparation for DGT deployment.

5.2.3 Statistical analysis

Statistical analysis was carried out to determine the effects of treatments on soil available P (C_{DGT} and Soil solution P), extractable P (Olsen P), and ryegrass characteristics (DMY, TP_{uptake}, TP_{plant}). This was achieved using repeated measures

analysis of variance (ANOVA). Post-hoc analysis was conducted using Fisher least significant difference (LSD) Homogeneous groups (0.005) where there is no significant difference; the same lower case letter is used. Error bars on each graph represent ± standard error. Ryegrass response to treatment addition was carried out using simple linear regression analysis. All statistical analysis was carried out using STATISTICA 11. Residuals were all normally distributed. Results of ANOVA analysis are displayed in Appendix Table C.1-1to Table C.1-12.

5.3 Results

5.3.1 DMY and TP_{uptake} Stage 1

Ryegrass was established in the glasshouse in October 2010 and grown until April 2011 (6 months), data reported in this section relates to dry matter yield (DMY). ANOVA was carried out to investigate the relationship between DMY*treatment source*time and the results displayed in Figure 5-3(a). Results showed a significant difference ($p<0.01$) in DMY between organic amendments and Cont ADAS-QC with time, with organic amendments being significantly greater. However there was no significant difference between ryegrass yields grown on soils receiving organic amendments.

The pattern of DMY over time decreased with time ($p<0.001$). Fisher and Jewkes (2009) explained that the timing of the first nitrogen application to grass in the season is important for optimum DMY and reducing N loss. The soil temperature must be sufficiently high to optimise ryegrass response from the applied amendments. Therefore establishment of DMY and application of amendments in October 2010 was not ideal for optimum ryegrass growth early in the experiment.

ANOVA was carried out to investigate the relationship between TP_{uptake}*treatment source*time and results are displayed in Figure 5-3(b). Results show a significant difference ($p<0.01$) in TP_{uptake} between organic amendments and Control ADAS-QC over time. There was also a significant difference between all treatments receiving organic amendments over time. The overal pattern of TP_{uptake} was FYM>SLRY>GW>Control ADAS-QC. TP_{uptake} decreased with time ($p<0.001$).

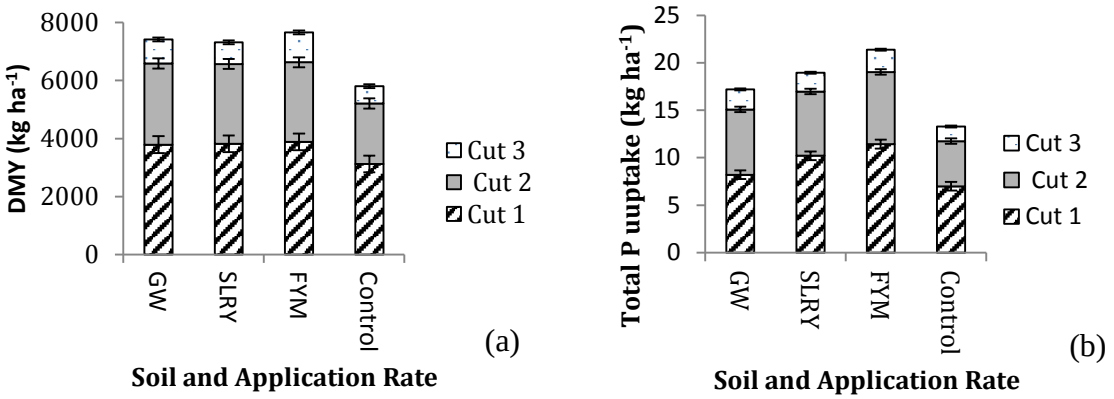


Figure 5-3: (a) DMY and (b) TP_{uptake} of ryegrass for three cuts in a 6 month period for Stage 1 of experiment. Data are means *n=4*. Error bars represent ± standard error. There was a significant difference between time and soil treatments p=0.008.

5.3.2 DMY and TP_{uptake} Stage 2

Treatments were incorporated into the surface layer of pots which had ryegrass established as described in Section 5.2. The data reported in this section relates to DMY which is shown in Figure 5-4 and Figure 5-5. ANOVA was carried out to investigate the relationship between DMY*treatment source*application rate*time and results are displayed in Figure 5-4. Results showed a significant difference ($p<0.01$) in DMY between soils receiving addition of organic amendments and SP overall, and with time, however there was no significant difference between soils receiving addition of organic amendments overall with time. Total DMY for each treatment decreases with time (Figure 5-4).

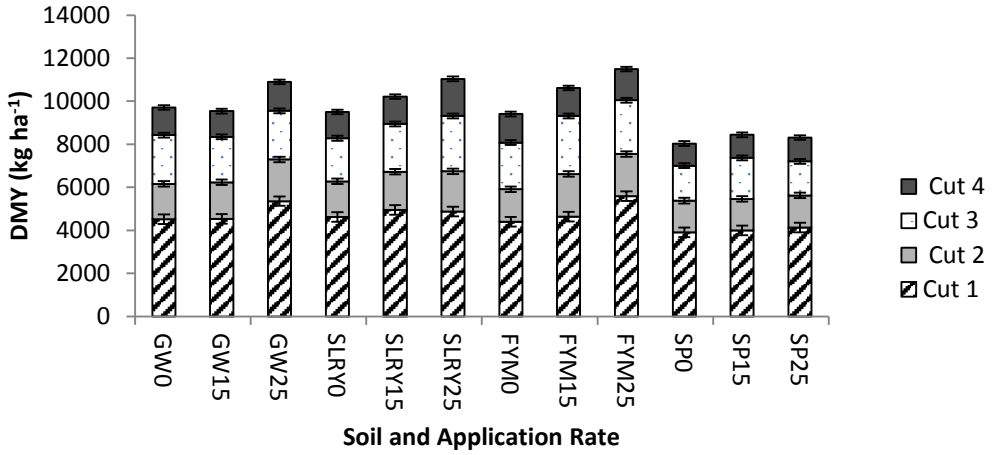


Figure 5-4: DMY of ryegrass showing four cuts in a 12 month period period for Stage 2 of the experiment. Data are means *n=4*. Error bars represent ± standard error. There was a significant difference between time and soil treatments p<0.001.

There is a significant increase in DMY with increasing treatment amount for all soils which had received organic amendments, however there is no significant increase in SP with increasing application rate. Analysis of covariance indicated the slope of the regression line, for organic and SP treated soils (Figure 5-5).

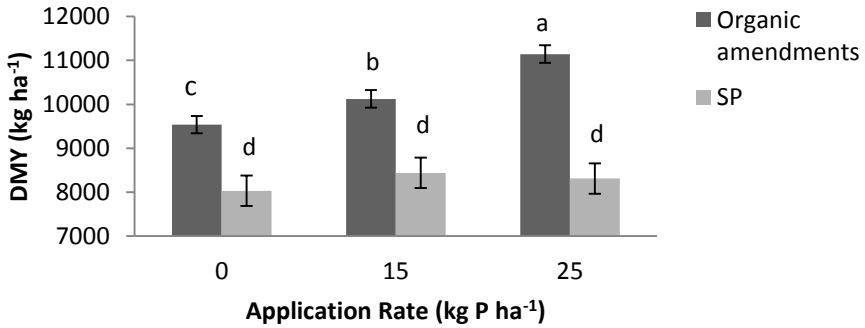


Figure 5-5: Change in DMY of ryegrass at application rates 15 and 25 kg ha^{-1} for each treatment-organic refers to mean of GW, FYM and SLRY, whereas inorganic refers to SP. Organic amendments increased with increasing application rate (p <0.001) and there was no change for SP (p=0.572); (Organic amendments *n=12, SP n=4*). Error bars represent ± standard error. Significant differences denoted by lower case letters (Fisher LSD).

The data reported in this section relates to TP $_{uptake}$ which is shown in Figure 5-6. ANOVA was carried out to investigate the relationship between TP $_{uptake}$*treatment source*application rate*time (Figure 5-6).

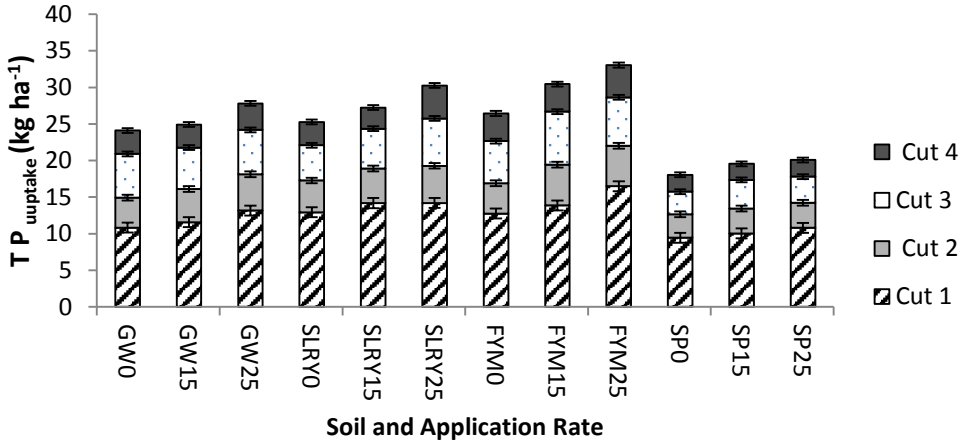


Figure 5-6: TP$_{uptake}$ of ryegrass showing four cuts in a 12 month period for Stage 2 of experiment. Data are means *n=4*. Error bars represent ± standard error.

There was a significant difference ($p<0.01$) in TP $_{uptake}$ between soils receiving organic amendments and soils receiving SP overall and over time. Furthermore there is a significant difference in TP$_{uptake}$ between all organic amendents overall and over time. The pattern of TP $_{uptake}$ followed GW>SLRY>FYM>SP overall.

There was a significant increase in TP$_{uptake}$ with increasing application rate, for the mean of all treatments. Furthermore there was a significant increase in TP$_{uptake}$ for all treatments between 0 and 25 kg P ha^{-1} (Figure 5-5). There was an increase in TP $_{uptake}$ for all treatments between 0 and 15 kg P ha^{-1} however this increase was not significant for all treatments. The slope of the regression line for each treatment is displayed in Table 5-3.

Table 5-3: Linear regression analysis relationships between mean TP_{uptake} v P application rate (0, 15 and 25 kg ha^{-1}). *n=4* for each treatment at each application rate.

Treatment	Regression equation	R^2	p-value
GW	y = 0.26x + 26.45	0.99	<0.001
SLRY	y = 0.19x + 24.99	0.94	<0.001
FYM	y = 0.14x + 23.73	0.82	0.038
SP	y = 0.08x + 18.13	0.97	0.073

5.3.3 Soil Analysis Stage 1

The data reported in this section relates to $C_{DGT.}$ ANOVA was carried out to investigate the relationship between C_{DGT} *treatment source*time (Figure 5-7(a)). Results showed a significant difference ($p<0.01$) in C_{DGT} between organic amendments and Control ADAS-QC over time. There was also a significant difference in C_{DGT} between all treatments receiving organic amendments over time. The overal pattern was GW>FYM>SLRY>Control ADAS-QC ($p<0.001$). C_{DGT} decreased with time ($p<0.001$) between control and soils which had received of organic amendments, however there was no significant difference between soils which had received organic amendments (Figure 5-7(a)).

An additional ANOVA was carried out to investigate the % of initial C_{DGT} P change over time (Figure 5-7(b)). This showed a significant difference between soils which had received application of organic amendments, and those which had received inorganic fertilisation overall. However there was no significant difference between soils receiving organic amendments overall. Soils which had received organic amendments showed a progressive overall decrease in % C_{DGT} however control soils had an initial increase in % C_{DGT} at each sampling dates for 6 months.

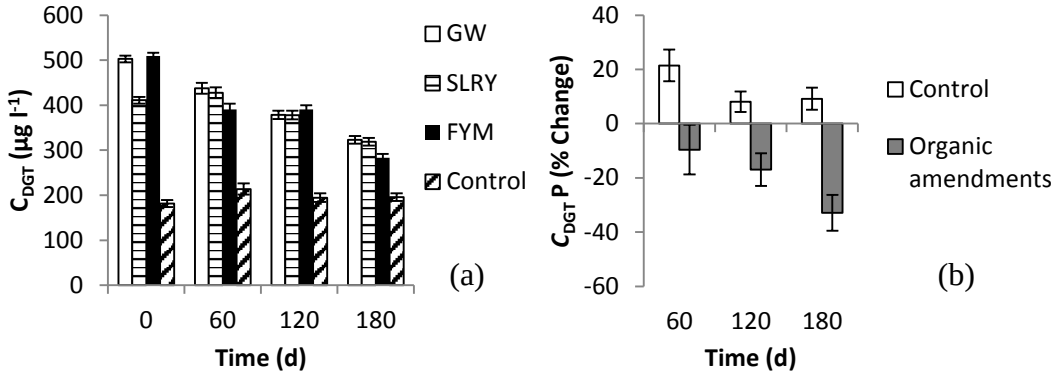


Figure 5-7: (a) C_{DGT} change with time over a 6 month period for Stage 1 of experiment. Data are means (Control *n=4* Treated *n=8*) (b) % C_{DGT} change with time over a 6 month period for the whole experiment with no addition of treatments. % change at day x = C_{DGT} (day x)-C_{DGT} (day0)/ C_{DGT} (day0)*100. Control *n=4* Treated *n=12*. Error bars represent ± standard error.

5.3.4 Soil Analysis Stage 2

The data reported in this section relates to C_{DGT}. ANOVA was carried out to investigate the relationship between C_{DGT}*treatment source*application rate*time. Figure 5-8(a) showed a significant difference ($p<0.01$) in C_{DGT}, between soils receiving orgnic amendments and SP overall and over time. Furthermore there is significant difference in C_{DGT} between all organic amendents overall and over time except between GW and SLRY. C_{DGT} shows a significant decrease over time for all treatments and controls (Figure 5-8(a)). The pattern of C_{DGT} followed GW>SLRY>FYM>SP.

Analysis of covariance indicated no significant change in C_{DGT} with increasing application rates ($p=0.068$) for organic amendments. However SP shows a significant increase with increasing application rate Figure 5-8(b)).

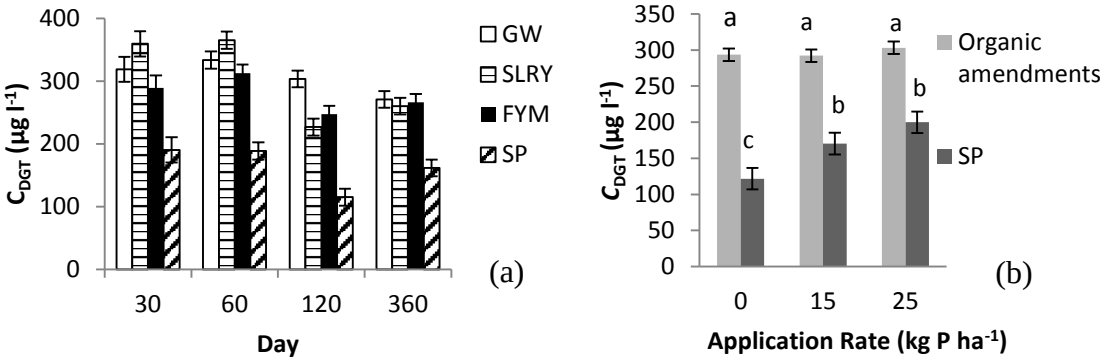


Figure 5-8: (a) C_{DGT} change with time over a 12 month period for Stage 2 of experiment. Data are means (Control *n=4* Treated *n=8*). There was a significant difference (p<0.01) between soils receiving orgnic amendments and SP overall and over time. (b) Increase in C_{DGT} at application rates 15 and 25 kg P ha^{-1} for each tretment organic refers to mean of GW, FYM and SLRY, whereas inorganic refers to SP.SP increased with increasing application rate (p=<0.05), there was no change for organic amendments (p=0.23). (Organic amendments *n=12, SP n=4*). Error bars represent ± standard error. Significant differences denoted by lower case letters (Fisher LSD).

ANOVA for treatment source*application rate*time was carried out to investigate the % of initial C_{DGT} P change over time. Results indicated that there was no significant difference between treatment source or rate. However there was a significant effect of time on all treatment sources. Soil C_{DGT} increased over the first 60 days following treatment incorporation then decreased significantly to day 120, however by day 360 the decrease had reduced (Figure 5-9).

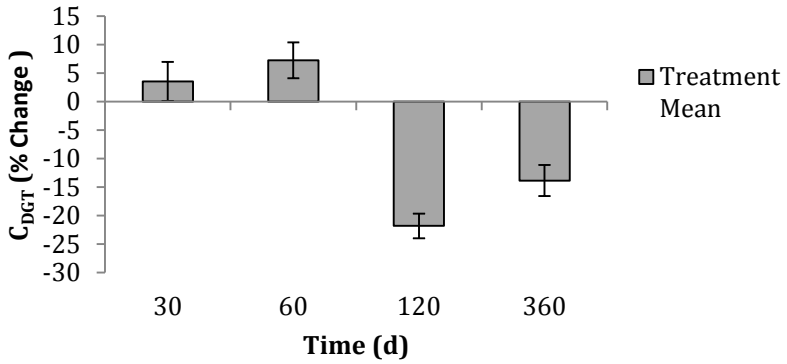


Figure 5-9: % C_{DGT} change with time over a 12 month period following treatment application.Values are means *n=12.* There was a significant change with time (p<0.01). Error bars represent ± standard error.

5.3.5 Soil pH

The data reported in this section relates to pH which is shown in Table 5-4. ANOVA was carried out to investigate the relationship between pH*treatment source*application rate*time. Results showed a significant difference (p<0.001) in pH for all analysis, pH *treatment source*application rate*time. Table 5-4 shows increases in pH from start to end. This follows the pattern SLRY>SP>GW>FYM.

Table 5-4: Displaying pH change from the start of the experiment to the end. Std error represents ± standard error. Data are means *n=8*

Treatment	Start	End
GW	6.50	7.22
SLRY	6.61	6.88
FYM	6.62	7.32
SP	6.31	6.90
Std error	**0.004**	**0.033**

5.3.6 Depth profile study

DGT devices were deployed 4 different depths within pots, in order to establish the range of variability with depth and between pots within samples taken with an auger for standard measurements throughout this experiment.

The data reported in this section relates to C_{DGT} which is shown in Figure 5-10(a). ANOVA was carried out to investigate the relationship between C_{DGT}*treatment source*application rate*depth. Measurements were carried out at the end of Stage 2, by the method developed for depth profile measurements (Section 2.6.3). There was no significant difference in C_{DGT} between treatment source*depth or treatment source*application rate. However there was a significant difference in overall C_{DGT}*depth.

ANOVA was carried out to investigate the relationship between Olsen P*treatment source*application rate*depth. There was no significant difference in Olsen P between treatment source*depth or treatment source*application rate. However there was a significant difference in overall Olsen P*depth Figure 5-10(b).

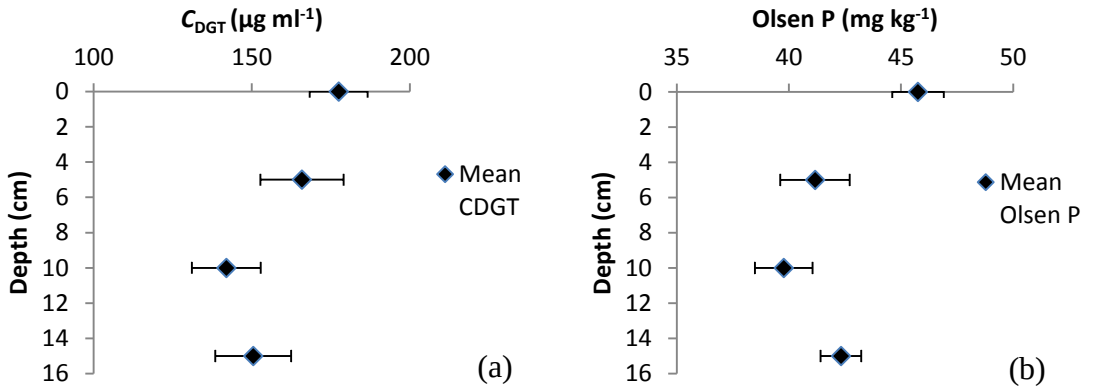


Figure 5-10: Change in (a) C_{DGT} and (b) Olsen P with depth for the mean of all soils following application of treatments (FYM and SLRY) at 0 and 25 kg P ha^{-1}, at the end of Stage 2. There are overall significant decreases with depth for C_{DGT} p=0.025 and Olsen P p=0.01. Data are means *n=8*. Error bars represent ± standard error.

5.4 Discussion

5.4.1 Stage 1 DMY and TP_{uptake}

Historical treatment additions are important at determining yield and TP_{uptake}. There are significant differences in DMY/TP_{uptake} from soils which had historically received application of organic amendments and Control ADAS-QC. The P status of the soils

which had received application of organic amendments was significantly greater than the control which may be expected to be responsible for the lower DMY/TP_{uptake} (Figure 5-3 (a) and (b) respectively).

It was established in the RB209 fertiliser manual (Defra, 2010) that between 16-25 mg P l^{-1} (Index 2) was sufficient for optimum ryegrass growth. As the control was above this range it can be expected that the P status of the soil was not a limiting factor in growth and uptake. It is therefore expected that other soil properties must be responsible for the increased yield of the soils, which had received organic amendment application. The application of organic amendments to soil can improve soil physical, chemical and biological properties, which can improve soil fertility and thus crop growth (Sharpley, 1996). However the focus of this study was on P, therefore the specific factor limiting ryegrass growth and uptake was not determined experimentally. As sufficient mineral N was added to the soil for ryegrass growth (150kg N ha^{-1}), it was also expected that N was not limiting initially in this experiment. However, Read *et al*, (2007) explained that the application of a combination of organic amendments and inorganic fertilisers to soil could improve crop DMY more than inorganic or organic alone, through improving availability of added N to the plant for longer. This may help to explain in part why soils, which had received organic amendments, had a greater DMY (Figure 5-3(a)).

There is no significant difference in DMY between the soils, which had historically received application of organic amendments (Figure 5-3(a)). Therefore, it is expected that the repeated application of different organic amendments, with different properties, has increased the overall fertility of the soils to levels, which produce similar yields.

Ryegrass DMY reduces significantly with each cut (Figure 5-3(a)). It is expected that this occurs as a result of N loss from the soil. Antille, (2011) explains that N is the most important nutrient limiting plant growth. Following its application to soil, N is extremely mobile, and undergoes a number of transformations, as time commences within a cropping season, losses from the soil system can occur from volatilization, denitrification, mineralisation and leaching. Antille *et al*, (2011) found a similar pattern of DMY following ryegrass establishment and attributed this to N losses described above.

Read *et al,* (2007) explained that the soil N status significantly affects TP_{uptake}. Table 2-2 shows that the soil N status of the soil before the experiment followed the pattern (FYM>GW>Cont ADAS-QC>SLRY). Although a blanket application of N was added to the soil to eliminate the influence of N on P dynamics, it is likely that the N chemistry of the soil was significantly different for each of the soils following application of urea. Fujita *et al,* (2010) explained that addition of N stimulates phosphatase activity via N:P stoichiometry effects, which potentially increases TP_{uptake}. However analysis of N dynamics was not within the scope of this work and was not investigated.

The pattern of TP_{uptake} is much different to that of the soil P status. TP_{uptake} = FYM>SLRY>GW>Cont ADAS-QC whereas the soil pattern is GW>SLRY>FYM>Cont ADAS-QC. This is further evidence that the P status of the soil is so high that there is sufficient P for uptake by ryegrass in each soil.

5.4.2 Stage 2 DMY and TP_{uptake}

Historical treatment additions are more important at determining yield and P uptake than fresh additions. It is evident that following a fresh application of organic amendments to soil, there is a significant increase in both DMY and TP_{uptake} (Figure 5-4 and Figure 5-6). The soil P is not thought to be limiting in this experiment, therefore it is expected that the overall increase in DMY and TP_{uptake} resulted from overall soil fertility improvement resulting from incorporation of organic amendments.

DMY increases with increasing application rate, however there is no significant increase for the soil receiving SP (Figure 5-5), this gives further evidence that the increase in DMY in soils receiving organic amendments is a result of benefits derived from treatment properties and not increased P released into the soil solution.

Despite the improved DMY following treatment incorporation, there is no significant difference ($p>0.05$) in DMY between the soils, which had received organic amendments (Figure 5-5), it is expected that following five years of application to soil, addition of fresh treatments at such low application rates was insufficient to bring about significant changes in DMY between treatments.

TP_{uptake} however is significantly ($p<0.05$) different between all treatments and increases with increasing application rate (Figure 5-6). As P is non-limiting, TP_{uptake} differences between the different treatments and different application rates were due to luxury consumption by ryegrass. Similar trends were found by Bennett *et al,* (2001) and Kratochvil *et al,* (2012).

Read *et al,* (2007) observed ryegrass TP_{uptake} values in the region of 11.6 to 23 kg ha^{-1}. By adding broiler litter at between ~4.5 and 36 kg ha^{-1}/ yr^{-1}, an increase in ryegrass TP_{uptake} by ~108 -333% could be expected. Ylivainio *et al,* (2008) found that increases in the range 27 and 141% for meat and bone meal and dairy manure at 25 and 100 mg P kg^{-1} respectively. This experiment has found maximum ryegrass TP_{uptake} values in the region of between 25-30 for SLRY at 15 and 25 kg P ha^{-1} addition, this represents a increase of 20% which was the maximum increase. Minimum ryegrass values of between 18 and 20 kg ha^{-1} were found for SP but this represented no significant increase.

Read *et al,* (2007) found annual ryegrass DMY values in the region of 5000 (control) to 14 000 (treated) kg ha^{-1} for the first year following application representing an 180% increase. Antille, (2011) found ryegrass DMY to range between 2000 (control) and 9000 (treated) kg ha^{-1}, in glasshouse pot experiments, representing a 350% increase. In this experiment DMY % increase is relatively low for each treatment. There was no significant difference between treatments, however overall DMY increased from 9162 to 10435 kg ha^{-1} between controls and treatments respectively which represents a 14% increase. The low increase can be explained by a number of factors influencing DMY.

The main reason for the low increase is thought to result from the relatively low rates of P application to a soil of relatively high P status. The P status of the soil is 4 (3 for SP) (Table 2-2), as a result, the RB209 fertiliser manual (Defra, 2010) recommends application of P is not necessary to improve crop growth. As a result low application rates of organic amendment were used, and therefore had limited influence on DMY**.** In addition previous applications of organic amendments were significantly higher than application rates used in this experiment (Appendix Table A.2-1), therefore it is likely that the residual effects of the five years of application on each of the soils which did not receive amendment application (0 kg ha^{-1}), may have had a large influence on the

soil, meaning that changes from the fresh application were relatively subtle. However a key message which can be taken from these findings is that even at a high P index, further addition of organic amendments can increase DMY and TP_{uptake}, associated with improved physical, chemical and biological soil properties following addition of each organic amendment.

5.4.3 Stage 1 Soil analysis

This section relates to changes to soil available P, measured by C_{DGT} and identifies the mechanisms responsible for the changes. It then considers changes to P measured by Olsen P and solution P and how these differ from C_{DGT}.

Historical addition of organic amendments to meet N demands leads to a build-up of P measured by DGT compared to control soils. It was previously observed that prior to establishment of the experiment the soils, which had historically received repeated application of organic amendments for the ADAS-QC studies (Bhogal *et al*, 2011), had a significantly greater P status than the control soils. Furthermore, there were significant differences between soils, which had received different organic amendments. This was investigated in Section 3.2.1, and mechanisms responsible for this trend were highlighted. However to summarise, it was established that regular addition of organic amendments resulted in a build-up of available P, compared to the control and each previously amended soil had a significantly different P status. Mechanisms responsible were attributed to (a) differences in the total mass of P added in to soil between the control and different amendments, which influenced the quantity of P added to the soil. (b) Differences in the total mass of C added to the soil differed between amendments, which primarily influences P mineralisation-immobilisation patterns, which have an important role in determining P availability. (c) Historical organic amendment additions were based on crop N requirement. This resulted in P supply in excess of plant demand, leading to a build-up of available P.

With the mechanisms for the initial differences in P status already established it was important to determine how soil P availability responded following ryegrass establishment for each historically-amended soil. It was observed that the differences in C_{DGT}, which were observed between the historically-amended soils at the start of the

experiment, maintained a similar trend (GW≥SLRY>FYM>CONT) for the duration of Stage 1.

It was observed that over time the soil C_{DGT} decreased for all historically-amended soils at each sampling date for 6 months (Figure 5-7(b)). However there was no significant difference ($p>0.05$) in the % decrease between each soil which received organic amendments. There was a progressive decrease in C_{DGT} with time for each treated soil. However there was a significantly different trend for the control, which showed an increase from day 0 for all sampling dates over 6 months.

It is expected that the increase in C_{DGT} for the control at each sampling date compared to day 0 is a result of two major processes. Firstly the influence of the urea fertiliser has been shown to be important in influencing soil P through stimulation of phosphatase activity via N:P stoichiometry (Fujita *et al*, 2010). Secondly, establishment and growth of plant roots significantly influences soil chemistry, through root exudates. Shen *et al*, (2011) explained that physiological activities in the rhizosphere, such as the exudation of organic compounds like mucilage, organic acids, phosphatases determine mobilization and acquisition of soil nutrients. Phosphorus is mobilized from the bulk soil to the rhizosphere to meet plant demand. It is expected that a combination of the two factors outlined above, have brought about the increase in C_{DGT} of the control soil, despite there being no additional P supplied to the soil.

A number of mechanisms can explain the decrease in C_{DGT} for all soils, which had received organic amendment application compared to day 0. Firstly, measurement of P, at day 0 came after drying and rewetting soil, collected from the field. Soinne *et al*, (2010) explained that rewetting of dried soils enhanced the mineralisation of organic matter. Nutrient bursts originate from solubilisation of organic matter and the disruption of aggregates revealing fresh new surfaces and through microorganisms broken down during drying or rewetting. Turner and Haygarth, (2001) found that air drying increased water, and sodium bicarbonate ($NaHCO_3$)–extractable P (Soinne *et al*, 2010). Therefore following this first dry-re wet phase there was a release of P from the organic matter which built up over the previous 5 years of the ADAS-QC trial, which resulted in a nutrient burst. Following this release, available P was then subject to processes, which remove available P from solution, such as immobilisation and sorption reactions.

Although the mechanisms described above are expected to influence both the control ADAS-QC soil and those receiving organic amendments, it is expected that the influence of the organic amendments on these nutrient bursts following re-wetting of soil, was so influential that they mask any effects of the urea fertiliser on N:P stoichiometry and plant root exudates, which were described in the paragraph above.

It was expected that differences in % P changes with time would provide valuable information on the organic amendment's ability to maintain P status following growth without application of further P. However there was no significant difference between treatments, suggesting that the five years of addition can result in differences in C_{DGT} P, however when no further amendments are applied, each treated soil decreases at a similar rate.

5.4.4 Stage 2 Soil Analysis

Treatment application history was significant in determining P release from fresh treatment additions. In addition soils which receive SP will show a greater response (C_{DGT}) than those which received addition of organic amendments, due to the slow release of P from organic amendments.

Overall organic amendment addition results in no significant increase in C_{DGT}, whereas addition of SP results in a significant increase in C_{DGT} with increasing application rate (Figure 5-8(b)). There are a number of reasons why the P in the SP amended soil would have been expected to increase. Firstly Stevenson and Cole, (1999) explained that inorganic P is more water soluble and therefore readily available than organic amendment P. Secondly, at the time of treatment addition, the P status of the SP soil was lower than that of the soils receiving addition of organic amendments. Therefore it is possible that this soil been more responsive to P application than soils at a higher P status.

It would have been expected that the addition of organic amendments to soil would have resulted in an increase in C_{DGT} however there was no significant increase for any organic amendment (Figure 5-8(b)). Prasad, (2009) suggested that the addition of organic amendments is in a slow release form and takes place over a number of years following application. For this reason, it can be expected that little change in available P

(Figure 5-8(b)), would occur as P from previous years is still being released into the soil, and a large portion of the P in the amendment added would be expected to be released to future crops. Application rates were very low, due to the high initial P status of the soil; therefore it is likely that the effects from previous year's applications were stronger than in this application.

Results of C_{DGT} for each soil treatment provided valuable information about amendment application history on the P status of each soil. However as the P status of each soil was different at the start of the experiment, data had to be processed in a way, which would allow comparisons to be made between the treatments, in order to establish the effect of individual treatments on C_{DGT}. This was achieved by calculating the % change in soil P (Figure 5-9) there was no significant difference between organic amendment sources for % P changes over time. Therefore although the historical amendment additions have resulted in a build-up of soil P which is different for each treatment, their addition to soil in this experiment do not have the ability to cause significant changes between amendments.

5.4.5 Relationship between soil and ryegrass

5.4.5.1 Stage 1

Overall there is a poor relationship between soil and plant characteristics. Table 5-5 shows R^2 values, highlighting correlations between soil available P (C_{DGT}, Soil solution P), extractable P (Olsen P), and plant characteristics (DMY, TP_{plant}). Analysis was carried out to establish the relationship between each of these in order to improve understanding of how years of repeated application of organic amendments to soil influences P dynamics and in turn plant growth (DMY) and P uptake (TP_{uptake}). It is expected that the repeated application of organic amendments in the ADAS –QC trials (Bhogal *et al*, 2011) has added sufficient P to each soil receiving organic amendments over 5 years so that P is high enough not to limit DMY and TP_{uptake}. C_{DGT} shows a slightly stronger correlation with plant factors than any of the other soil P tests. This suggests the P it is measuring has a greater influence on TP_{uptake} than what is being measured by Olsen P and soil solution P, when P is not the factor limiting plant growth most. Further work needs to be done to establish if this is the case when P is a limiting factor. Previous authors (Mason *et al*, 2008, Menzies *et al*, 2005, Mcbeath *et al*, 2007)

observed a strong correlation between soil's C_{DGT} and DMY/TP_{uptake}. It was expected that this strong correlation was an indication of DGT being an accurate indicator of the soil P status. It was also observed that C_{DGT} showed a better correlation with DMY or P_{uptake} than extraction procedures or anion exchange resin P.

Table 5-5: Regression analysis of C_{DGT}, Olsen P and Soil solution P vs Plant factors (DMY and TP_{uptake}) for Stage 1. Values represent the correlation coefficient displayed as R^2. Values in red indicate a significant relationship for the test p>0.05.

	Olsen P	C_{DGT}	Soil Solution
Olsen P			
C_{DGT}	0.85		
Soil Solution	0.74	0.70	
DMY	0.47	0.54	0.40
TP_{uptake}	0.44	0.48	0.33

A log graph of Olsen P, Soil solution P and C_{DGT} over time is displayed Figure 5-11. This highlights a similar trend between the three measurement methods, suggesting that they undergo similar transformations when ryegrass is grown on each soil without addition of additional P. This also emphasises the difference in scale between measurements made by each technique. It can be seen that the soil solution P is ~10.5 times greater than DGT, and Olsen P is ~15 times greater than soil solution.

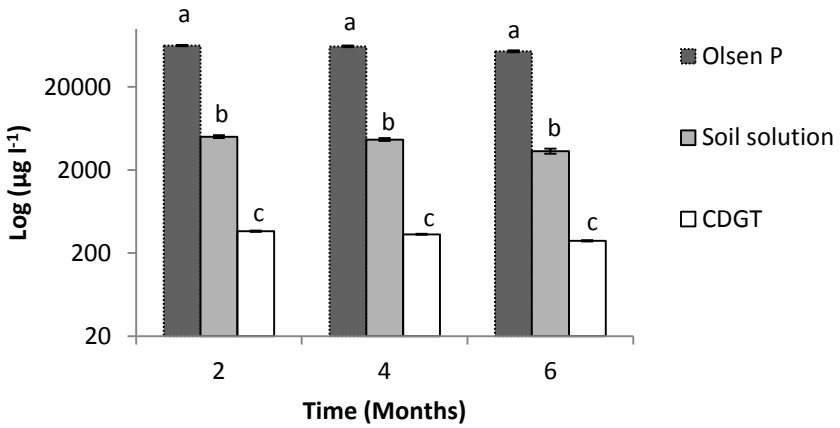


Figure 5-11: Log graph (Base 10) of relationship between all P pools with time for the mean of all soils with no treatment addition. The same lower case letters indicate no

significant difference (Fisher LSD). There was a significant difference between each pool over time p<0.001.

5.4.5.2 Stage 2

Historical treatment additions are more important at determining DMY and TP_{uptake} than fresh additions. Table 5-6 shows R^2 values, for correlations between C_{DGT}, Soil solution P and Olsen P, with plant characteristics (TP_{uptake} and DMY) and organic amendment characteristics ($C:P_{treatment}$, $TP_{treatment}$). Analysis was carried out to establish relationships between each of these to improve understanding of how fresh application of organic amendments to soil, which had received repeated application of these amendments, influences P dynamics and in turn plant growth and P uptake. Results between soil and plant factors for the Stage 2 showed similar trends to the first year. The additional factor considered in the second stage was treatment characteristics. Results show no significant relationship between $C:P_{treatment}$ and soil P or plant factors. There is a good relationship between $TP_{treatment}$ vs. Olsen P and C_{DGT}. As P was not limiting ryegrass growth there was not a strong correlation between soil and plant factors. The depletion in soil P over the first growing season before treatment addition was insufficient to deplete soil P enough so it was a limiting factor for plant characteristics.

Regression analysis was carried out between organic amendment characteristics vs. soil available and extractable P, and plant factors. $C:P_{treatment}$ showed a poor relationship with soil and plant factors, suggesting that this had no influence on addition of P to soil, and no influence on plant characteristics (Table 5-6). It is therefore established that an accurate analysis of the relationship between organic amendment characteristics and soil factors and plant factors was difficult, primarily because each treated soil had a different P availability at the start of the experiment, therefore the effects of the previous treatment applications over the years would be more influential than this fresh application.

Table 5-6: Regression analysis of P measurements (C_{DGT}, Olsen P and Soil solution P) vs plant factors (DMY, TP_{uptake}) and organic amendment characteristics ($TP_{treatment}$ and $C:P_{treatment}$) for Stage2. Values represent the correlation coefficient displayed as R^2. Values in red indicate a significant relationship for the test p>0.05.

	Olsen P	C_{DGT}	Soil Solution	DMY	TP_{uptake}
Olsen P					
C_{DGT}	0.69				
Soil Solution	0.68	0.67			
DMY	0.47	0.54	0.48		
TP_{uptake}	0.49	0.57	0.54	0.90	
$C:P_{treatment}$	0.21	0.20	0.13	0.23	0.42
$TP_{treatment}$	0.73	0.70	0.57	0.46	0.55

5.4.6 Soil pH

Soil pH is important in determining P availability. Mechanisms are described in detail in Section5.3.5. Ryegrass growth can also have an influence on soil pH. The release of root exudates and organic acid anions can enhance mineral nutrient solubility, and liberate H^+ and OH^- in order to counterbalance cations and anions entering the root (Hinsinger, 2003; Waldrip 2010). Results show that the soil pH increases following the addition of all treatments. Treated soils increase pH more than control soils (Table 5-4). The increase from the start to the end of the experiment was a result of the growth of ryegrass, and the additional change for each of the amended soils was thought to be due to the amendment itself. However as the soil pH experienced relatively small changes following amendment application, and the changes are within pH values optimum for P speciation (Waldrip 2010) it is expected that these changes had limited influence on P changes between treatments.

5.4.7 Depth Profile

Koopmans *et al*, (2007) showed soil extractable P is not uniform across a soil profile. Following long term application of phosphorus with animal manure in amounts exceeding removal by crops, extractable P was seen to build-up in different concentrations at different depths. It was found that extractable P decreased with depth; however they also found that P was leached from upper layers through the soil profile

over time. As soil sampling was taken using an auger, there were concerns that the P being measured with the DGT did not take into consideration this variability. Furthermore there was a desire to determine how well the DGT device could perform when deployed in situ in pots compared to standard protocol described in Section 2.2.3. As a result an experiment was conducted where DGT was deployed in situ at different depths in the soil profile to assess the variability. Olsen P measurements were also taken to compare each depth.

Results show a similar trend for Olsen P and C_{DGT} (Figure 5-10(a) and (b)). It is expected that there is no significant difference between the treatment amounts with depth, due to mechanisms explained previously (Section 5.4.4). The application of treatments was at a low application rate, which did not have the ability to significantly increase the soil DGT status overall, and had a relatively small effect on Olsen P with increasing application rate. Therefore as P release from treatments to soil was relatively low, downward movement of P through the soil profile did not occur. Furthermore as there was only one application of treatments over an 18 month period, it is unlikely that a significant build and release of P with depth is likely to materialise, previous experiments have measured changes over a number of years. It is expected that there is no significant difference between the different treatment sources as a result of the high variability of deploying the devices at different depths. In addition, there is limitations to the methodology used. When cutting open the pot, the soil is exposed to O_2 which is likely to have significantly influenced results.

However overall, there is a significant difference in both Olsen P and DGT with depth. As there is no effect of treatment on P change with depth the same factor is influencing all soils tested. It is expected that the growth of ryegrass over an 18 month period has resulted in an accumulation of organic matter at the surface of pots, which contains P measured by Olsen P and DGT (Figure 5-10(a) and (b)). Whereas this build-up did not take place with depth throughout the profile.

5.5 Conclusions

Stage 1.

- Addition of organic amendments to meet N demands leads to addition of P in excess of plant requirements. This resulted in a build-up of C_{DGT} in historically treated soils compared to ADAS-QC control soils by 88, 86 and 76% for FYM, GW and SLRY respectively, although there was no significant difference between the treatments.
- Results show a poor relationship between soil and plant characteristics. It is expected that the repeated application of organic amendments in the ADAS –QC trials added sufficient P to each soil receiving organic amendments over 5 years so that P is high enough not to limit plant growth and P uptake.

Stage 2

- SP was more efficient at increasing C_{DGT} than all other treatments. SP addition increased C_{DGT} over control soils by 52 % overall, there was no significant difference between control and treated soils for all organic amendments.
- Despite the greater increase in C_{DGT} following SP addition than all organic amendments, SP addition did not significant increase in yield of treated soils over control soils overall. However organic amendment addition resulted in increases over the control of 12 and 18 % for SLRY and FYM, respectively. There was no significant increase for GW. Following (5 year) application of historical organic amendment additions in ADAS-QC studies, influence of previous treatments is still influential. Addition of treatments in this experiment at such low application rates was insufficient to bring about significant changes in DMY between treatments.
- SP and GW addition resulted in no significant increase in TP_{uptake} whereas organic amendment addition resulted in increases over the control of 13 and 21 % for SLRY and FYM respectively.
- Although the historical amendment additions have resulted in a build-up of soil P which is different for each treatment, their addition to soil in this experiment do not have the ability to cause significant changes between amendments.

- As P was not limiting ryegrass growth there was not a strong correlation between soil and plant factors. The depletion in soil P over the first growing season before treatment addition was insufficient to deplete soil P enough so it was a limiting factor for plant characteristics.

6 Kincraigie pot experiments

6.1 Introduction

Pot experiments were set up as a natural progression to the incubation studies, with the advantage of being able to assess plant response to treatment addition, under controlled conditions. The experiment was designed to understand changes in P availability in soils, which are deficient in P (index 0), following addition of treatments at agronomic application rates, and the subsequent transfer of P from soil to plant.

The main aim of the chapter is to understand how fresh application of organic amendments and inorganic fertilisers influence C_{DGT}, Soil solution P, Olsen P and their subsequent influence on plant characteristics (root and shoot (DMY) and (TP_{uptake})). The objectives are outlined below.

Objectives

1. To determine P availability patterns in soils deficient in plant available P following addition of the aforementioned treatments, at agronomic application rates, and determine the impact on plant yield and P uptake.

2. To investigate how organic amendments perform compared to SP in the aforementioned soils.

Hypotheses

- $C{:}P_{treatment}$ will be a better proxy for P availability than $TP_{treatment}$.
- C_{DGT} will be a more accurate indicator of ryegrass root and shoot DMY and TP_{uptake} than Olsen P and Soil solution P.
- SP will be responsible for greater P release than organic amendments, and in turn show greater DMY and TP_{uptake}.

6.2 Methodology

6.2.1 Experiment details

Experiments were conducted in the Cranfield University glasshouse facility, (Figure C.3-1). The soil type used was categorised as a loam Table 3-1. Soil analysis is displayed in detail in Table 3-1. Pots were of 2.5 litre capacity and were filled with 1.5 kg of air dried soil which was sieved to <2mm. The base of the soil was also filled with >25mm

gravel, to stop soil loss, but allowed free drainage. When filling the pots with soil, urea was applied 2 cm below the ryegrass seeds in order to restrict direct contact during germination. Tap water was added to soils gradually to avoid leaching of N. Ryegrass seeds were spread evenly across the soil surface at a rate of 4g m^{-2}. A pot diagram is displayed (Figure 6-1). Photographs of the setup are shown Figure 6-2.

GW, FMY, SP and SLRY were added at application rates of 80 and 120 kg P ha^{-1} with an unamended control. In addition a blanket application of urea was applied to all pots at a rate of 150 kg N ha^{-1}. A randomised block design was used with four repetitions of each treatment. Treatments were incorporated into the top 5 cm of the pot. Crops were established in November 2011 and grown until April 2012, in which time three harvests were taken, based on the growth of 4 leaves between harvests (EBLEX, 2013). Weeds were removed manually from pots; all treatments were added to the soil manually.

It must be established here that when reference is made to treatments throughout this chapter, this means all materials which were added to supply P (FYM, GW, SP and SLRY), however when reference is made to organic amendments this is (FYM, GW, and SLRY)

6.2.2 Soil and crop measurement and analysis

Soil sampling was conducted with an auger 15cm length*1.5 cm diameter. Sub samples (x3) were taken from the pot on each sampling date, and holes re-filled with soil from the initial batch. Grass was cut 2cm above the soil surface (Antille, 2011, Cordovil *et al*, 2007) at each sampling date, and at the end plant root and shoot material were separated by cutting at soil level (Waldrip *et al*, 2011) roots were washed to remove any adhering soil particles. Both root and shoot material were oven dried at 60°C for 48hours (MAFF, 1986; Method No.: 1), weighed and reported as DMY (kg ha^{-1}) (root and shoot separately). Determination of total P in plant material (TP_{plant}) for roots and shoots was required for the estimation of total P uptake (TP_{uptake}). To do this plant material was ground using an electric grinder, and TP_{plant} was determined using acid digestion, followed by P determination with a spectrophotometer (USEPA Method No.:3051).

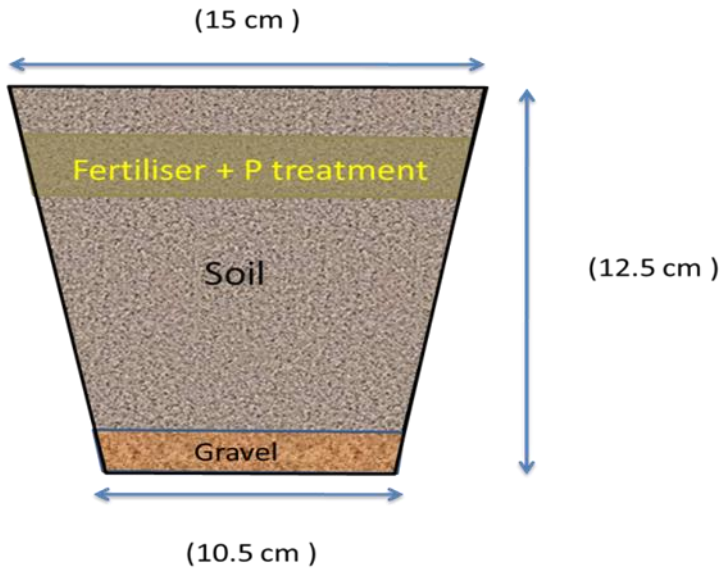


Figure 6-1: Schematic diagram of pot profile used in the Kincraigie glasshouse experiment

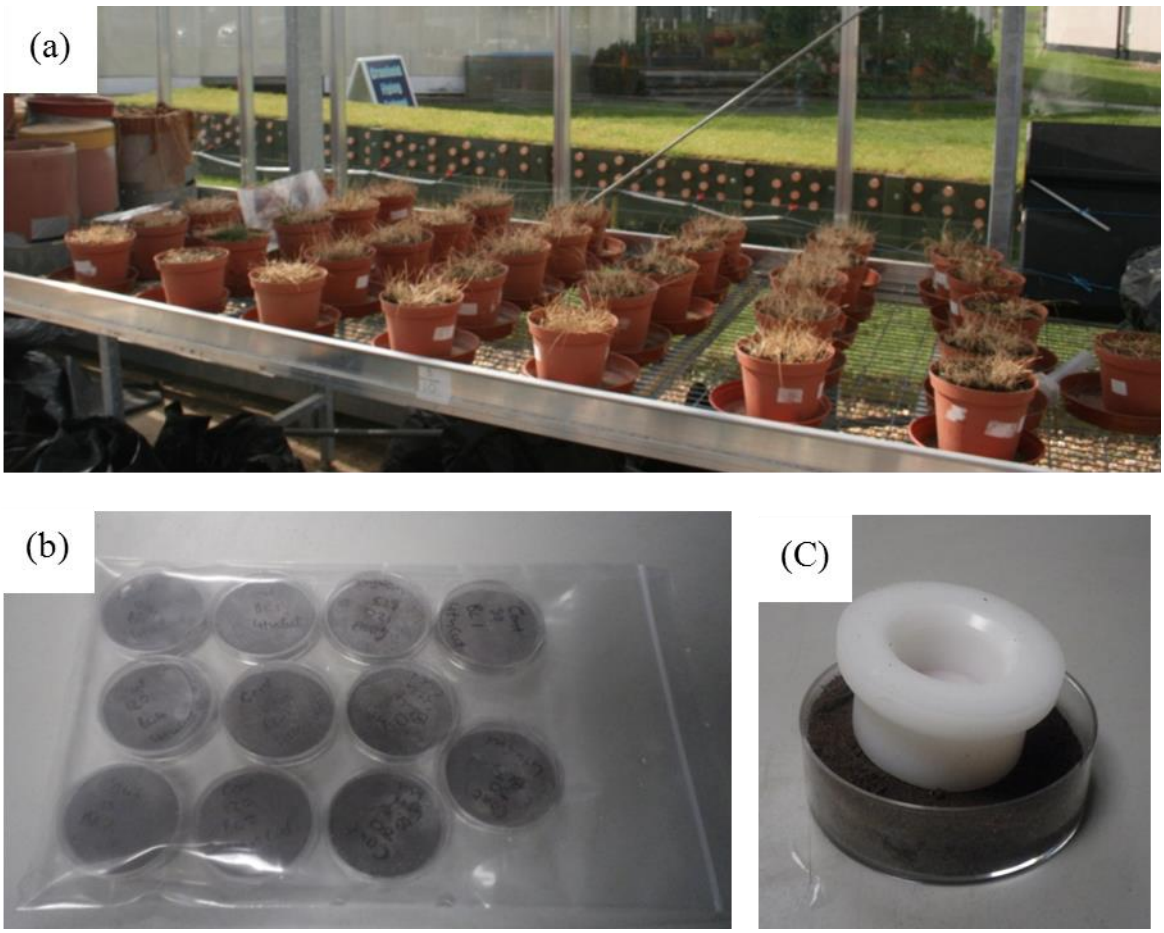


Figure 6-2: Photographs of experimental setup. (a) Shows the pot randomised block design (b) shows the homogenised soil in petri dishes awaiting DGT deployment (c) shows a DGT device deployed on the soil in a petri dish.

Table 6-1: Details of which treatment was added to the corresponding soil taken from ADAS-QC plots, for the incubation experiment.

	Treatment(g)			
	FYM	**SLRY**	**GW**	**SP**
App rate (kg P ha^{-1})				
0	0	0	0	0
80	35.4	148.8	35.4	0.98
120	53	223.2	53	1.47

6.2.3 Statistical analysis

Statistical analysis was carried out to determine the effects of treatments on soil available P (C_{DGT}, Soil Solution P), extractable P (Olsen P) and plant characteristics (root and shoot (DMY) and (TP_{uptake})). This was achieved using repeated measures analysis of variance (ANOVA). Post-hoc analysis was conducted using Fisher least significant difference (LSD) Homogeneous groups (0.005) where there is no significant difference; the same lower case letter is used. Error bars on each graph represent ± standard error. Ryegrass response to treatment addition was carried out using simple linear regression analysis. All statistical analysis was carried out using STATISTICA 11. Residuals were all normally distributed. Results of ANOVA analysis are displayed in Appendix Table D.1-1to Table D.1-7.

6.3 Results

6.3.1 Influence of treatments on C_{DGT}

Figure 6-3 (a) and (b) show significant differences for soil treatment*treatment amount*time (p=0.046). There is a significant difference between the control and treated soils and between the soil treatments. Following treatment addition there is an increase in C_{DGT} until day 30, followed by a decrease to day 120, where C_{DGT} then remains at a similar level for the duration of the experiment.

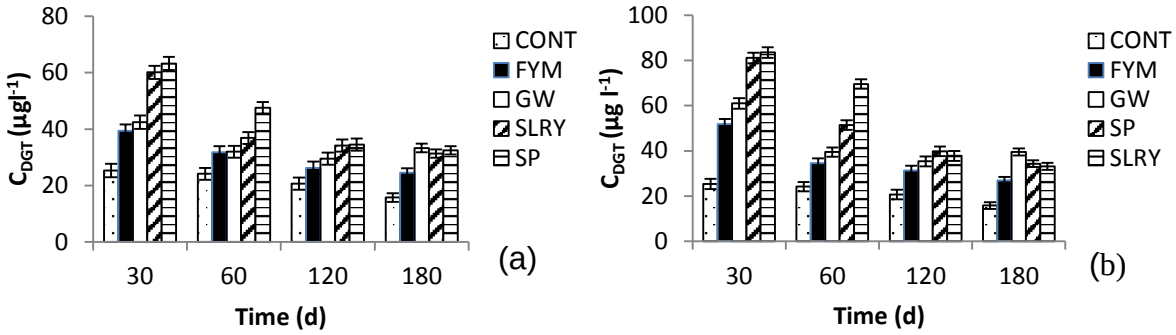


Figure 6-3: Mean C_{DGT} values over time across experiment for all treatments at (a) 80 and (b) 120 kg P ha^{-1} as well as the control soil. Control *n=4* and treated *n=8*. Error bars represent ± standard error. There was a significant difference between the control and both application reates p=0.046.

Figure 6-3 showed a significant difference ($p<0.001$) in C_{DGT} P between treatments and rates for each sampling date. The application of treatments increased soil C_{DGT} in the pattern SP>SLRY>GW>FYM. SP had a mean increase over the control of (134%), SLRY had a mean increase of (115%), GW had a mean increase of (82%) FYM had a mean increase of (55%).

Koenig *et al*, (2008) determined that the response of extractable P to increasing P addition follows a linear trend for applications between 50 and 350 kg P ha^{-1}, to a soil low in extractable P. In this experiment, regression analysis indicated that the effect of application rate on C_{DGT} exhibited a significant linear relationships for all treatments (Table 3-1).

Table 6-2: Linear regression analysis relationships between mean C_{DGT} v P application rate (kg P ha^{-1}). R^2 values represent the correlation coefficient. *n=4* for each treatment at each application rate.

Treatment	Regression equation	R^2	p-value
SP	y = 0.29x + 21.6	0.99	>0.001
SLRY	y = 0.22x + 21.9	0.99	>0.001
GW	y = 0.18x + 21	0.97	>0.001
FYM	y = 0.11x + 21.5	0.99	>0.001

The slope of the regression equations indicates the increase in C_{DGT} for every additional unit of phosphorus applied with each treatment. Subba Rao *et al*, (1996), explained that the larger slope value reflected a more efficient utilisation of applied fertiliser.

It is improtant to note that regression analysis Table 6-8, has been carried out for treatments only (excluding SP), for regression analysis between plant factors and soil and

treatment analysis. This is because the properties of the SP are so different to the treatments, (total P content, C:P ratio, extractable P content) therefore it was not possible to carry out accurate statistical analysis.

The difference in C_{DGT} between organic amendment sources decreased with increasing C:P ratio of the treatment added ($C:P_{treatment}$). The slope of the regression equations shown in Figure 6-4, indicates the increase in C_{DGT} for every incremental decrease in $C:P_{treatment}$. This increases with increasing application rate.

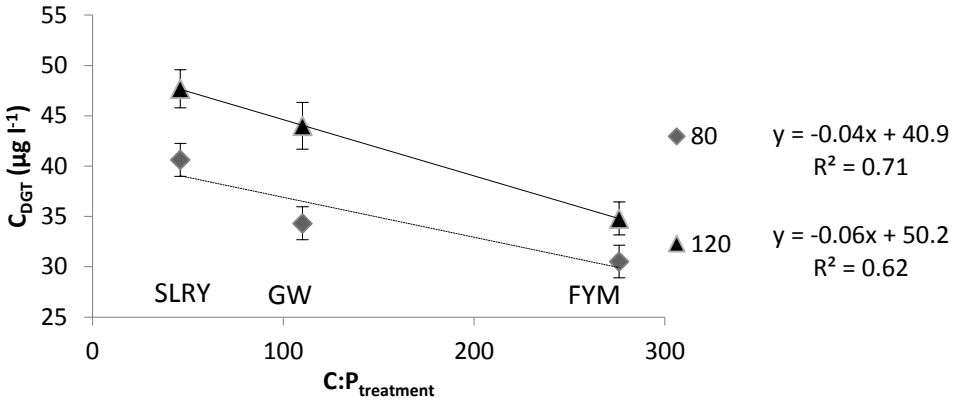


Figure 6-4: Linear regression analysis relationships between C_{DGT} v $C:P_{treatment}$ for each application rate (kg P ha^{-1}). R^2 values represent the correlation coefficient. Data are means *n=4* for each treatment at each application rate. p values are >0.001 and >0.05 for 80 and 120 kg P ha^{-1} respectively.

Table 6-8, shows significant relationship ($p<0.001$) between the TP of the treatment ($TP_{treatment}$) and the soil $C_{DGT.}$ However the relationship follows the inverse of what would be expected as it shows a C_{DGT} increase with decreasing $TP_{treatment}$. Results show no significant relationship ($p>0.05$) between treatment extractable P P ($AVP_{treatment}$) and $C_{DGT.}$

6.3.2 Olsen P

ANOVA analysis for Olsen P showed a significant difference for soil treatment*treatment amount*time ($P<0.001$). There was a significant difference between the control and treated soils and between soil treatments.

There was a significant difference ($p<0.001$) in Olsen P between treatments and rates for each sampling date (Figure 6-5). Treatment addition increased Olsen P in the pattern SP>SLRY>GW>FYM. SP had a mean increase over the control of (123 and 141%), SLRY (49 and 94%), GW (22 and 66%) and FYM (21 and 36%) for 80 and 120 kg P ha^{-1}

respectively. Figure 6-5 showed a trend of an initial increase in Olsen P, until day 30, followed by a decrease to day 120, where the Olsen P then remains at a similar level for the duration of the experiment.

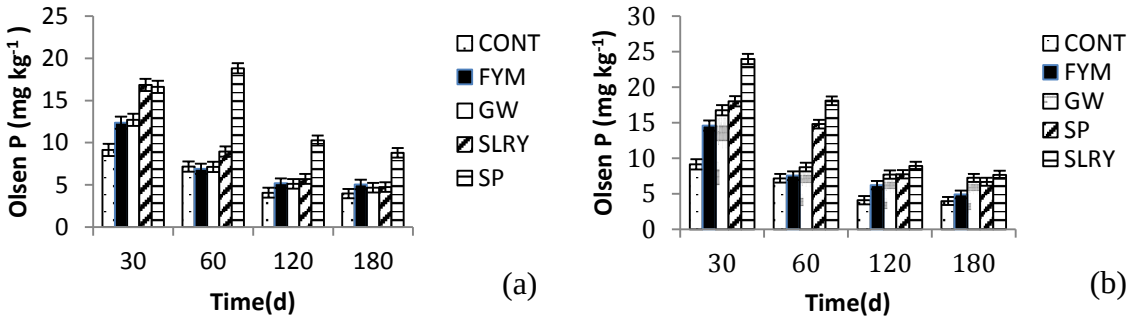


Figure 6-5: Mean Olsen P values over time across experiment for all treatments at (a) 80 and (b) 120 kg P ha^{-1} as well as the control soil. Control *n=4* and treated *n=8*. Error bars represent ± standard error. There was a significant difference between the control and both application rates p<0.05.

Soil solution P

There was no significant difference between soil treatment*treatment amount*time for soil solution P (P<0.001) (Appendix Table D.1-3). There was a significant difference between the control and treated soils but no significant difference between treatments. There was a significant difference (p<0.01) in soil solution P over time for mean of all treatments. Figure 6-6 highlights the increase in soil solution P compared to the control for the mean of all treatments.

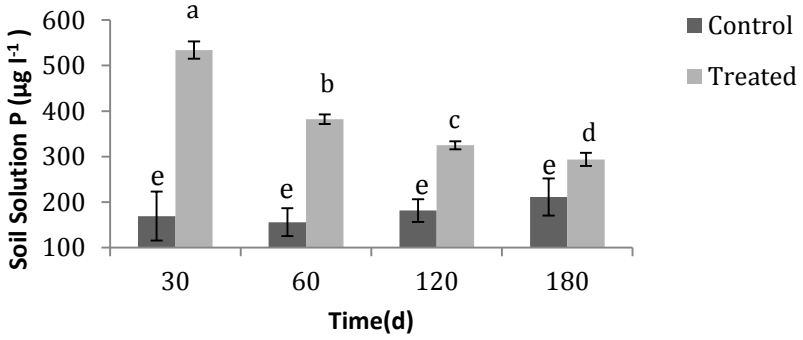


Figure 6-6: Mean Soil solution P values over time across experiment for mean of all treatments as well as the control soil (Control *n=4* Treated *n=32*). There was a significant difference (p<0.01) between control and treated soils overall and over time. Error bars

represent ± standard error. Significant differences between soils are denoted by lower case letters (Fisher LSD).

Regression analysis was carried out between C_{DGT}, Soil Solution P and Olsen P (Mean of all sampling dates) and plant characteristics (root and shoot (DMY) and (TP_{uptake}) sum of all samples). There is a significant correlation between the mean Olsen P and DMY/ TP_{uptake} (Table 6-8). There is also a significant correlation between C_{DGT} and DMY/ TP_{uptake}. There is a significant but poor correlation between mean Soil solution P and sum of DMY/ TP_{uptake}. The regression analysis shows C_{DGT} has the strongest relationship with DMY/ TP_{uptake} (Table 6-8).

6.3.3 Shoot DMY

The data reported in this section relates to shoot DMY which is shown in (Figure 6-7). ANOVA was carried out to investigate the relationship between DMY*treatment source*treatment application rate*time. Figure 6-7 shows a significant difference ($p<0.001$) in shoot DMY between treatments and application rates for each cut. The application of treatments improved DMY performance over the control by 196, 152, 121 and 57 % for SLRY, GW, FYM, and SP respectively.

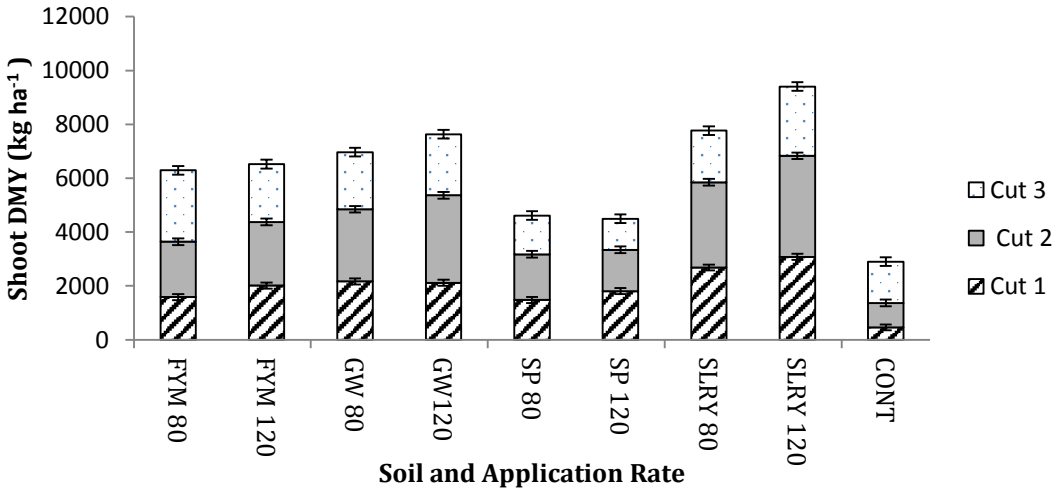


Figure 6-7: DMY of ryegrass showing 3 cuts in a 6 month period (November 2011-April 2012). Data are means *n=4*. Error bars represent ± standard error. There was a significant difference between all soil treatments and application rates for each cut p<0.001.

Regression indicated that the effect of application rate on shoot DMY exhibited statistically significant linear relationships for all treatments. Analysis of covariance

indicated the slope of the regression line follwoed the pattern SLRY> GW> FYM> SP (Table 6-3).

Table 6-3: Linear regression analysis relationships between mean shoot DMY v P application rate (kg P ha^{-1}). R^2 values represent the correlation coefficient. *n=4* for each treatment at each application rate.

Treatment	Regression equation	R^2	p-value
SLRY	y = 55.2x + 3013.2	0.99	>0.001
GW	y = 41.1x + 3095.1	0.94	>0.001
FYM	y = 31.9x + 3109	0.92	>0.001
SP	y = 14.5x + 3038.1	0.82	>0.001

The pattern of shoot DMY in this experiment is different to that observed in previous studies. Typically it would be expected that shoot DMY would decrease with each cut (Sikora and Enkiri *et al*, 2005; Antille *et al*, 2011). However in this experiment, yields are smilar for each cut. The trend observed in this experiment can be attributed to the experiment setup, where ryegrass was established in the glasshouse in November. Fisher and Jewkes (2009) explained that the timing of the first nitrogen application to grass in the season is important for optimum DMY and reducing N loss. The soil temperature must be sufficiently high to optimise ryegrass response from the applied treatments. Therefore establishment of shoot DMY and application of treatments in November was not ideal for optimum ryegrass growth early in the experiment.

6.3.4 Root DMY

The data reported in this section relates to root DMY which is shown in (Figure 6-8). ANOVA was carried out to investigate the relationship between root DMY*treatment source*application rate. Figure 6-8 shows a significant difference ($p<0.001$) in root biomass between treatments. The application of treatments improved root biomass performance over the control by 155, 99, 70, 49 % for SLRY, GW, FYM, and SP respectively.

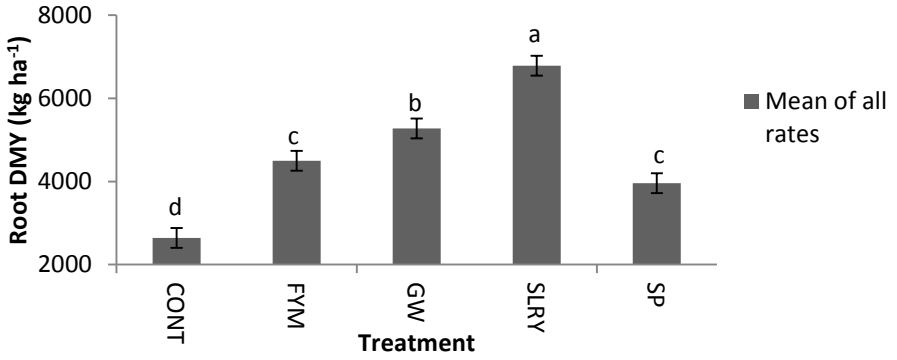


Figure 6-8: Root DMY of ryegrass over a 6 month period (November 2011-April 2012);. Data are means Control *n=4* Treated *n=8*. Error bars represent ± standard error. There was a significant difference between all soil treatments overall p<0.001. Significant differences between soils are denoted by lower case letters (Fisher LSD).

Regression indicated that the effect of application rate on root DMY exhibited statistically significant linear relationships for all traetments (Table 6-4).

Table 6-4: Linear regression analysis relationships between root DMY v P application rate (kg P ha^{-1}). R^2 values represent the correlation coefficient. *n=4* for each treatment at each application rate.

Treatment	Regression equation	R^2	p-value
SLRY	y = 40.8x+2658.5	0.93	<0.001
GW	y = 25.7x+2677.2	0.87	<0.001
FYM	y = 19.4x+2588	0.86	<0.001
SP	y = 13.1x+2646.7	0.84	<0.001

Figure 6-9 shows a scatterplot of ryegrass root and shoot DMY. There is a strong relationship (R^2=0.82) between root and shoot DMY, suggesting that soil factors which influence roots is also influencing shoot DMY.

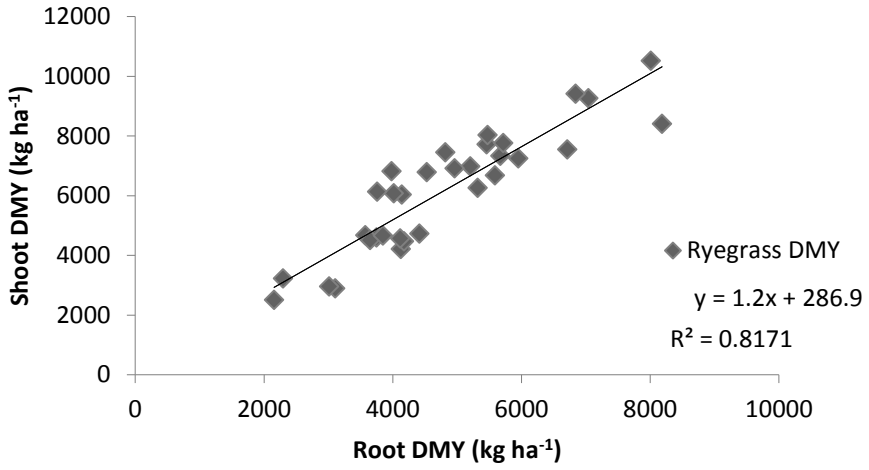


Figure 6-9: Scatterplot with simple linear regression analysis displaying a strong relationship between root and shoot DMY for ryegrass for all treatments; p<0.001. R^2 represents the correlation coefficient.

6.3.5 Shoot TP_{uptake}

The concentration of P in harvested ryegrass (TP_{plant}) was determined for each cut in the experiment according to the principal outlined in Table 6-8. TP_{uptake} for each cut was determined based on TP_{plant} and DMY. The sum of all cuts for the experiment was determined as Sum $TP_{uptake.}$ The data reported in this section relates to TP_{uptake} which is shown in (Figure 6-10). The results of the regression analysis was carried out to investigate the relationship between TP_{uptake}*treatment source*treatment application rate*time is displayed in Appendix Table D.1-5.

Figure 6-10 shows a significant difference ($p<0.001$) between treatments over time, and between treatment amounts. TP_{uptake} for each treatment followed the pattern SP>SLRY>GW>FYM. Values for each cut are compared for each treatment and rate. Regression analysis was carried out for mean TP_{uptake} versus treatment application rate . Regression equations, R^2 and p values are displayed in Table 6-5.

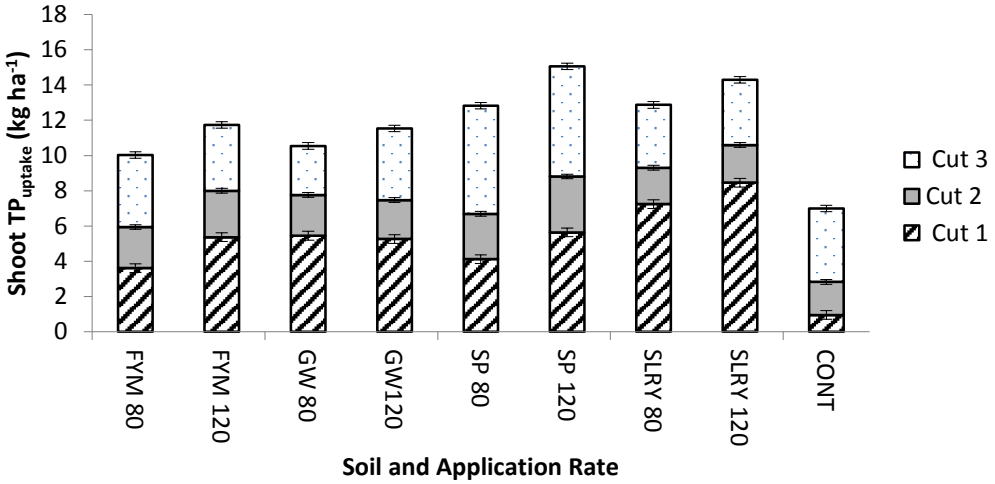


Figure 6-10: Shoot TP_{uptake} of ryegrass showing four cuts in a 6 month period (November 2011-April 2012). Data are means *n=4*. Error bars represent ± standard error. There was a significant difference between all soil treatments and application rates for each cut p<0.001.

Regression indicated that the effect of application rate on ryegrass TP_{uptake} exhibited statistically significant linear relationships for all treatments. Analysis of covariance indicated the slope of the regression line for each treatent (Table 6-5). Regression analysis showed that P uptake was related to the $C:P_{treatment}$. (Table 6-8) shows that TP_{uptake} increases with decreasing $C:P_{treatment}$. The slope of the regression line also increased with treatments with a lower $C:P_{treatment}$. Table 6-8 also shows no significant relationship existes between plant TP_{uptake} and, $TP_{treatment}$, $AVP_{treatment}$.

Table 6-5: Linear regression analysis relationships between mean shoot TP_{uptake} v P application rate (kg P ha^{-1}). R^2 values represent the correlation coefficient. *n=4* for each treatment at each application rate.

Treatment	Regression equation	R^2	p-value
SP	y = 0.07x + 7.1	0.99	>0.001
SLRY	y = 0.06x + 7.2	0.98	>0.001
GW	y = 0.04x + 7.1	0.96	>0.001
FYM	y = 0.04x + 7	0.98	>0.001

6.3.6 Root TP_{uptake}

The data reported in this section relates to root TP_{uptake} which is shown in (Figure 6-11). ANOVA was carried out to investigate the relationship between root TP_{uptake}*amendmen

source*treatment application rate. Figure 6-11 shows a significant difference ($p<0.001$) in root TP_{uptake} between treatments.

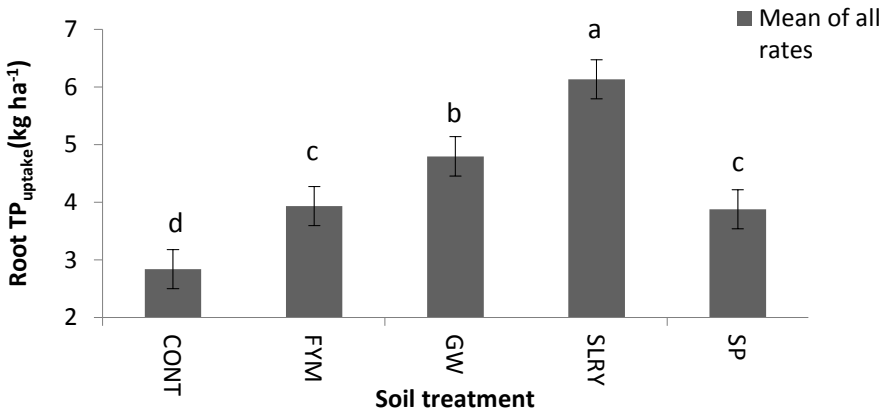


Figure 6-11: Root TP_{uptake} of ryegrass of ryegrass over a 6 month period (November 2011-April 2012). Data are means Control *n=4* Treated *n=8*. Error bars represent ± standard error. There was a significant difference between all soil treatments overall p<0.001. Significant differences between soils are denoted by lower case letters (Fisher LSD).

Regression indicated that the effect of application rate on root TP_{uptake} exhibited statistically significant linear relationships for all traetments. Analysis of covariance indicated the slope of the regression line for each treatment (Table 6-6). The application of treatments improved root biomass performance compared to the control by 114, 69, 39, 37% for SLRY,GW, FYM, and SP respectively.

Table 6-6: Linear regression analysis relationships between mean root TP_{uptake} v P application rate (kg P ha^{-1}). R^2 values represent the correlation coefficient. *n=4* for each treatment at each application rate.

Treatment	Regression equation	R^2	p-value
SLRY	y = 0.03x + 2.27	0.38	>0.001
SP	y = 0.02x + 2.75	0.66	>0.001
GW	y = 0.01x + 2.73	0.68	>0.001
FYM	y = 0.01x + 2.79	0.53	>0.001

Figure 6-12 displays a scatterplot of ryegrass root and shoot TP_{uptake}. There is a relationship between root and shoot TP_{uptake}. Subsequent analysis suggests the trend between aboveground and root TP_{uptake} is similar for all treatments, however the control shows a different trend, which lowers the correlation.

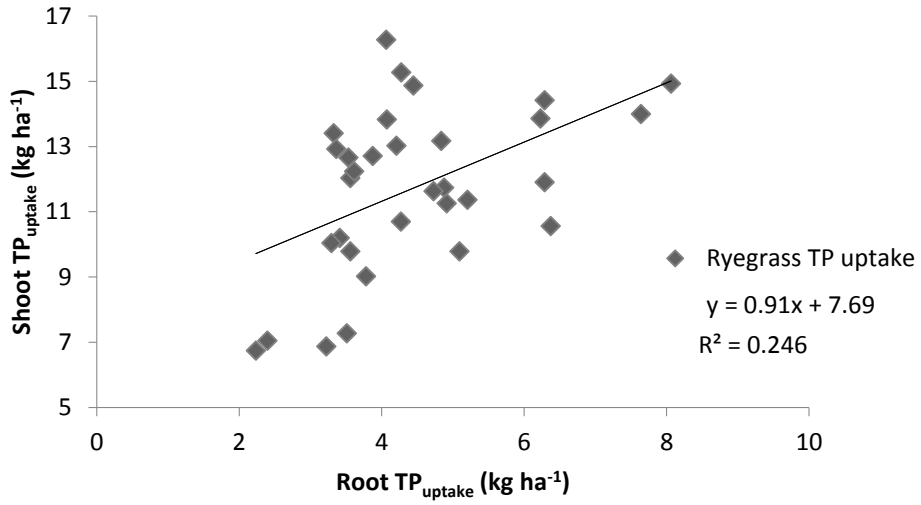


Figure 6-12: Scatterplot with simple linear regression analysis displaying a strong relationship between root and shoot TP_{uptake} for ryegrass for all treatments; p<0.001. R^2 represents the correlation coefficient.

6.3.7 Shoot TP_{plant}

The concentration of P in harvested ryegrass (TP_{plant}) was determined for each cut in the experiment according to the principal outlined in Table 6-8.The data reported in this section relates to TP_{plant} which is shown in Figure 6-13. The results of the regression analysis was carried out to investigate the relationship between TP_{uptake}*treatment source*treatment application rate is displayed in Figure 6-13**.** There was a significant difference (p<0.001) between treatmentssources amounts. TP_{plant} for each treatment followed the pattern SLRY> SP>GW>FYM. Values for each cut are compared for each treatment and rate. Regression analysis was carried out for mean TP_{plant} versus treatment application rate. Regression equations, R^2 and p values are displayed in Table 6-7**.**

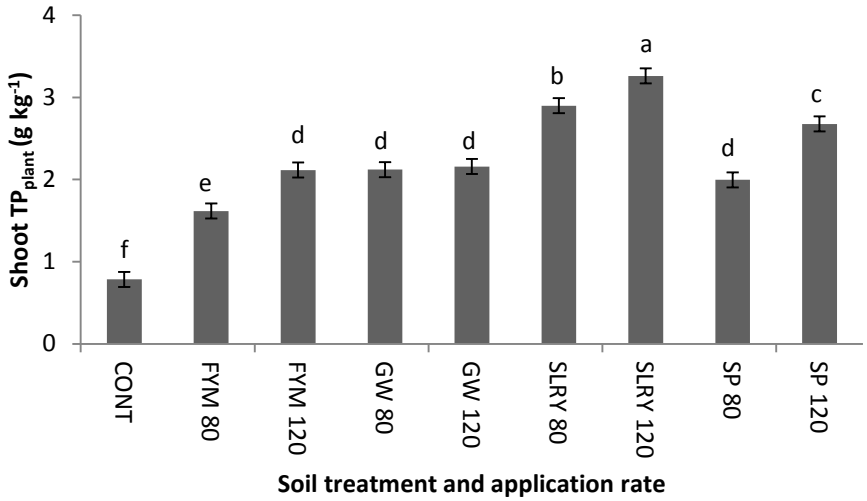


Figure 6-13: Shoot TP_{plant} of ryegrass over a 6 month period (November 2011-April 2012). Data are means *n=4*. Error bars represent ± standard error. There was a significant difference between all soil treatments overall p<0.012. Significant differences between soils are denoted by lower case letters (Fisher LSD).

Regression indicated that the effect of application rate on shoot TP_{plant} exhibited statistically significant linear relationships for all traetments. Analysis of covariance indicated the slope of the regression line for each treatment (Table 6-7).

Table 6-7: Linear regression analysis relationships between mean root TP_{plant} v P application rate (kg P ha^{-1}). R^2 values represent the correlation coefficient. *n=4* for each treatment at each application rate.

Treatment	Regression equation	R^2	p-value
SLRY	y = 0.09x + 3.5	0.96	>0.001
SP	y = 0.06x + 3	0.99	>0.001
GW	y = 0.05x + 3.5	0.93	>0.001
FYM	y = 0.04x + 3.1	0.99	>0.001

6.3.8 Root TP_{plant}

The data reported in this section relates to root TP_{plant} which is shown in (Figure 6-14). ANOVA was carried out to investigate the relationship between root TP_{plant}*treatment source*treatment application rate. Results show a significant difference (p<0.001) in root TP_{uptake} between treatments.

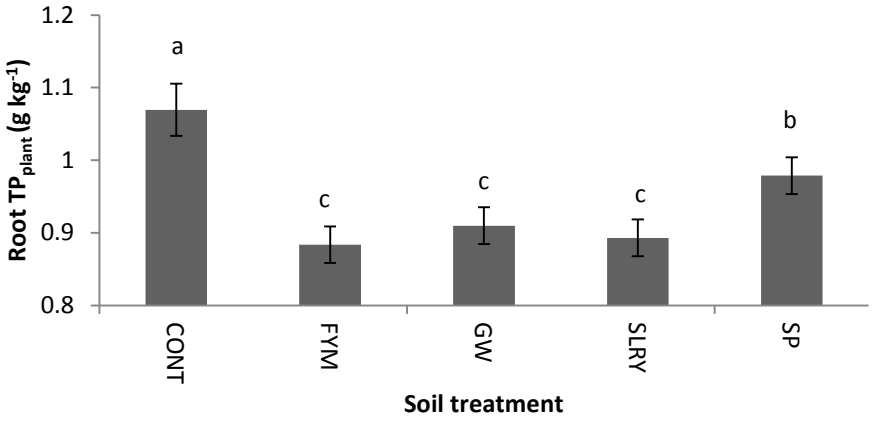


Figure 6-14: Root TP_{plant} of ryegrass over a 6 month period (November 2011-April 2012);. Data are means Control *n=4* Treated *n=8*. Error bars represent ± standard error. There was a significant difference between all soil treatments overall p<0.001. Significant differences between soils are denoted by lower case letters (Fisher LSD).

6.3.9 Depth profile

It was established in Section 2.6, that soil sampling in this experiment, which assesses P in soil removed from the full 12.5cm depth of the pot, is homogenising a portion of soil which may be spatially heterogeneous with depth, it was therefore decided that an analysis of the pot experiments with depth should be undertaken to determine this heterogeneity. It must be established that FYM and SLRY were the only treatments measured, for the purpose of preliminary analysis being conducted, and if significant findings were evident, further analysis would be conducted.

The data reported in this section relates to C_{DGT} which is shown in Figure 6-15(a). ANOVA was carried out to investigate the relationship between C_{DGT}*treatment source*application rate*depth. There is no significant difference ($p<0.001$) in C_{DGT} between treatment source* depth or treatment source*application rate. However there was a significant difference in overall $C_{DGT.}$

The data reported in this section relates to Olsen P which is shown in Figure 6-15(b). ANOVA was carried out to investigate the relationship between Olsen P*amendmen source*application rate*depth. There is no significant difference ($p<0.001$) in Olsen P between treatment source*depth or treatment source*application rate. However there was a significant difference between the control and treated soils.

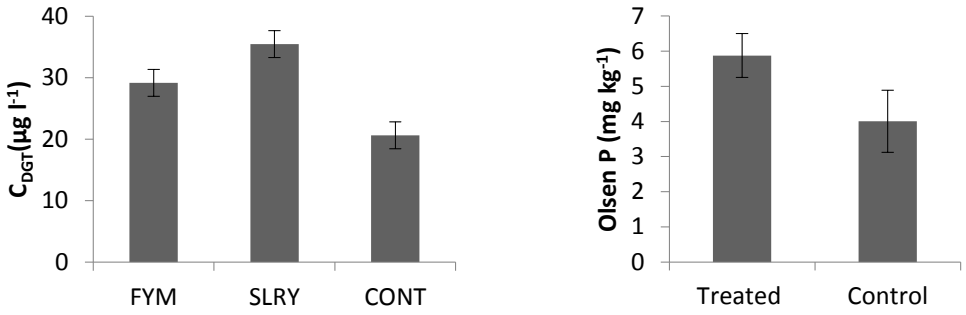


Figure 6-15: Change in (a) C_{DGT} and (b) Olsen P with depth for the mean of all soils following application of treatments (FYM and SLRY) at 0 and 120 kg P ha^{-1}. There was no overall significant decreases with depth for C_{DGT} and Olsen P p>0.05. Data are means *n=4*. Error bars represent ± standard error.

6.4 Discussion

6.4.1 Influence of treatments on C_{DGT}

This section involves a general discussion of how treatments influence C_{DGT}. The pattern of P release into the soil following treatment addition followed SP>SLRY>GW>FYM (Figure 6-3). This pattern of P release (inorganic >organic), has been identified by previous authors (Sharpley and Sisak, 1997; Igelesias- Jiménez *et al*, 1993; Griffin *et al*, 2003; Bar Tal *et al*, 2004). Results in this experiment suggest that the organic amendments are not as effective at releasing P into the soil solution as SP (Table 6-2).

This study established that C:$P_{treatment}$ was a better proxy for P availability than $TP_{treatment}$. Previous studies on P mineralisation have suggested treatment characteristics significantly influence P release (Griffin *et al*, 2003; Azeez *et al*, 2009; Miller *et al*, 2010). Authors have established that the C:$P_{treatment}$ affects its availability to soil (Nwoke *et al*, 2004; Nziguheba *et al*, 2000; Gagnon and Simmard, 2003) where P availability to soil decreases with an increased C:$P_{treatment}$. The principal is that the higher C:$P_{treatment}$ will cause immobilisation of P and reduce its availability in soil. Table 6-8 shows a significant relationship between treatment C:$P_{treatment}$ and the P release into soil, where FYM with the highest C:$P_{treatment}$ has the lowest P availability, and SLRY with the lowest C:$P_{treatment}$ has the highest P availability (Figure 6-4) (SP was not included in analysis). Linking this information with results found in **Chapter 4**, it can be expected that a similar pattern of immobilisation is occurring in the pot experiment as the incubation, suggesting

immobilisation of P is limiting P availability and hence plant growth in the early stages of this experiment for FYM in particular. An important factor influencing this behaviour is the time of sampling. The first soil sample was taken on day 30. It was suggested in **Chapter 4** that by day 30 much of the available P from treatments had already undergone transformations with the soil organic and inorganic constituents, reducing the availability of P. However it must be highlighted that the time of grass establishment (November 2011) meant that soil temperatures differed between pot and incubation experiments. Incubation temperatures were a constant 25°C, whereas in the glasshouse temperatures ranged from a minimum of 10°C and rarely exceeded 15°C, which would be expected to have been responsible for reduced MBP production in the glasshouse compared to incubation studies.

This has important implications for analysis of the relationship between C_{DGT} and extractable P content of the treatment ($AVP_{treatment}$). Previous studies (Eghball, 2002; Sneller and Laboski, 2009) explained that the soluble P content of the organic amendment is the factor limiting P release into the soil. Regression analysis in this study showed no significant relationship between $AVP_{treatment}$ and P release (Table 6-8). It is observed that this is due to the $AVP_{treatment}$ of the FYM, and low C_{DGT} throughout the experiment compared to other treatments. Despite having a higher $AVP_{treatment}$ than GW, the P released was less over the experiment. It was established in **Chapter 4** that following treatment application FYM released significantly more P in the first 14 days, than all other treatments. However for the remainder of the experiment lower C_{DGT} values were recorded for FYM than other treatments. In this study the first soil samples were recorded after day 30, therefore if a similar pattern of P release from FYM occurred, it was not recorded due to the sampling regime. Therefore although there is no significant relationship in this experiment between $AVP_{treatment}$ and C_{DGT} for the timescale measured, previous experiments show this is an important factor influencing P release over a different timescale. It is expected that the low $AVP_{treatment}$ of the GW is a factor, limiting P release into the soil over the 6 months of this experiment. The high $AVP_{treatment}$ of SLRY is also expected to combine with low C:P as an important factor determining the relatively higher P availability. Although the low $AVP_{treatment}$ of the GW is thought to be a factor limiting P release in this experiment, it must be established that the duration of 6-months was relatively short. Sneller and laboski (2009) have shown that the benefits of compost are

realised over a number of years following application to soil. Therefore if this experiment was carried out over a longer period, a different pattern may be observed.

It was highlighted in **Chapter 1** that the critical P content of organic amendments above which there is no net immobilisation is 0.2-0.3 % (Laboski and Lamb, 2003). So it would be expected that the treatments with a greater $TP_{reatment}$ (Table 3-1) would be responsible for greater C_{DGT} in soil, however regression analysis shows the opposite trend (Table 6-8), where SLRY with the lowest $TP_{treatment}$ (Table 3-1) increased C_{DGT} most, over the timescale of the experiment (Figure 6-3). It is expected that other treatment characteristics such as $C:P_{treatment}$ are more important at determining C_{DGT} over the timescale of this experiment than amendment $TP_{treatment}$.

The rapid decrease in C_{DGT} following treatment addition was expected to have resulted from distribution of P amongst forms which are not plant available; a principal mechanism is adsorption of P to inorganic soil constituents. Miller *et al*, (2010) suggested this when assessing P dynamics with Mehlich-3 extraction. The soil is very high in Ca, Mg, Fe and Al as shown in Table 4-3.This suggests that the fixation capacity of this soil is high, and could be responsible for the initial decrease in C_{DGT} following application to soil. Previous studies (Iyamuremye *et al*, 1996; Iglesias Jimenez *et al*, 1993) highlighted that organic amendments can block P adsorption sites, improving the availability of P to the plant. This study did not measure P adsorption /desorption behaviour following application of treatments, however as SP is more available than organic amendments, therefore it is unlikely that the blocking of adsorption sites following organic amendment addition was sufficient to increase C_{DGT} to levels achieved by SP application.

6.4.2 Relationship between C_{DGT}, Olsen P and Soil solution P

Previous authors have investigated the relationship between application of treatments (organic and inorganic) and the resulting distribution of P between the relative pools, based on availability (Hedley *et al*, 1982*)*. Some studies have found that P in the soil solution (H_2O or resin P) behaves in a similar way to the $NaHCO_3$ pool (Read *et al*, 2007; Ayaga *et al*, 2006) however others found that the two pools can behave in different ways following application of different treatments (Kashem *et al*, 2003). Olsen P represents extractable P. Soil solution represents forced extraction of the soil pore water by centrifugation; therefore represents plant available P in the soil solution. C_{DGT} represents the portion of the soil solution which is available by diffusive supply, as a time averaged

concentration. Results show a similar trend between Olsen P and C_{DGT} and Soil solution over time. This is the first time the pattern of change has been compared between C_{DGT} and extraction solutions. Therefore it is expected that the pattern is dependent on a number of soil specific factors.

Previous authors (Mason *et al,* 2008; Menzies *et al,* 2005; Mcbeath *et al,* 2007; Mason *et al*, 2010) have stated that C_{DGT} is a more accurate indicator of plant available P than extraction solutions. This is based on C_{DGT} showing a better correlation (R^2=0.74 for DMY and grain) with plant uptake than the extraction solutions (Colwell P had no significant relationship) (Mason *et al*, 2010). However (Humphreys *et al,* 2001*)* stated that different extraction solutions are used in different countries based on the suitability for the soils of that particular country. In previous comparisons, the correlation with the extraction solutions and DMY/TP_{uptake} was relatively poor, suggesting the extraction solution was unsuitable for this particular soil (Mason *et al,* 2008). However Mcbeath *et al,* (2007) found a better relationship between some extraction solutions (R^2=0.53-0.82) and plant response to fertiliser addition, than C_{DGT} (R^2=0.74-0.82).

Olsen P is a suitable extraction solution, for use on soils in the U.K. according to the RB209 fertiliser manual, (Defra, 2010) representing a good relationship with root/shoot DMY. It is therefore not surprising that Olsen P showed a good correlation with DMY and TP_{uptake} (Table 6-8). However the stronger correlation between C_{DGT} and combined DMY and combined TP_{uptake}, confirms that relationships identified in previous studies are similar to those found in this study; DGT is a more accurate representation of plant available P than Olsen P. It was explained in **Chapter 1** that extraction solutions represent P in forms which are not readily plant available, as well as plant available P. However C_{DGT} measures the P available in the soil by diffusive supply therefore representing a more readily available pool of P within the soil. This work confirms this and also suggests that the accuracy of the C_{DGT} technique is still greater on soils, which have received organic amendments.

Table 6-8: Regression analysis of C_{DGT}, Olsen P and Soil solution P vs Plant factors (DMY and TP_{uptake}). Values represent the correlation coefficient displayed as R^2. Values in red indicate a significant relationship for the test p>0.05. *Indicates analysis which was carried out without SP. ^Indicates combined shoot and root. N/S –Indicates no significant relationship (p>0.05). x – Not appropriate to calculate.

	Olsen P	C_{DGT}	Soil Solution	Shoot DMY*	RootDMY*	Shoot TP_{uptake}*	Root TP_{uptake}*	Combined DMY	Combined TP_{uptake}
C_{DGT}	0.73								
Soil Solution	0.57	0.6							
Shoot DMY*	0.63	0.73	0.24						
Root DMY*	0.65	0.59	0.45	0.69					
Shoot TP_{uptake}*	0.73	0.77	0.27	0.8	0.5				
Root TP_{uptake}*	0.65	0.53	0.53	0.54	0.91	0.38			
Combined^ DMY*	0.71	0.80	0.75	x	x	x			
Combined^ TP_{uptake}*	0.52	0.72	0.75	x	x	x			
$TP_{treatment}$*	0.65	0.56	N/S	0.61	0.53	0.5	0.37	0.6	0.62
$AVP_{treatment*}$	0.4	0.4	N/S	0.28	N/S	N/S	N/S	N/S	N/S
$C:P_{treatment}$*	0.66	0.72	N/S	0.68	0.46	0.75	0.35	0.54	0.38

6.4.3 Root and shoot DMY

Previous authors have highlighted the importance of treatment properties on shoot DMY. Kwabiah *et al,* (2003) and Nziguheba *et al,* (2000), explained that the $C:P_{treatment}$ can predict TP_{uptake} (when N is non limiting). However (Laboski and Lamb, 2003; Gagnon and Simmard, 1999) explained that $TP_{treatment}$ can be used to predict soil extractable P. Kuligowski *et al,* (2010) found soil extractable P increases following organic amendment application, to show a good relationship with TP_{uptake} and DMY. Ylivainio *et al,* (2008) and Nwoke *et al,* (2004) found that shoot DMY did not increase with increasing $TP_{treatment}$ or decreasing $C:P_{treatment}$. Umrit and Friesen (1994) stated that using the treatment $C:P_{treatment}$ to predict immobilisation would be misleading and Nwoke *et al,* (2004) suggested that attempts to elucidate the relationship between $C:P_{treatment}$ and extractable P following treatment addition to soil have been inconsistent.

In a review of the literature, it was determined that studies into the effect of treatments on ryegrass DMY and TP_{uptake} following treatment addition, focuss on aboveground biomass, and neglect the important effect on root DMY and $TP_{uptake.}$ A few studies (Chen *et al*, 2002; Pederson *et al*, 2002) investigated root TP_{uptake}, and Waldrip *et al,* (2011) assessed the effect of poultry manure on root TP_{uptake} and total biomass production.

In this study, total biomass was partitioned relatively evenly between roots (44%) and shoots (56%). This is relatively comparable to a study by Waldrip *et al,* (2011) who fould root biomass (57%) and aboveground biomass (43%). Although it differs slightly in that their study shows more of the biomass is contained in roots than aboveground, which is opposite to this study.

The treatment characteristic with the strongest influence on DMY is the $C:P_{treatment}$ (Table 6-8). $C:P_{treatment}$ values are provided in Table 3-1. Nwoke *et al,* (2004) explained that as $C:P_{treatment}$ increases aboveground DMY decreases, and a C:P ratio >200 produced lower shoot DMY than those with lower ratios. This is similar to the patterns identified in this experiment (Table 6-8). Stevenson and Cole (1999) explained that a C:P > 300 is responsible for immobilisation and a C:P <200 is responsible for mineralisation. It is therefore expected that the higher $C:P_{treatment}$ was responsible for

lower rates of P mineralisation particularly from FYM and and to a lesser extent GW, when compared to SLRY which had the highest P C_{DGT} following incorporation to the soil (Figure 6-3). This increased soil P following treatment application is therefore responsible for increasing the ryegrass DMY.

It is expected that the C concentration of treatments had a similar effect on N mineralisation / immobilisation patterns as P. Stevenson and Cole, (1999) explained that N and P mineralisation and immobilisation patterns are linked and show similar trends. Therefore the addition of treatments would have influenced the availability of the mineral N which was added to the soil along with the treatments affecting its availability within the experiment. It is unlikely that this would have had a significant influence in the first cut as its availability would have been sufficient for optimal growth, causing P to be the limiting nutrient, however with time the interaction of the mineral N with the treatments in soil may have caused differences in available N, resulting in N becoming more limiting than P. Antille, (2011) and Simic *et al,* (2012), explain that N is the most important nutrient limiting plant growth. Following its application to soil, N is extremely mobile, and undergoes a number of transformations, as time commences within a cropping season, losses from the soil system can occur from volatilisation, denitrification, mineralisation and leaching. Antille *et al*, (2011) found a similar pattern of shoot DMY following ryegrass establishment and attributed this to N losses described above.

Despite a positive correlation between DMY and $TP_{treatment}$ (R^2=0.61), $TP_{treatment}$ did not significantly influence DMY in this experiment (Table 6-8). Laboski and Lamb, (2003) explained that $TP_{treatment}$ can accurately explain differences in extractablee P following treatment application. Authors have reported that increases in extractable P following application of treatment can increase ryegrass shoot DMY (Waldrip *et al,* 2011, Yilvanio *et al,* 2008). The main reason it does not explain DMY, is because the SLRY has a lower P content than other treatments, yet it is responsible for a greater yield. Kuligowski *et al,* (2010) explained that treatments exhibit significantly different behavioural patterns, whether applied in a solid or liquid form. It was observed treatments applied in liquid form were responsible for greater P utilisation efficiency. This may help explain in part why SLRY despite having a lower total P content than the

other treatments produced greater DMY(along with the effect of $C:P_{treatment}$ detailed above (Stevenson and Cole, 1999).

$AVP_{treatment}$ also has a poor relationship with DMY, Ylivainio *et al,* (2008) explained that the % of $TP_{treatment}$ available as $AVP_{treatment}$ influences soil P and DMY. However there was no relationship between $AVP_{treatment}$ and DMY (Table 6-8). SLRY produced greater DMY than FYM and GW (Figure 6-7 and Figure 6-8), which is in agreement with the principal of increasing $AVP_{treatment}$ giving a greater DMY, however, $AVP_{treatment}$ in FYM is greater than that in the GW, yet GW has a greater yield. It is thought that soil biological factors had an important role in transforming the high $AVP_{treatment}$ in FYM into less available P forms. It is likely that a portion of the AVP in the FYM was rapidly immobilised after application to the soil, due to its high $C:P_{treatment}$ (Table 3-1), resulting in a lower P availabliity and hence a lower DMY production. This is in agreement with results in **Chapter 4**, which show rapid increase in Olsen P and C_{DGT} FYM in the first week after application to soil, followed by a rapid immobilisation by the soil microbial biomass. It is likely that a similar trend occurred in this experiment, which is responsible for the lower yield despite high $AVP_{treatment}$.

Read *et al,* (2007) found annual ryegrass shoot DMY values in the region of 5000 (control) to 14 000 (treated) kg ha^{-1} for the first year following application. Antille, (2011) found values between 2000 (control) and 9000 (treated) kg ha^{-1}. In this experiment values for shoot DMY vary between 2900 and 9400 kg ha^{-1} within 6 months and are within a similar range as those aforementioned experiments (Figure 6-7).

Ylivainio *et al,* (2008) found that by adding P rich by products at 100 mg P kg^{-1}, an increase in ryegrass shoot DMY of between 27 and 120 % could be expected. This study shows that adding treatments to soil can increase ryegrass shoot DMY between 59 and 168 % for 80 kg P ha^{-1} and 55 and 224 % for 120 kg P ha^{-1} overall. Subba Roa *et al,* (1996) previously established that the response of crops to applied P in P deficient soils was outstanding, as is confirmed by these findings.

It was hypothesised that SP would be responsible for greater P release than organic amendments, and in turn shows greater DMY and TP_{uptake}.SP was indeed responsible for greater P release than organic amendments however Figure 6-7 and Figure 6-8, show a greater DMY in soils which received organic amendment application over SP for root

and shoot, despite a greater C_{DGT} and Olsen P (Figure 6-3 and Figure 6-5) for soils which received SP. This trend is unexpected, Oberson *et al,* (2010) found that ryegrass produced more shoot DMY when fertilised with mineral P than manure P, this happened in the prescence of a higher extractable P in the soil. However Sharpley (1996) suggested that organic fertilisers may have equivalent or better effects on crop yields than inorganic sources. It was suggested that increased DMY resulted from improved soil chemical, physical and biological property changes following the addition of treatments, which have the effect of increasing available P (Eichler–Lobermann, *et al,* 2007). C_{DGT} is higher following SP addition than organic amendments. Therefore these properties which are altered following treatment application may still be responsible for increasing DMY, however it is unlikely that P is limiting in this case.

The slope of the regression lines displayed in Table 6-3 and Table 6-4 are a reflection of the efficiency of each of the treatments to supply P (shoot and root respectively). Subba Rao *et al,* (1996), explained that the larger slope value reflected a more efficient utilisation of applied fertiliser. The efficiency of the treatments in this experiment followed SLRY>GW>FYM>SP which was the same pattern for overall DMY. Kuligowski *et al,* (2010) showed that liquid fertilisers (organic) had 3 times the efficiency of solid ones. This may explain why SLRY was more efficient than GW and FYM. The efficiency of each treatment shows the same pattern as overall availabiltiy, it is expected that the factors influencing efficiency and availability are the same.

It is likely that DMY may be influenced by soil K. Read *et al,* (2007) and Pettigrew, (2008) explained that K deficiency can have a significant impact on crop yield, when K levels are insuficient, reduced production of grain fiber or biomass occurs. This is thought to be due to the reduced overall production of photosynthetic assimilates. Table 6-8 shows that the initial K content of the soil is extremely low and is categorised as deficient, based on RB209 fertiliser manual recommendations (Defra, 2010). The addition of treatments is thought to have increased soil K over the control and SP amended soil. This increased K available from the treatments v SP is likely to play a role in improving the yield of the organically amended soils over the SP soil.

6.4.4 Root and shoot TP_{uptake} and TP_{plant}.

TP_{uptake} is partly determined by DMY and TP_{plant} , therefore mechanisms which are important in influencing DMY are equally important for TP_{uptake} determination. To avoid repetition, the factors which influence TP_{uptake} but were previously mentioned in the DMY section will be highlighted.

It was established in Section 6.4.3 that a lack of information exists about influences of different treatments (specifically organic amendments) on ryegrass shoot TP_{uptake}, and there is even less information available on root TP_{uptake}. In addition Figure 6-12 shows there is a weak relationship (R^2=0.25) between overall root and shoot $TP_{uptake.}$ Results show the overall general pattern of root and shoot TP_{uptake} is similar for all organic amendments however a different behaviour for the control between roots and shoots, makes this relationship weak. It is expected that this occurs as a result of different patterns of overall TP_{plant} of roots and shoots. The TP_{plant} of the roots for the control was significantly greater than the treatments in the roots (Figure 6-14), however this trend was not identified in the TP_{plant} of shoots (Figure 6-13). It has been established that when there is sufficient P in the soil for plant uptake, P absorbed by the plant roots is transported in the xylem to the younger leaves. There is also significant retranslocation of P in the phloem from older leaves to the growing shoots and from the shoots to the roots. However when P is deficient the supply from roots to shoots by the xylem is restricted, P is then mobilised from stored P in old leaves and retranslocated to younger ones and to roots for further growth (Schachtman *et al*, 1998). It is anticipated that this mechanism is responsible for the higher P concentration of plant roots in the control than treated for experiment.

However consideration of alternative mechanisms must be conveyed. Pang *et al* (2010) found high root (perennial legumes) P concentrations in P –impoverished environments, may be related to a low capacity of the plant to down-regulate P uptake. Teng et al, (2013) highlighted that when soils are deficient in P, plants have developed specialized morphological, physiological, and biochemical adaptive mechanisms to modify the rhizosphere and, hence, increase the ability of their root systems to utilize Pi from soils. Mechanisms include: (1) investment of a greater proportion of photosynthates in the roots, alterations in root morphology, and establishment of symbiotic relations with

arbusular-mycorrhizal (AM) fungi to increase exploration of the soil volume; (2) increased proton release and secretion of organic anions and phosphatase enzymes into the soil to mobilize Pi from inorganic and organic P sources in the rhizosphere ; and (3) enhancing the capacity of root cells to take up Pi by increasing the employment of highaffinity Pi transporters. Therefore it must be considered that each of the mechanisms described above may play an important role in providing the ryegrass root with a greater P concentration than the roots of the soils which had received P supply from treatments.

As a result of the mechanisms described above discussion of results of treatment influences on root and shoot TP_{uptake} must be investigated separately. The important influence of $C:P_{treatment}$ on yield was described previously (Section 6.4.3) The treatment characteristic with the greatest relationship with shoot TP_{uptake} is $C:P_{treatment}$ (Table 6-8). It is expected that the high C content of the FYM and GW was responsible for greater immobilisation of C_{DGT} than SLRY (**Chapter 4**). Hence the uptake of P for SLRY amended soils is greater.

$TP_{treatment}$ has a significant influence on shoot TP_{uptake} (Table 6-8) in this experiment. However the relationship between overall shoot TP_{uptake} and $TP_{treatment}$ showed that, the trend opposite to what would be expected based on the theory suggested by (Zvomuya *et al*, 2006) which states that treatments applied with a higher TP content release more extractable P and $TP_{treatment}$ could describe 81% of the variation in TP_{plant}. It is likely that the correlation with TP_{uptake} does not accurately explain a relationhsip between $TP_{tretment}$ and TP_{uptake}. It is expected that other aspects of the treatment chactacteristics have a stronger influence on the ryegrass TP_{uptake} (described above). $AVP_{treatment}$ also has a weak relationship with TP_{uptake}, Table 6-8, the mechanism described in detail in **Chapter 4**, where the available P in the FYM is rapidly immobilised causing less to become available in the soil, is thought to be responsible for this poor relationship with TP_{uptake}.

Sikora and Enkiri *et al*,(2005) and Ylivainio *et al*, (2008) found that the highest shoot TP_{uptake} was obtained in the first cut following application of treatment and the following cuts progressively decline, as the N is removed from the system. This trend is observed for all soils, which received application of organic amendments, however for

the soil receiving SP and the control, the final cut had the greatest TP_{uptake} (Figure 6-10). It is likely that the initial poor yield was due to insufficient P and K for ryegrass establishment. Consequently less of the mineral N which was applied to all soils at the same rate at the start was removed from the soil by uptake, which meant that as environmental conditions improved later in the experiment, there was more N available, which helped improve DMY, and in turn TP_{uptake} later in the experiments. The extra N in the soil would have helped mobilise the residual soil P under improved environemntal conditions in both the SP soil and the control, which is likely why these soils have a much higher TP_{plant} than other experiments in the last cut. A similar effect is likely for SP, which was deficient in K at the start of the experiment, therefore despite high P and N concentrations, establishment was initially restricted, however when established there was additional N and P in soil to improve TP_{uptake} for subsequent cuts.

Read *et al,* (2007) observed ryegrass shoot TP_{uptake} values in the region of 11.6 to 23 kg ha^{-1}. By adding broiler litter at between ~4.5 and 36 kg ha^{-1} /yr^{-1}, an increase in ryegrass TP_{uptake} by ~108 -333% could be expected. Ylivainio *et al,* (2008) found that increases in the range 27 and 141% for meat and bone meal and dairy manure at 25 and 100 mg P kg^{-1} respectively. Values in this experiment range between 7 and 15.1 kg ha^{-1} for half a year, are within a similar range as those aforementioned experiments. This study shows that adding different treatments to soil can increase ryegrass TP_{uptake}, between 44 and 84% for 80 kg P ha^{-1} and 68 and 105 % for 120 kg P ha^{-1} following treatment application and ryegrass establishment. Subba Roa *et al,* (1996) previously established that the response of crops to applied P in P deficient soils was outstanding, this is confirmed by these findings.

The slope of the regression lines displayed in Table 6-5 and Table 6-6 are a reflection of the efficiency of each of the treatments. Subba Rao *et al,* (1996), explained that the larger slope value reflected a more efficient utilisation of applied fertiliser. The efficiency of the treatments in this experiment followed SP>SLRY>GW>FYM which was the same pattern for overall TP_{uptake}. The shoot TP_{uptake} of the ryegrass treated with SP is greater than all other sources (Figure 6-10), this despite it having a lower DMY than the organic amendments. This adds to the point made in Section 6.4.3 that P is not the main factor limiting plant DMYfor SP.

6.4.5 Depth profile

Results show a similar trend for Olsen P and DGT. It is expected that there is no significant difference between the treatment amounts with depth for the following reasons.

- Because it was the end of the experiment, most of the P added had been removed from the system by mechanisms described in Section 6.4.
- In previous studies, where authors have identified changes in P with depth, the soil has received repeated applications of P over a period of time. It is expected that the short term nature and single application of P has contributed to the low P availability.
- It is possible that the method of disturbing the pot exposed the soil to O_2 this would be expected to have significantly influenced results.

6.5 Soil pH

This section relates to pH which is shown in Table 6-9. ANOVA was carried out to investigate the relationship between pH*treatment source*application rate*time. There was a significant difference ($p<0.001$) in pH for, treatment source* time. However there is no significant difference in pH when applied at different application rates. Each soil increases in pH from start to end. The increase follows the pattern GW>SP>FYM>SLRY>CONT. Soil pH increases following the addition of all treatments (inorganic and organic) treatments increase pH more than the control. It is expected that the increase from start to the end is mainly influenced by the addition on the treatments, as is evident in the greater pH increase over the control soil. However the pattern of change for all P measurements is different to that of pH change, it is therefore expected that although pH may have had an effect on P availability, it was not the most important factor in this experiment. It is expected that this is the case because changes are within pH values optimum for P speciation (Waldrip 2010).

Table 6-9: Displaying pH change from the start of the experiment to the end. Std error represents ± standard error. Data are means *n= 4* for control, *n=8* for treated.

Treatment	Start	End
CONT	6.6	6.6
FYM	6.6	6.7
GW	6.6	6.9
SLRY	6.6	6.7
SP	6.6	6.8
Std error	0.002	0.021

6.6 Appraisal of DGT

The DGT technique is a soil P test which is in its relative infancy, as there have been few studies on its application to soil as a P test. However those studies have shown it to be a promising tool for P analysis (Mason *et al*, 2008; Menzies *et al*, 2005; Mcbeath *et al*, 2007; Mason *et al*, 2010). Despite the promising results, it has never been used to a) study the effects of application of organic amendments to soil. b) Assess the relationship between soil available P and plant growth and uptake characteristics over time. c) Assess the effects of different treatments on the same soil.

C_{DGT} was a more accurate indicator of ryegrass root and shoot DMY and TP_{uptake} than Olsen P and Soil solution P. Regression analysis of C_{DGT} and treatment characteristics is highlighted Table 6-8. C_{DGT} accurately predicts a relationship between $C:P_{treatment}$ and P release from treatment to soil. This is the first time analysis like this has been conducted; therefore there are no previous studies to compare results to. This shows that the $C:P_{treatment}$ can influence the P in soil which is readily available by diffusive supply, adding to the idea put forth by (Gagnon and Simmard, 1999) that the $C:P_{treatment}$ influences soil available P. Although this work highlights the importance of $C:P_{treatment}$ at increasing C_{DGT}, it must be noted that this is specific to this experiment, and it is likely that under different experimental conditions other characteristics of organic amendments may be the factor most important in determining C_{DGT}.

Regression analysis between C_{DGT} and TP_{uptake}, DMY (root and shoot) is shown in Table 6-8. DGT accurately predicts the ability of this soil pool to contribute P to the plant. Previous studies have found a good correlation between DGT and TP_{uptake}/ DMY (Mason *et al,* 2008; Menzies *et al,* 2005; Mcbeath *et al,* 2007). However the primary focus was to assess a range of soils to assess how accurate the technique could predict TP_{uptake}. The regression analysis, which showed an accurate relationship between C_{DGT} and the plant factors, gives confidence that DGT, can be used to carry out this type of analysis. This confirms that when treatments are added to the soil, they undergo transformations, which affect C_{DGT}, and thus the P available by diffusive supply for plant uptake. This approach assessing the value of different materials added to a specific soil has value in agronomy, as it allows a farmer to assess the value of different materials and application rates on a specific soil.

It is also the first time DGT has been used to measure the contribution of P to the soil then plant from organic amendments over time. Specifically it is the first time an analysis of the root DMY or TP_{uptake} has been related to C_{DGT}. This was important to assess, as the principals of DGT mean that it is supposed to simulate a plant root. Work in this experiment confirms that this is indeed the case. This experiment is the first time C_{DGT} has been used to assess the slope of the relationship between application rate and different treatments and P availability. Results suggest that C_{DGT} increased over the control for treated soils. The gradient of the slope suggests a greater efficiency of released P. The pattern of efficiency for C_{DGT} follows SP>SLRY>GW>FYM. This information firstly shows that the C_{DGT} pool is responsive to different application rates of different organic amendments, and that the effect of each amendment is not equal when added to the soil. This suggests that the pool of P readily available for plant uptake (C_{DGT}) is sensitive to different organic amendments at different application rates.

6.7 Conclusions

- The pattern of P release from each treatment, and its subsequent influence on C_{DGT} represented an increase with decreasing $C:P_{treatment}$ (R^2=0.72). $C:P_{treatment}$ was more important than $TP_{treatment}$ (R^2= 0.56) as a proxy for P availability. This is because the C content of the treatment applied significantly influences microbial immobilisation.

- Higher P availability does not necessarily lead to an increased DMY. Despite the greater C_{DGT} following SP addition than all other treatments, there was a lower root and shoot DMY than on soils, which received organic amendments. SP addition increased shoot DMY over the control by 57% overall, compared to 121, 152 and 196 % for FYM, GW and SLRY respectively. It is likely that the improvements in biological, physical and chemical properties organic amendments supply to soil, which SP does not, was responsible for this trend.
- There was an increase in shoot TP_{uptake} for all treatments, SP increased shoot TP_{uptake} over the control, by 100% overall, compared to 53, 58 and 94% for FYM, GW and SLRY respectively.
- There is a strong relationship between C_{DGT} and DMY for both roots (R^2=0.59) and shoots (R^2=0.73). Overall this relationship is stronger for roots and shoots combined than roots or shoots alone (R^2=0.73). Similarly there is a strong relationship between C_{DGT} and TP_{uptake} for both roots and shoots (R^2=0.53) and shoots (R^2=0.77). Overall this relationship also strong for roots and shoots combined (R^2=0.72).The aforementioned R^2 values are generally greater for C_{DGT} than Olsen P or soil solution P, indicating that C_{DGT} is the more accurate indicator of DMY and TP_{uptake} in this study.
- The trend between Olsen P, Soil solution P and C_{DGT} is similar over time, suggesting that at a low P status P pools undergo similar transformations following treatment application and P uptake.

7 Soil kinetic parameters

7.1 Introduction

It has been established that P is supplied to plant roots from soil solution. Soil solution is resupplied from the solid phase as P concentration is depleted adjacent to the root, according to nutrient uptake models (Barber, 1995; Zhang *et al*, 2004). If supply from solid phase to solution in kinetically limited, this can influence plant P uptake. Determining rates of P transfer from the solid phase to solution at a timescale relevant to that which occurs in the rhizosphere generates information on the role of this kinetic control (Zhang *et al*, 2006).

DGT induced fluxes in soils (DIFS) is a dynamic numerical model of P transfer from the soil to the DGT device has been developed (Harper *et al*, 1998). It represents exchange of P between solid phase and solution using first order rate equations, with the equilibrium partition between the two phases described by a distribution coefficient for labile phosphorus K_d. Measuring the ratio of the DGT measured concentration to the soil solution concentration, R, allows the calculation of kinetic parameters. Kinetic parameters are the response time to perturbation of P removal, T_c, which is related to the rate constants k_1 and k_{-1} (Zhang *et al*, 2006).

Extension of this approach to soils which have received application of treatments is necessary to identify their effects on kinetic processes which control phosphorus supply to plants. Using DIFS advances conceptual understanding of the dynamic response of the soil to perturbations that locally lower concentrations e.g. DGT and plants.

This chapter uses data from soils collected from glasshouse experiments outlined in **Chapters 5 and 6**, which received addition of P in organic form as FYM, GW, SLRY and in inorganic form as superphosphate (SP) to two different soils at different application rates in order to assess their relative effect on soil P availability.

The main aim of the chapter is to understand how addition of treatments to soil affects the quantitative relationship for the distribution of P between solid and solution phases, in relation to the DGT device.

Objective

To investigate the effects of treatment addition to each of the aforementioned soils on soil kinetic parameters, in order to understand how kinetic limitations influence P supply.

Hypotheses

- Addition of treatments to soil will result in a reduction in response time (T_c) of the soil phase to resupply soil solution for Gleadthorpe and Kincraigie soils.
- Addition of treatments will result in increased dissociation rate constant k_{-1} values overall.
- Addition of treatments will increase the soils ability to resupply P in response to lowering of solution concentration compared to control soils.

7.2 Methods

7.2.1 Glasshouse experiments

This analysis was conducted using data from glasshouse experiments which are described in detail in **Chapter 5 and 6**. Soil analysis was carried out before each experiment started (Day 0), following treatment application (Day 30) and at the end of the experiment (Day 180 and 360 for Kincraigie and Gleadthorpe respectively), are used in this experiment. These sample days were selected in order to understand the soil P kinetics before and after treatment application (Day and month scale). Table 7-1 gives an overview of the experimental setup.

It must be established that the two soils represent different treatment application histories, different texture and therefore a direct comparison of them based in this experiment would be impractical.

Gleadthorpe soils have had historical application of organic amendments from ADAS-QC trials which are described in Section 3.2.1. Therefore day 0 corresponding to each soil treatment represents the culmination of the ADAS-QC study. The following sampling dates (day 30 and 360) represent a fresh application of the corresponding treatment at the rates described. Each corresponding soil also has an unamended control which received no fresh treatment application and therefore represents only the historical treatment applications.

Application history was not a factor taken into consideration for Kincraigie soils, therefore treatments were applied to exactly the same soil. Sampling day 0 represents the control before treatment application and days 30 and 180 are after treatment application.

For the sake of clarity definitions will be established herein. When reference is made to treated soils this means all soils which have received application of P in this experiment, whether it is an organic amendment or SP. Control ADAS-QC refers to the control of the ADAS-QC experiment described in which is represented by Gleadthorpe day 0. Gleadthorpe control soils are those which have been taken from corresponding treatment plots from ADAS-QC sites and used in the pot experiment of this study but received no further treatment addition (Table 7-1).

Table 7-1: Details of experimental setup.

	Application rates (kg P ha^{-1})	Sampling days	Treatments	Reps
Gleadthorpe	0*, 15 and 25	0,30, 360	FYM, GW, SP, SLRY	4
Kincraigie	0, 80, 120	0,30, 180	FYM, GW, SP, SLRY	4

*0 (Gleadthorpe) Represents the soil which had received the historical treatment addition (ADAS-QC) however received no additional application in this study. There was therefore an untreated control for each historically treated soil.

7.2.2 DIFS

The procedure for analysing the soil characteristics, Soil solution P, C_{DGT}, and Olsen P have been described in **Chapters 5 and 6**. In this chapter, Olsen P is displayed as $C_{ls,}$ which is short for concentration of labile phosphorus on soil solids. Soil solution P will be displayed as C_{soln} and C_{DGT} will remain the same.

It is important to establish that the quality of the input data has been ensured. Input parameters were derived from established methods of soil analysis with at least three repetitions taken for each sample. Table 7-2 highlights a range of variables and parameters which were required for DIFS and describes methods of analysis.

The program flowchart is illustrated in Figure 7-1. DIFS simulations require necessary parameters as inputs and predict DGT response. A text file is used to supply DIFS with the necessary parameters. A list of input parameters for the DIFS model is detailed in Table 7-3 and Table 7-4 for Gleadthorpe and Kincraigie respectively.

Table 7-2: Key parameters and variables used for DIFS modelling.

Symbol	Units	Description	Type	Reference
C_{DGT}	µg l^{-1}	DGT measured concentration	DGT	Davison and Zhang, 1994
C_{soln}	µg l^{-1}	Soil solution concentration	Variable	Centrifugation method described in (Zhang *et al*, 2006)
C_{ls}	µg l^{-1}	Soil solid phase concentration	Variable	Olsen *et* al, (1954); BS 7755 Section 3.6
P_c	g cm^{-3}	Particle concentration	Parameter	Harper *et al*, 2000
D	cm^2 s^{-1}	Diffusion layer	Parameter	Davison and Zhang, (1994)
D_s	cm^2 s^{-2}	Soil diffusion coefficient	Parameter	Harper *et al*, 2000
ϕ	-	Diffusion layer / soil porosity	Parameter	Baber, 2005
θ^2	-	Tortuosity	Parameter	Boudreau, 1996
d_p	(g cm^{-3})	Particle density	Parameter	BS 7755 Section 5.6 (1999)
$k_1 + k_{-1}$	s^{-1}	Sorption and desorption rate constants	Parameter	Zhang *et al*, (2006)
t	s	Time	Independent variable	

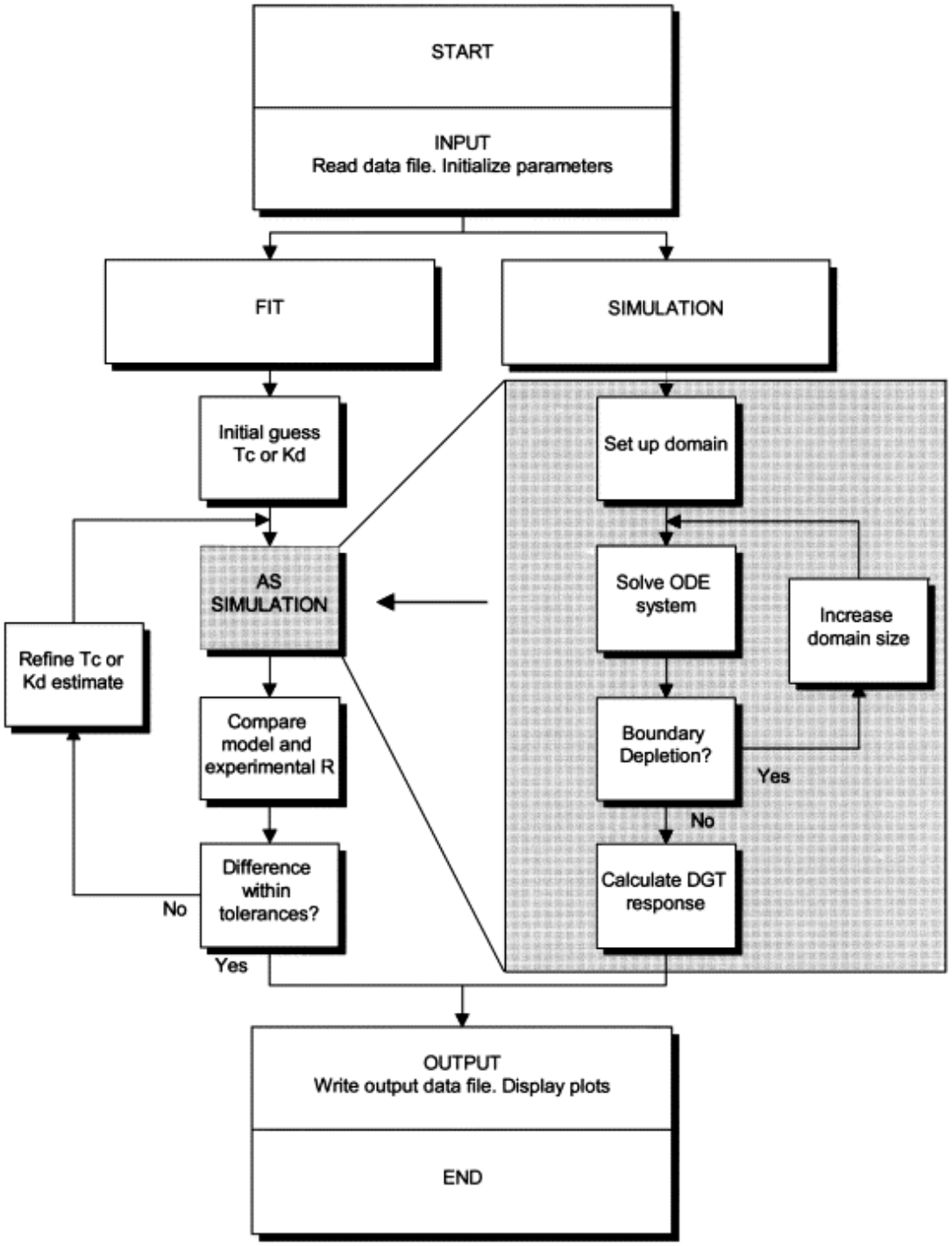


Figure 7-1: Flowchart of DIFS program design adapted from Harper *et al,* (2000).

Table 7-3: Input parameters for Gleadthorpe DIFS modelling

Input parameter		R			k_d			D	D_s	ϕ	P_c	θ^2	d_p
Day		0	30	180	0	30	180						
Treatment	Rate												
SP	0	0.05	0.07	0.11	11.1	20.1	24.9	6.05E-06	2.05E-06	0.38	2.95E+00	1.95	1.18
	15	0.04	0.10	0.07	11.3	15.0	15.8	6.05E-06	2.05E-06	0.38	2.95E+00	1.95	1.18
	25	0.05	0.11	0.05	11.5	14.4	16.0	6.05E-06	2.05E-06	0.38	2.95E+00	1.95	1.18
GW	0	0.07	0.09	0.10	10.0	12.3	19.5	6.05E-06	2.14E-06	0.40	2.83E+00	1.76	1.18
	15	0.07	0.11	0.06	9.0	15.5	15.1	6.05E-06	2E-06	0.36	3.02E+00	1.87	1.07
	25	0.07	0.10	0.05	9.1	15.3	12.0	6.05E-06	2E-06	0.36	3.02E+00	1.87	1.07
SLRY	0	0.06	0.12	0.08	9.0	16.3	12.7	6.05E-06	2.08E-06	0.39	2.91E+00	1.71	1.07
	15	0.06	0.11	0.07	9.7	15.4	12.2	6.05E-06	2.08E-06	0.39	2.91E+00	1.71	1.07
	25	0.05	0.11	0.07	10.1	13.0	11.3	6.05E-06	2.08E-06	0.39	2.91E+00	1.71	1.07
FYM	0	0.05	0.09	0.11	6.8	16.0	18.1	6.05E-06	2.16E-06	0.41	2.80E+00	1.56	1.07
	15	0.05	0.08	0.07	6.2	11.7	11.6	6.05E-06	2.16E-06	0.41	2.80E+00	1.56	1.07
	25	0.05	0.11	0.06	6.5	15.8	12.4	6.05E-06	2.16E-06	0.41	2.80E+00	1.56	1.07

Table 7-4: Input parameters for Kincraigie DIFS modelling

Input parameter		R			k_d			D	D_s	ϕ	P_c	θ^2	d_p
Day		0	30	180	0	30	180						
Treatment	Rate												
SP	80	0.10	0.12	0.11	25.3	33.0	37.8	6.05E-06	2.61E-06	0.52	1.02	2.32	1.09
	120	0.10	0.13	0.11	25.3	39.5	28.7	6.05E-06	2.61E-06	0.52	1.02	2.32	1.09
GW	80	0.10	0.08	0.12	26.7	27.8	21.3	6.05E-06	2.53E-06	0.50	1.06	2.39	1.05
	120	0.10	0.14	0.16	25.8	37.5	29.4	6.05E-06	2.77E-06	0.55	0.85	2.18	1.05
SLRY	80	0.10	0.11	0.08	24.2	32.3	11.8	6.05E-06	2.65E-06	0.53	0.95	2.28	1.05
	120	0.10	0.12	0.12	26.7	26.0	27.6	6.05E-06	2.64E-06	0.52	0.91	2.29	0.99
FYM	80	0.10	0.08	0.09	23.3	26.7	17.3	6.05E-06	2.64E-06	0.52	0.91	2.29	0.99
	120	0.10	0.11	0.08	24.2	28.2	17.5	6.05E-06	2.73E-06	0.55	0.79	2.21	0.94
CONT	0	0.10	0.15	0.08	25.3	60.6	21.5	6.05E-06	2.67E-06	0.53	0.99	2.27	1.12

Table 7-5: Description of input parameters for DIFS model

Parameter	Units	Input line	Description
R	-	1	Experimental *R* value
T_c	s	2	Exchange process response time
K_d	$cm^3\ g^{-1}$	3	Distribution coefficient
P_c	$g\ cm^{-3}$	4	Particle concentration
D	$cm^2\ s^{-1}$	5	Diffusion layer/ soil diffusion coefficient
ϕ	-	6	Diffusion layer / soil porosity
C_{soln}	$mol\ cm^{-3}$	7	Initial C_{soln}
t	hours	8	DGT device deployment time
Δ_g	cm	9	Diffusion layer thickness
Times	-	10	No. of solution times, or list (Default=40), logarithmically spaced
Domsize	cm	11	Initial domain size (Default=0.01)
Rtol	-	12	Relative tolerance for ODE solver (Default=$1x10^{-3}$)
Atoll	-	13	Absolute tolerance for ODE solver (Default=$1x10^{-6}$)

*Amended table originally from: (Harper *et al*, 2000)

*ODE stands for Ordinary Differential Equations

7.2.2.1 Defining kinetic parameters

Table 7-3 and Table 7-4, show the format of the input file and a description of the parameters used for the DIFS model. An explanation for each of these parameters is detailed as follows.

The DIFS model was used to determine kinetic parameters from measurements of K_d and R. R is defined as the ratio of DGT measured concentration, C_{DGT}, to the independently measured soil solution concentration, C_{soln} (Equation 7-1).

$$R = C_{DGT}/C_{soln} \qquad \textbf{Equation 7-1}$$

K_d is related to the rate constant for association with (k_1) and dissociation from (k_{-1}) the solid phase (Equation 7-2).

$$K_d = C_{ls}/C_{soln} = k_{-1}/(P_c\, k_{-1}) \qquad \textbf{Equation 7-2}$$

P_c is the weight of the solid phase in a unit volume of soil divided by the volume of pore water (Equation 7-5). Equations are coupled in the soil compartment to diffusion to the

device and are linked to P diffusion across the gel layer to the Fe oxide sink. *R* is calculated by the model if K_d and kinetic parameters are known. The model was therefore run in inverse mode to calculate the kinetic parameters based on (Zhang *et al*, 2006). It is configured to provide a value of the systems response time to perturbation of P removal, T_c which is related to the rate constants k_1 and k_{-1} (Equation 7-3).

$$T_c = 1/(k_1 + k_{-1}) = 1/(k_{-1}[1 + K_d P_c])$$ **Equation 7-3**

Porosity ϕ of the soil was calculated using (Equation 7-4), (Babar, 2005)

$$\phi = \frac{V_v}{V_T} = \frac{V_v}{V_s + V_v}$$ **Equation 7-4**

Where V_v, V_s and V_T are the volumes of the void spaces, matrix solids, and total volume, respectively. Porosity of the diffusive gel is 0.95 which was determined previously (Harper *et al*, 2000).

Particle concentration P_c (g cm^{-3}) is the ratio of the mass of particles to volume of pore water in a given volume of soil. It can be calculated from porosity, ϕ, and the density of the particulate matter, d_p (g cm^{-3}), using (Equation 7-5) (Harper *et al*, 2000).

$$P_c = \frac{d_p (1 - \phi)}{\phi}$$ **Equation 7-5**

It has been found diffusion coefficients D_s (cm^2 s^{-1}) in soils and other porous media are related to the self-diffusion coefficients, D_o, by tortuosity, θ^2: (Equation 7-6)

$$D_s = \frac{D_o}{\theta_2}$$ **Equation 7-6**

Tortuosity, θ^2 is measured based on empirical relationships described in (Equation 7-7) (Boudreau, 1996).

$$\theta^2 = 1 - ln(\phi^2)$$ **Equation 7-7**

The diffusion coefficient of the diffusive gel, *D* has been found experimentally (Davison *et al*, 1994) to be represented by equation as tortuosity was equal to 1. (Equation 7-8)

$$D = D_o$$ **Equation 7-8**

7.2.3 Statistical analysis

Statistical analysis was carried out to determine the effects of treatments on *R*, K_d, T_c and k_{-1}. This was achieved using repeated measures analysis of variance (ANOVA). All statistical analysis was carried out using STATISTICA 11 software.

7.3 Results and discussion

7.3.1 Temporal *R*

Figure 7-2 shows temporal variations for *R* at day 0, 30 and 360 for Gleadthorpe. The pattern of *R* over time changed for Gleadthorpe soils. All sampling days showed a rapid increase in *R* from 0 to ~0.18 in the first hour, and then decreased to a plateau, which differed for each sampling date. The lowest temporal *R* pattern was at the start of the experiment, (~0.06), to the highest following treatment application (~0.010) before decreasing by the end of the experiment (~0.08), which was still higher than day 0 (Figure 7-2).

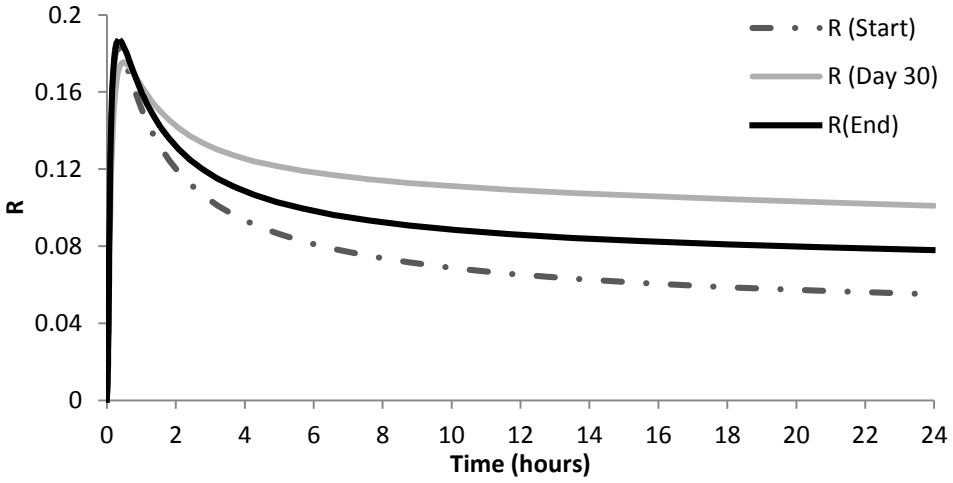


Figure 7-2: Mean temporal *R* values over a 24 hour period for Gleadthorpe

The R value indicates the ratio between C_{DGT} and C_{soln}, and thus provides information about the ability of the soild phase to buffer P to soil solution following P uptake by DGT. The range of R values for the Gleadthorpe soils were between, 0.040 and 0.123 indicating poor buffering from solid phase resupply of the concentration in soil solution (Table 7-6). The R value of soils which received treatment additions is higher than the control soils before application of additional treatments. This indicates that the long term addition of organic amendments can improve soil buffering capacity. From day 0 to 30 there is an increase in the R value of all soils. However following treatment addition, there was no significant difference in R between all soils, treatments and rates. However by day 360 R values are lower for treated soils than controls. This indicates that immediately following treatment application there is no effect on soil buffering capacity, however by 360 days of addition results in an a reduced soil buffering capacity (Table 7-6).

The K_d value of soils receiving organic amendments is lower than control soils before application of additional treatments indicating that the 5 year addition in ADAS-QC studies lowers the available solid phase pool of P (Table 7-6). Following treatment application the K_d value increases for all soils including controls, there is no significant difference between all treatments or application rates. However by the end of the experiment soils which received treatment application had a lower K_d than controls, and soils which received addition of (FYM, GW and SLRY) was lower than soils receiving SP (Table 7-6). Indicating that following application of P there is a decrease in the solid phase P pool, and (FYM, GW and SLRY) reduce the solid phase P pool more than SP.

Table 7-6: Details of *R* and K_d values for control and treated soils at day 0, 30 and 360 for Gleadthorpe pot experiments. SE represents ± standard error. Data are means *n=4*.

Day	0		30		360	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**
R						
SP	0.04	0.04	0.08	0.10	0.12	0.07
GW	0.07	0.07	0.09	0.10	0.11	0.06
SLRY	0.06	0.06	0.12	0.11	0.08	0.07
FYM	0.05	0.05	0.09	0.11	0.11	0.06
SE	0.003	0.002	0.009	0.007	0.011	0.008
K_d						
SP	10.4	11.2	21.5	14.1	30.7	15.9
GW	10.0	9.3	12.3	14.7	19.5	14.0
SLRY	9.0	9.9	16.3	15.7	12.7	12.6
FYM	6.5	6.6	15.6	15.3	17.6	12.6
SE	0.4	0.3	1.5	1.1	2.7	2.1

Figure 7-3 shows temporal variations for *R* at day 0, 30 and 180 for Kincraigie. The temporal pattern of *R* over a 24 hour period -An initial rapid increase from 0 to a peak ~ 0.25 in the first hour, then decreased to a plateau~0.1 - was similar for Kincraigie soils at day 0, 30 and 180. This suggests the application of the treatments has little effect on Kincraigie soils over time.

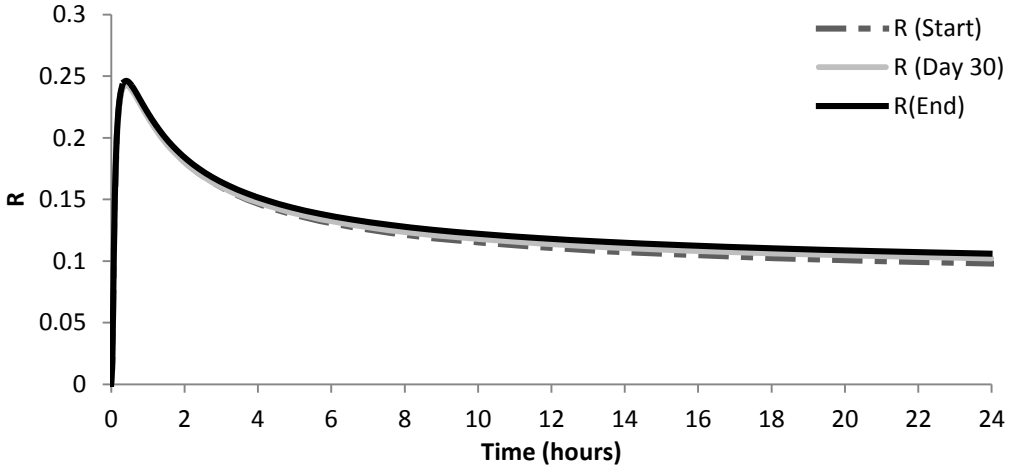


Figure 7-3: Mean temporal *R* values over a 24 hour period for Kincraigie soils.*n=4*.

The increase in the first hour results from establishment of a linear diffusion gradient in the diffusion layer. It is influenced by the rate of P release from the solid phase adjacent to the device, as this and diffusion determine the supply from the soil. After the peak *R*

progressively declines, if there was an unlimited P supply from the solid phase *R* would remain constant. However it decreases because the reservoir of P in the soil adjacent to the device is consumed, increasing the effective diffusive pathway, and therefore lowering the flux (Ernstberger *et al*, 2005).

The range of *R* values for the Kincraigie soils were between 0.063 and 0.167 indicating poor buffering from solid phase resupply of the concentration in soil solution (Table 7-7). Following treatment addition (Day 30), there was no significant difference in *R* values between all treatments, however control was higher than treated soils, indicating poorer buffering of soils following application of P. By day 180, the control soil had a lower *R* value than treated soils indicating reduced buffering capacity of soils following treatment addition was temporary, and the treatments improved P buffering capacity (Table 7-7).

The K_d of soils receiving FYM, GW and SLRY showed no significant difference at day 30, however the K_d of soils receiving SP was significantly greater (Table 7-7). The K_d of soils receiving treatment addition was significantly lower than the control at day 30 indicating a decrease in the solid phase P. By day 180 there was no significant difference in K_d of soils receiving FYM, GW and SLRY, and no significant difference these and control soils. However the soil receiving SP was greater than all other soils indicating that addition of SP increases the soils solid phase P pool; however FYM, GW and SLRY do not.

Table 7-7: Details of *R* and K_d values for control and treated soils at day 0, 30 and 180 for Kincraigie pot experiments. SE represents ± standard error. Data are means *n=4*.

Day	0	30	180	0	30	180
		R			K_d	
SP	0.1	0.12	0.11	24.7	36.6	32.3
GW	0.1	0.11	0.13	24.7	32.2	22.7
SLRY	0.1	0.10	0.11	24.7	29.2	20.0
FYM	0.1	0.08	0.09	24.7	28.0	16.3
Control	0.1	0.15	0.08	24.7	60.5	21.1
SE	**0.002**	**0.015**	**0.007**	**1.5**	**2.2**	**3.1**

7.3.2 Solution and solid phase concentrations

The modelled distributions of the solution and solid phase concentrations of P through the diffusive layer and soil, with respect to distance from the interface are shown at 3, 6,

12, and 24 hours for Kincraigie at day 0 30 and 180 (Figure 7-4), and Gleadthorpe at day 0 30 and 360 (Figure 7-5).(a) Refers to the simulation of relative concentration of soil dissolved P (mol cm^{-3}) with respect to distance from the DGT filter (cm) over a 24 hour period. (b) Refers to simulation of the relative concentration of soil sorbed P (mol g-1)with respect to distance from the DGT filter (cm).

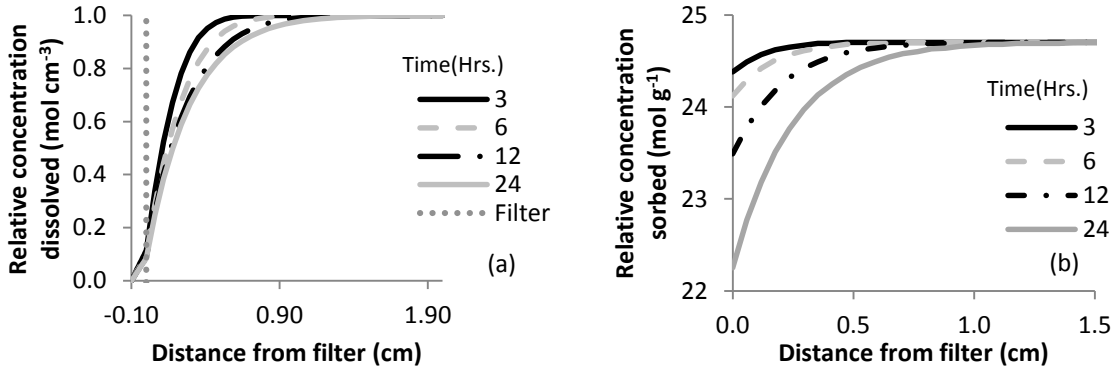
For Kincraigie soils, there is little change in the temporal pattern at each sampling date for dissolved concentration. However changes are apparent for the concentration sorbed. This can be linked to results of **Chapter 6**, which showed significant differences in Olsen P (C_{ls}), between sampling dates. At day 0 Olsen P values were very low, however were significantly increased following treatment addition, then by day 180 had again decreased significantly.

For Gleadthorpe soils the temporal dissolved pattern changes at each sampling date. At day 0 the soil has the greatest temporal depletion of solution concentration with deployment of DGT. However after application of treatments at day 30 there is less temporal depletion of P from the soil solution induced by DGT, consequently depletion does not occur as far into the soil as it did at day 0 (>0.90 cm for day 0 and <0.90 cm for day 30). By day 360, the temporal depletion of P in solution again increases, however the depletion is still less than at day 0, where depletion into the soil is (~0.90 cm). This trend is likely to have occurred as a result of temporarily increasing the soil solution P concentration following application of P.

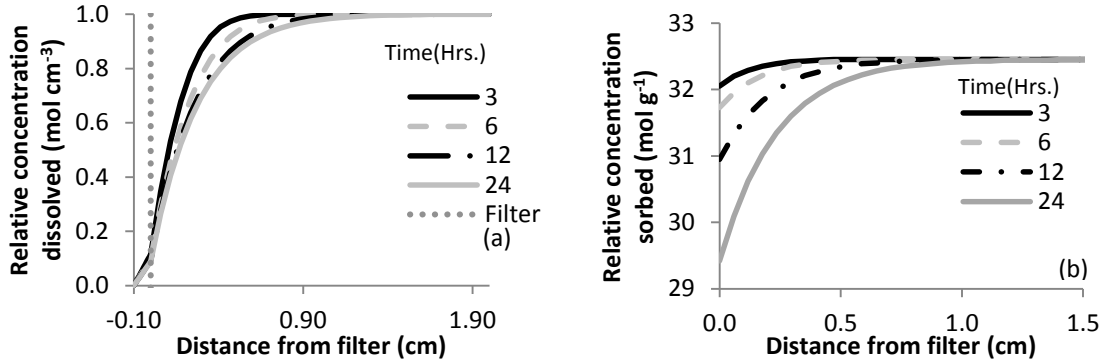
At each sampling date, for both Kincraigie and Gleadthorpe soils it is evident that there is depletion of the available pool of sorbed phosphorus. This results in resupply from, and hence depletion of the solid phase. The depletion of the solid phase P cannot be sustained, causing solid phase P to become depleted further away from the DGT device. This has previously been described as a partially sustained case (Harper *et al*, 2000) where the rate of resupply depletes the available pool of sorbed P. The resupply cannot be sustained and pore water concentrations become depleted further into the soil.

This shows P depletion is evident in the exchangeable and loosely sorbed P pools over the 24 hour timescale of the experiment. Soil solution concentrations were also depleted over the same deployment time. The desorption rate appears sufficient to sustain a low resupply rate.

Kincraigie: Day 0



Day30



Day 180

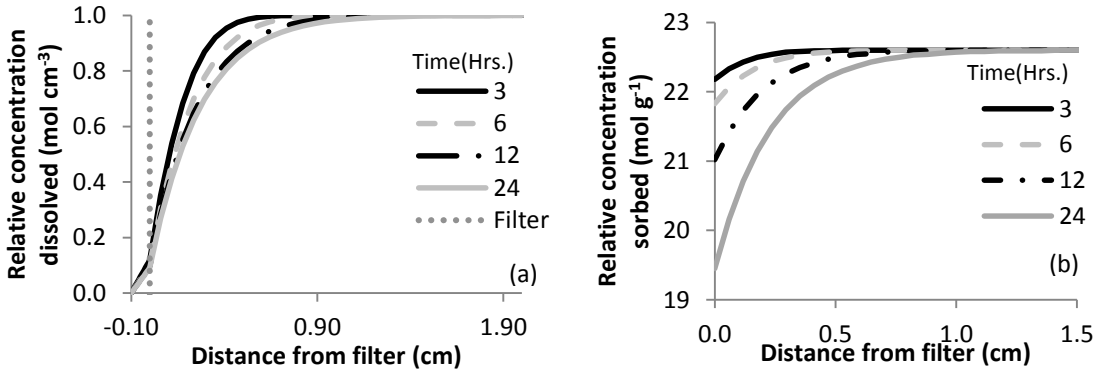
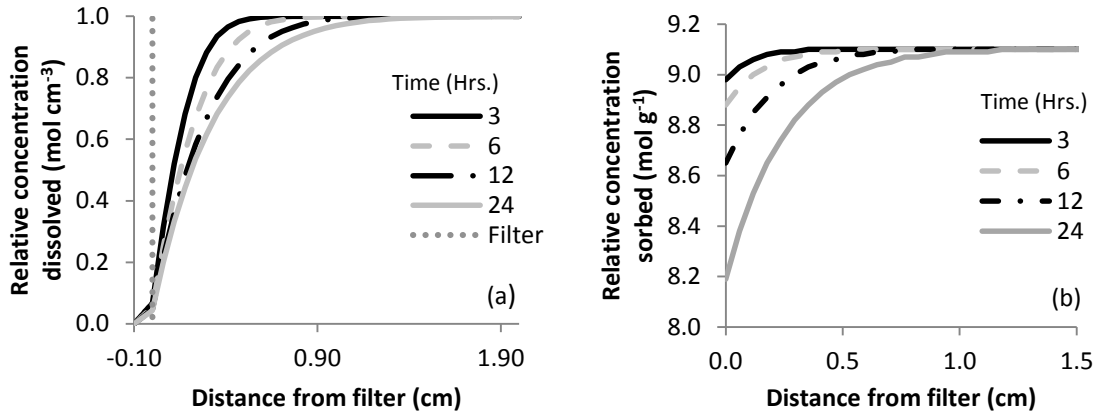
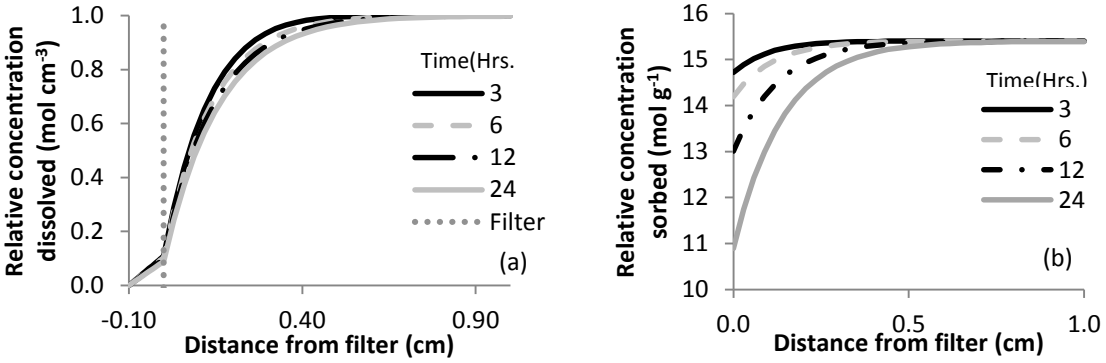


Figure 7-4: DIFS output illustrating the behaviour of (a) soil dissolved P (b) soil sorbed P, in the vicinity of the DGT device for Kincraigie start, day 30 and day 180.

Gleadthorpe: Day 0



Day 30



Day 360

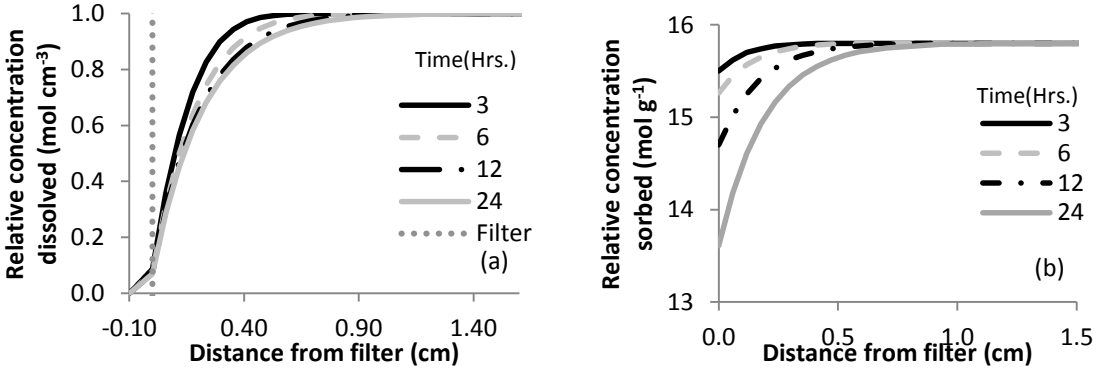


Figure 7-5: DIFS output illustrating the behaviour of (a) soil dissolved P (b) soil sorbed P, in the vicinity of the DGT device for Gleadthorpe start, day 30 and day 360.

7.3.3 Kinetic parameters

7.3.3.1 Gleadthorpe T_c

T_c represents the systems response time to perturbation of P removal. The results of the analysis of variance (ANOVA) for Gleadthorpe soils was carried out to investigate the relationship between T_c *treatment source *application rate * time, and is displayed in Figure 7-6. Day represents the culmination of ADAS-QC studies. There was a significant difference between treatments, which were all lower than the ADAS-QC control ($p=0.004$). Following treatment application there was a significant difference between control and treated soils ($p<0.001$) where the control was greater. However there was no significant difference between the treatments overall. Furthermore, there was no significant difference between application rates ($p=0.197$). At day 360 there was a significant difference between the control and treated soils ($p<0.001$) where the treated soils were greater. There was no significant difference between treated soils. Furthermore there was no significant difference in T_c values from different rates of application. Over time there was a significant difference in T_c values overall ($p<0.001$) T_c decreases from day 0 to 30, then increases from 30 to 360, however the value at the end is still less than than day 0.

T_c values represent a slow resupply from the solid phase to solution (Between 2 and 28 hours). The application of treatments over 5 years (ADAS-QC) significantly affected the soils T_c. At day 0 (Figure 7-6a) treated soils were lower (8-19 hours) than control soils (28 hours). This suggests a faster resupply for the treated soils. Between treated soils GW had a faster resupply than FYM and SLRY.

Following application of treatments in glasshouse pot experiments T_c values decrease significantly from ~18 to ~2 hours. Furthermore treated (~4hours) soils are significantly lower than control soils (~2hours) (Figure 7-6b) suggesting P addition results in faster resupply of P. However, there is no significant difference in T_c between the treatments suggesting the type of P applied does not influence on T_c.

By the end of the experiment (day 360) T_c values increased from values measured (day 30), however represent values lower than before treatment application (day 0). This suggests that there is an effect of time on overall T_c (Figure 7-6c), however overall treated soils have a higher T_c (10hours) than control soils (3.5 hours). This suggests

faster resupply of P following treatment addition is temporary. The effects of P application rate on T_c was measured, but there was no significant relationship, which is not surprising given the conclusions of **Chapter 5** which found that the treatments had little effect on P, except between control and treated soils.

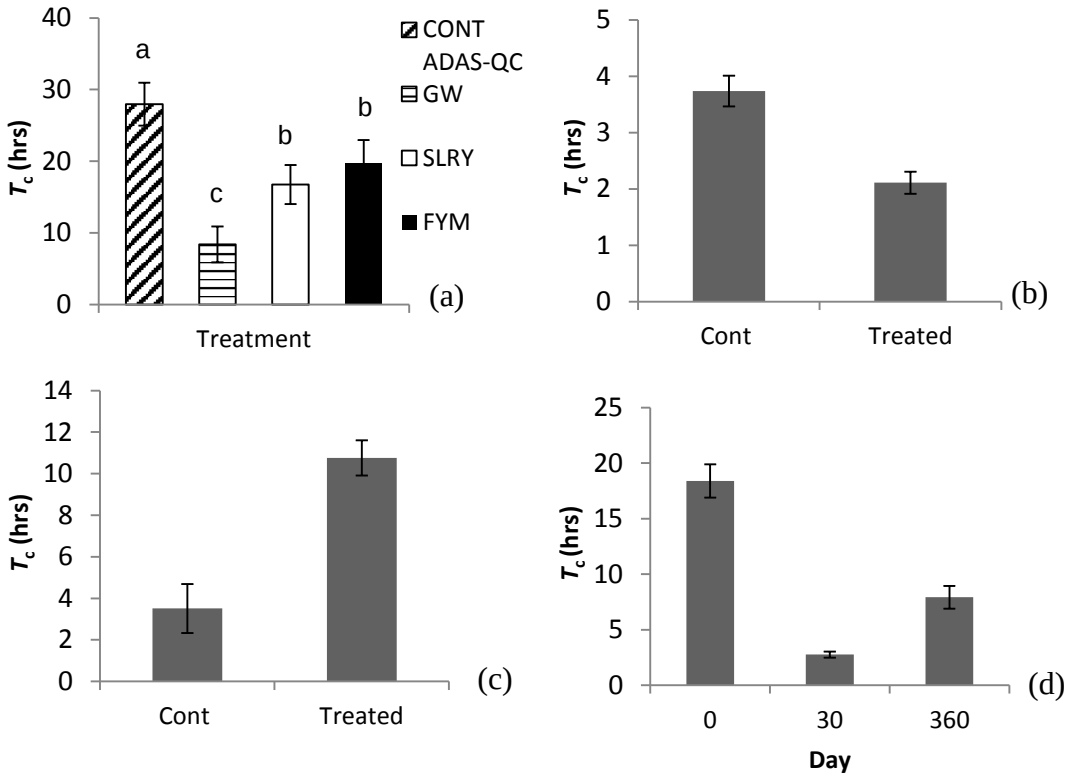


Figure 7-6: Response times, T_c estimated for P using DIFS at days (a) 0 (b) 30 (c) 360 and (d) mean of all Gleadthorpe soils over time. DIFS simulations based on experimental R and K_d values. Data are means ($n=4$). Error bars represent ± standard error. The same lower case letters indicate no significant difference (Fisher LSD). Overall there was a significant difference between Cont ADAS-QC and treated soils at day 0 ($p < 0.05$) (a). control soils were greater than treated soils at day 30 ($p<0.01$) (b) treated soils are greater than the control at day 360 ($p<0.001$) (c).

7.3.3.2 Gleadthorpe k_{-1}

ANOVA was carried out for Gleadthorpe soils to investigate the relationship between K_{-1}*treatment source * application rate * time, and is displayed in Figure 7-7. The highest T_c values produce the lowest k_{-1} values so k_{-1} results represent the inverse of the relationships for T_c. At day 0 there is a significant difference between treatment sources ($p<0.001$).

At day 30 there was no significant difference between control and treated soils (p=0.056). Furthermore, there was no significant difference in k_{-1} values from different rates of application (p=0.085). At day 360 there was a significant difference between the control and treated soils (p<0.001) where the control was greater. There was no significant difference between treated soils. Furthermore there was no significant difference in k_{-1} values from different rates of application. Over time there was a significant difference in k_{-1} values overall (p<0.001) k_{-1} increases from day 0 to 30, then decreases from 30 to 360, however the value at the end is still greater than day 0.

The dissociation rate constant k_{-1} was calculated from Equation 7-3. Maximum values of T_c produce minimum values of k_{-1}, therefore Figure 7-7 shows an inverse relationship when compared to Figure 7-6. T_c represents the inverse of a rate and therefore embraces the capacity of the labile P on the solid phase as well as its rate constant of release. However the rate constant is a purely kinetic term (Ernstberger *et al*, 2005). It therefore excludes the concentration effect (Zhang *et al*, 2006). Values for k_{-1} ranged between (4.3×10^{-3} s^{-1} and 1.5×10^{-4} s^{-1}), the pattern of k_{-1} values at day 0 (ADAS-QC studies) shows the pattern SLRY>GW>SP>FYM; however there was no significant difference between treatments except SLRY (Figure 7-7a). This suggests that the lower T_c for treated soils described above is likely to have resulted from different P concentrations of the soils, rather than due to kinetic effects.

k_{-1} values increase significantly from day 0 to day 30. Larger desorption rate constants suggest the intrinsic rate of P release is higher when there is less binding to surfaces of soil particles (corresponding to weaker binding for the adsorption site density) (Ernstberger *et al*, 2005). However the pattern of k_{-1} values at day 30 show all treated soils to have no change compared to control soil (Figure 7-7b). Therefore it is likely that changes to T_c following treatment addition described above are again due to the increased concentration of P rather than kinetic influences.

By day 360, control soils have a higher k_{-1} values than treated soils (Figure 7-7c). It is possible differences between control and treated soils can be explained by mechanisms described in Section 5.4.2. DMY and TP_{uptake} of ryegrass were found to be greater in treated soils, than control soils. It is likely that the increased P demand has resulted in greater solid phase resupply to replenish soil solution, contributing to greater depletion

of solid phase P. This has consequently led to the trend of dissociation rate constants in control soils than treated soils.

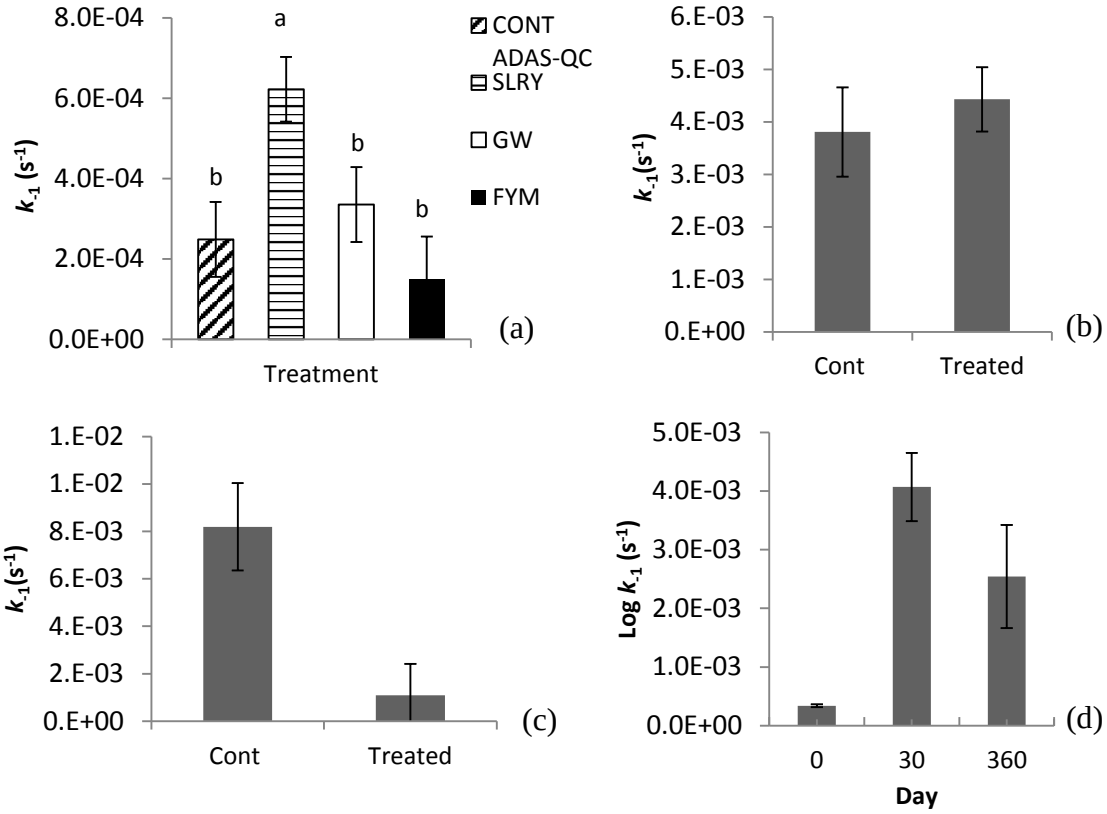


Figure 7-7: Dissociation rate constant, k_{-1} estimated for P using DIFS at days (a) 0, (b) 30 (c) 360 and (d) mean of all Gleadthorpe soils over time. Calculations were made based on Equation 7-3. Data are means (*n=4*). Error bars represent ± standard error. The same lower case letters indicate no significant difference (Fisher LSD). Overall there was no significant difference between Cont ADAS-QC and treated soils at day 0 ($p < 0.05$) (a). There was no difference between control and treated soils at day 30 ($p<0.56$) (b) Control soils are greater than the treated soils at day 360 ($p<0.001$) (c).

7.3.3.3 Kincraigie T_c

ANOVA for Kincraigie T_c values was carried out to investigate the relationship between T_c *treatment source * application rate * time, and is displayed in Figure 7-8. ANOVA showed that at day 30 there was a significant difference between treatments and the control (p=0.003) and a significant difference between treatments (p<0.001). The pattern of T_c was CONT>FYM>GW>SLRY>SP. There was no significant difference in T_c values from different rates of application (p=0.400). At day 180 there was a

significant difference between treatments ($p<0.001$) however there was no significant difference between the control and SLRY/GW. There was no significant difference in T_c values from different rates of application ($p=0.04$). Over time there was a significant difference in T_c values. Overall ($p<0.001$) T_c decreases from day 0 (~12 hours) to 30 (<1hour) then increases from 30 to 180 (~ 9hours). However the value at day 180 is still lower than at day 0.

T_c values represent slow resupply from solid phase to solution (Between 40 min. and 12 hrs.). The pattern of T_c values following treatment addition shows all treated soils (40-80 mins) have a lower T_c than control soils (97 mins) suggesting the application of P results in faster resupply. Between treated soils GW and FYM were higher than SP and SLRY (Figure 7-8a). It is expected that the higher P availability from SP and SLRY following treatment addition discussed in **Chapter 6** is responsible for this.

The pattern of T_c values day 180 show an overall increase in T_c compared to day 30, however T_c values are still lower than Day 0. Although there is a statistically significant difference between treatments at day 180, there is no obvious trend between control and treated soils (Figure 7-8b). It is likely that these results can be explained by the trend in **Chapter 6**. At the end of the glasshouse pot experiment C_{DGT} values were similar for all treatments and the control (Figure 6-3); therefore it is likely that these results are due to a similar overall P supply, for all soils measured. The effects of P application rate on T_c was measured, however there was no significant relationship found.

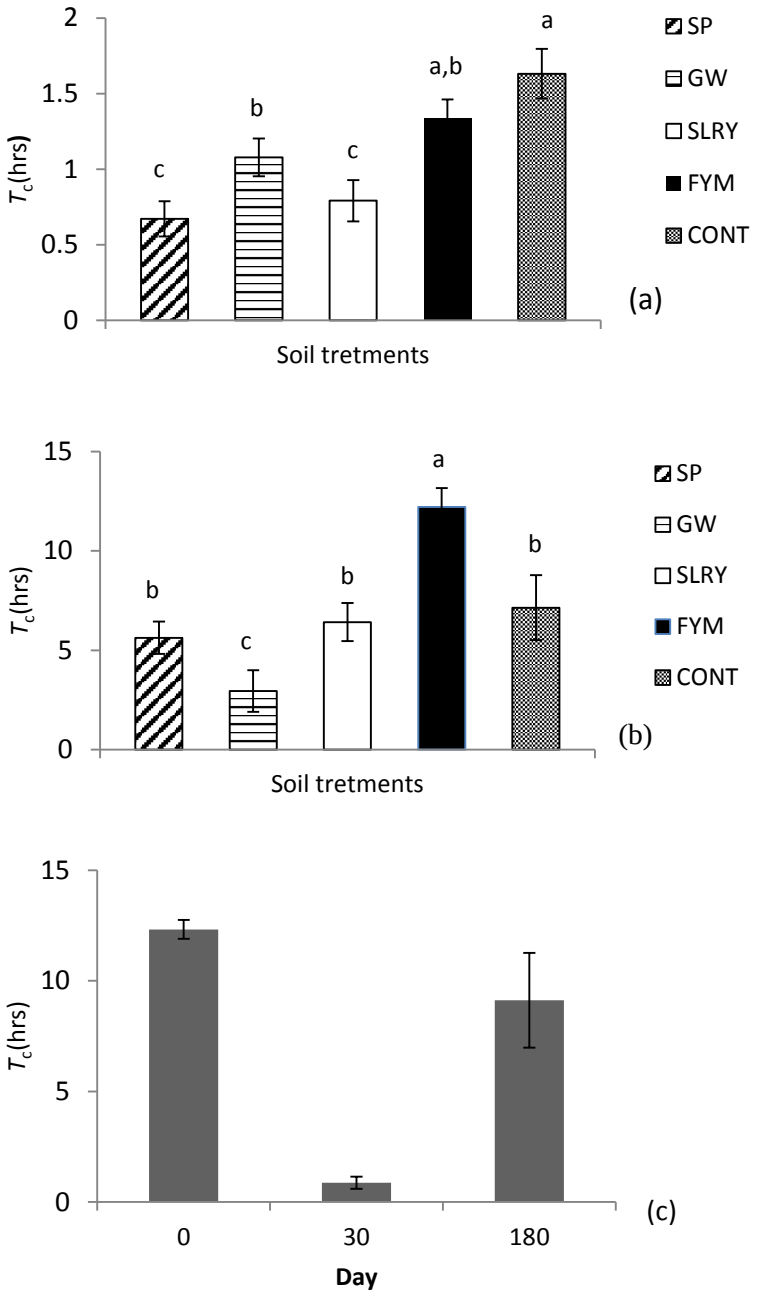


Figure 7-8: Response times, T_c estimated for P using DIFS at days (a) 30, (b) 180 (c) mean of all Kincraigie soils over time (days 0, 30 and 180). DIFS simulations based on experimental R and K_d values. Data are means ($n=4$). Error bars represent ± standard error. The same lower case letters indicate no significant difference (Fisher LSD). Control soils were greater than treated soils at day 30 ($p<0.001$) (a) there is no obvious trend at day 180 ($p<0.001$) (b). Overall there is a decrease from day 0 to 30 then an increase between day 30 and 180 ($p<0.001$) (c).

7.3.3.4 Kincraigie k_{-1}

ANOVA for Kincraigie k_{-1} values was carried out to investigate the relationship between k_{-1} treatment source *application rate* time, and is displayed in (Figure 7-9). ANOVA showed that at day 30 there was no significant difference between treatments and the control ($p<0.001$) and no significant difference difference in k_{-1} values from different rates of application ($p=0.161$). At day 180 there was a significant difference between treatments ($p<0.001$) in the pattern GW>SP>SLRY>CONT>FYM. There was no significant difference in k_{-1} values from different rates of application ($p=0.271$). Over time there was a signifcant difference in k_{-1} values overall ($p<0.001$) k_{-1} increases from day 0 to 30 then decreases from 30 to 180. However the value at day 180 is still greater than at day 0. There was no significant difference in k_{-1} values from different rates of application following application of treatments at each sampling date (Figure 7-9).

Values for k_{-1} ranged between (1.3×10^{-2} s^{-1} and 5.9×10^{-4} s^{-1}). The pattern of k_{-1} for Kincraigie at day 30 showed no significant difference in k_{-1} between soils which had received treatment application and the control soil (Figure 7-9a). This again suggests that the differences in T_c identified above are as a result of the increased concentration of P supplied by treatments rather than kinetic effects.

The trend changes at day 180 where soils which had received application of treatments have higher k_{-1} values than the control except FYM. However as no clear pattern of k_{-1} is evident there is no obvious influence of treatments on kinetic parameters (Figure 7-9b). It is expected that because the duration of the experiment is only 180 days, there was insufficient time for treatments to significantly influence kinetic parameters.

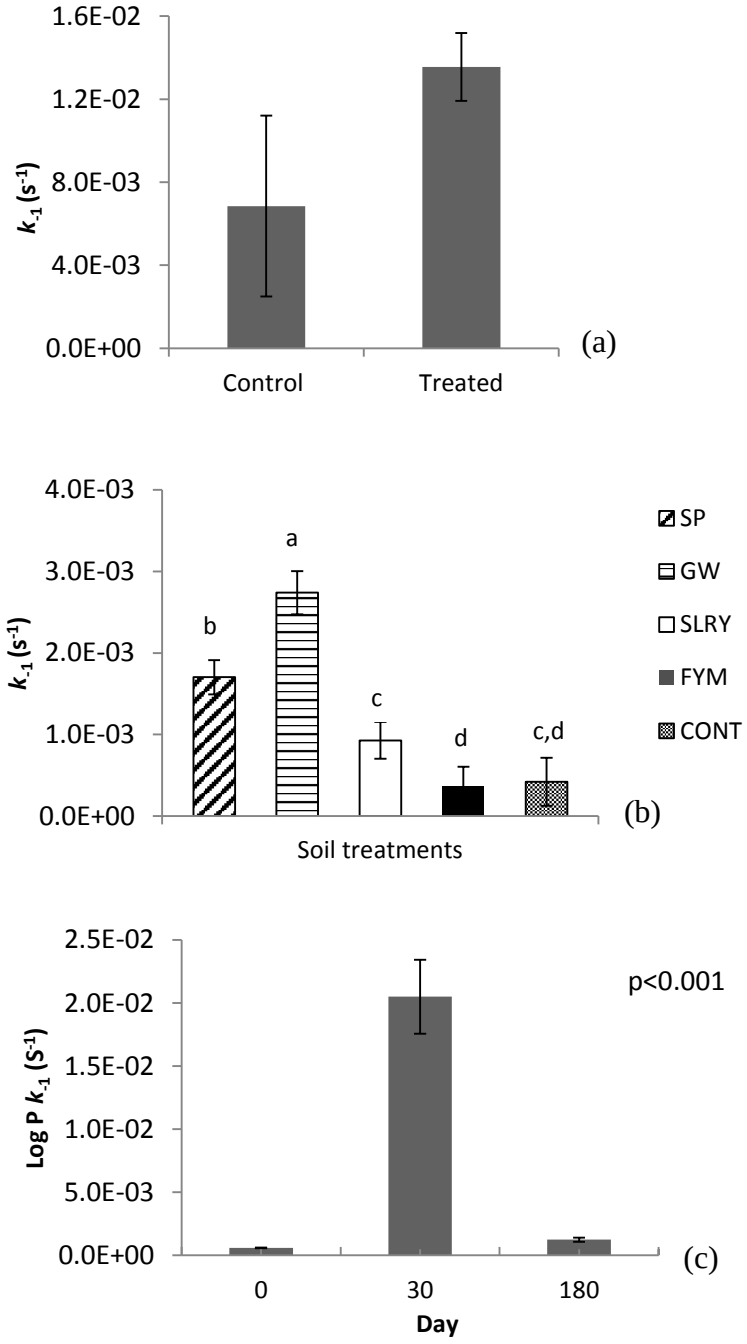


Figure 7-9: Dissociation rate constant, k_{-1} estimated for P using DIFS at days (a) 30, (b) 180 (c) mean of all Kincraigie soils over time (days 0, 30 and 180). Calculations made based on Equation 7-3. Data are means (*n=4*). Error bars represent ± standard error. The same lower case letters indicate no significant difference (Fisher LSD). There was no significant difference between control and treated soils at day 30 (p=0.161) (a) Treated soils are ≥ the control (p<0.001) (b). Overall there is an increase from day 0 to 30 then an decrease between day 30 and 180 (p<0.001) (c)

7.3.4 Implications of kinetic behaviour

Slow rates of P transfer from solid phase to solution for both soils measured suggest kinetic limitations may limit the rate of supply of P to plants in all soils (as fluxes of P from the soil to DGT are similar to fluxes to plants (Section6.6)). However both experiments show that P addition may increase rates of P transfer. It is likely that this may reflect larger P concentrations from treatment addition which is described in **Chapters 5 and 6**.

However the rate constant excludes the concentration effect. k_{-1} generally increases with treatment application, indicating that the treatments are responsible for greater dissociation from the solid phase. Previous authors have found that addition of organic matter to soil can result in inhibition of P adsorption or promotion of P desorption. Redding *et al*, (2006) suggested that organic anions are responsible for formation of stable organic complexes of Al and Fe on mineral surfaces or in solution through electrostatic attraction masking or occupation of P sorption site on soil particles. Although treatment addition is responsible for greater dissociation from the solid phase, between treatments there is no obvious trend when adding P as an organic amendment (GW, FYM, and SLRY) or as SP.

7.4 Conclusions

- Use of the DIFS model made it possible to determine rates of P transfer from the solid phase to solution at a timescale relevant to that which occurs in the rhizosphere and generates information on the role of this kinetic control.
- This study has shown that addition of treatments to soil can result in a significant reduction in response time (T_c) of the soil phase (Olsen P) to resupply soil solution (4- 2hours and 97-40 mins) for Gleadthorpe and Kincraigie respectively.
- k_{-1}, is a purely kinetic term which unlike T_c, excludes the concentration effect. Addition of treatments does not significantly influence k_{-1} values overall. Therefore increased resupply (T_c) of P was expected to be due to increases in P supply, as dissociation rate constant (k_{-1}) did not change following treatment addition.

- The interpretation of physiochemical processes controlling the DGT-soil system can help identify factors which control supply phosphorus to plants.
- Addition of treatments also increases the soils ability to resupply P in response to lowering of solution concentration compared to control soils. This suggests improved buffering from P on the solid phase.
- Controls on P supply are complicated. They are dependent on concentration in solution (C_{soln}), as well as solid phase capacity (C_{ls}), because for the fastest and slowest desorption kinetics, the soil solution adjacent to the DGT device becomes considerably depleted within a 24 hour period.
- It is expected that again T_c reductions come from increased P supply from treatment additions, and the short duration of the experiment meant that there was insufficient time for treatments to significantly influence $k_{-1.}$
- Rates of P transfer from solid phase to solution may limit the rate of supply of P to plants in all soils. As fluxes of P from the soil to DGT are similar to fluxes to plants, the rate of P transfer is expected to affect plants grown on these soils.

8 Integrated discussion and Conclusions

8.1 Introduction

There is scope for improved P use in agriculture, which can bring a range of benefits environmentally, and to farmers economically (Hilton *et al*, 2010). It is well established that recycling of organic wastes in agriculture, can benefit soil physical, chemical and biological properties, and thus represent a resource with potential to improve agricultural production and reduce environmental damage (Fuentes *et al*, 2006). However, there is a lack of knowledge about how much plant available P is supplied from different organic amendments following application to soil. A literature review was conducted to determine knowledge gaps which could contribute to a resolution for the aforementioned issues. These formulated the basis of this study, the conclusions are outlined below.

This chapter discusses results of all studies conducted in the laboratory and glasshouse. It also refers to literature review which identified knowledge gaps. In addition, it highlights areas in which a contribution to knowledge is thought to have been made. This chapter integrates results from this work in order to address the overall aims and objectives of the project.

8.2 Method development

This experiment was linked to objective 1, which set out to produce and use DGT in a consistent and reliable manner across a range of scales, on soils used in this study.

DGT gels could be produced in a consistent and reliable manner. Optimum deployment conditions were established by carrying out a range of tests at different times, water contents, temperatures, and with 10 reps on the same soil. It was established that saturation of the Fe-oxide gel was not an issue when DGT was deployed on soils in this experiment for 48 hours. However the optimum deployment time was 24 hours. In addition optimum conditions were established for DGT deployment in situ in pots by controlling the amount of soil water and accurately recording the soil temperature.

Results demonstrate that DGT is a robust and versatile tool which can accurately measure P at a range of scales on soils which have received application of organic

amendments. This gives confidence that the results derived from the technique are accurate and reflect diffusive supply of P regardless of scale of deployment.

Previous studies were conducted to determine the suitability of the DGT technique for use on different soils to measure P status. Zhang *et al*, (1998) determined that Fe oxide gel used in DGT was an effective sink for P measurement in water. Menzies *et al*, (2005) reported that DGT was an effective tool for measuring P in soil and was an accurate predictor of plant uptake in a laboratory setting results showed DGT measurements were not significantly affected by time and temperature, but was limited by soil water content under the range of conditions tested. Mason *et al*, (2010) showed that DGT was an effective predictor of wheat DMY at the field scale. However a gap in knowledge was established as no previous study had investigated the effects organic amendments had on soil P, using the DGT technique. Therefore before conducting research into the effects of organic amendments on P availability, assessments had to be conducted to establish if DGT could be used on soils which had received application of organic amendments. This study confirms that results found by Menzies *et al*, (2005) are similar for soils receiving application of organic amendments.

Findings from this chapter reveal that DGT can indeed be used on soils which have received application of organic amendments, and therefore provide a platform to carry out the research required to meet the objectives of the project. In addition, DGT could detect changes in soils which had received organic amendment application, which was not the case when using Olsen P. This demonstrates the differences in P measured by the two different methods, with DGT measuring the P available by natural diffusion, whereas Olsen P measures readily extractable P as well as potentially available forms.

8.3 Gleadthorpe soils

Gleadthorpe soils were investigated in both incubation and pot experiments. The objective was to determine P availability patterns in soils which have historically received application of organic amendment, and the subsequent impact on ryegrass yield and P uptake, with and without addition of further treatments (FYM, GW, SLRY and SP) at agronomic application rates.

This study successfully addressed objective 2. Prior to this study there was a lack of information about how repeated application of organic amendments to soil, influences P availability. In addition available knowledge mainly focussed on measurements by extraction techniques, which have been described by some authors as representing P in forms, which are not readily available to the plant (Menon, 1990; Menzies *et al*, 2005, Mason *et al*, 2010). There have been relatively few studies conducted on soils using DGT to measure P, and none on soils receiving application of organic amendments. In addition, previous work (Mason *et al*, 2008, Menzies *et al*, 2005, Mcbeath *et al*, 2007) investigating phosphorus in soils using the DGT technique have investigated a single application of fertiliser to soil and related the DGT results to plant P. This is the first time DGT has been used for repeated measures analysis for P measurement.

It was hypothesised that historical addition of organic amendments to meet N demands would lead to a build-up of P measured by DGT compared to control soils and pattern will be influenced by the total mass of C and P added in the organic amendments. It was already established that adding organic amendments to meet crop N demands can lead to a build-up in soil P, due to the low N:P of most organic amendments (Read *et al*, 2007; Evers, 2002). This study showed that adding organic amendments to meet N demands increases P available by diffusive supply as soil P status increases.

It was also hypothesised that treatment application history would be significant in determining P release from fresh treatment additions. Also soils which receive SP will show a greater response (C_{DGT}) than those which received addition of organic amendments, due to the slow release of P from organic amendments. Addition of fresh organic amendments in pot and incubation studies did not significantly increase soil P, however addition of SP did. Although the P status of the soil is at a level above which is required for optimum yield, (P index 3), addition of organic amendments increased DMY and $TP_{uptake.}$ However despite significant increases in soil P following SP addition, there was no significant improvement in DMY or TP_{uptake}.

Therefore addition of corresponding organic amendments at low P rates (15-25 kg P ha^{-1}) can improve soil fertility, associated with improved soil organic matter, without significantly increasing soil P (as Olsen P or C_{DGT}).

It was hypothesised that Olsen P and DGT would show a different trend as they are measuring different P pools. This was confirmed as DGT represents P available by diffusive supply, and Olsen P represents P which is both readily available, as well as a fraction of potentially extractable P.

The wider relevance of these findings is; the build-up of P in excess of plant needs, brought about by previous management practices pose a risk to the environment associated with high levels of soil P (> plant requirement), primarily potential for eutrophication (Tunney *et al,* 1997). However by adding organic amendments at low rates it is possible to improve or maintain soil organic matter, benefiting overall soil health whilst not increasing the risk of environmental damage posed by excess P. However this poses an additional issue associated with insufficient N supply for optimum crop growth, highlighting the problem of nutrient imbalance in organic amendments.

8.4 Kincraigie soils

8.4.1 Microbial biomass phosphorus

Soil microbial biomass P (MBP) was measured following treatment application in incubation experiments, to understand the relationship between soil microbial biomass and P availability.

It was hypothesised that following lysis of microbial biomass P, there will be a significant increase in C_{DGT} at the subsequent sampling date and Olsen P will not detect this increase. The study found that following lysis, there was a significant increase in C_{DGT} at the subsequent sampling date. This signifies a transfer of P from the soil microbial biomass to the soil solution, causing an increase in P available by diffusive supply. Olsen P does not detect this increase. This suggests that the DGT technique shows promise in measuring the P availability following lysis.

Using the improved understanding of how treatment properties influences soil MBP, and how MBP influences P available to the plant by diffusive supply can be useful in managing resource application to meet the needs of the plant. Applications can be made with potential mineralisation/immobilisation patterns in mind, and a better knowledge of how these will influence plant P availability.

8.4.2 Influence of treatment application to soil on root and shoot DMY and TP_{uptake}

In pot experiments perennial ryegrass was grown to assess the relationship between addition of organic amendments to soil with differences in DMY and TP_{uptake}. This was carried out to elucidate knowledge of transformations between different treatments, with the soil and plants, following treatment additions,

(1) Relationship between soil and root/shoot DMY.

Work in this section addressed objective 3. The intention was to determine P availability patterns in soils deficient in plant available P following addition of the aforementioned treatments, at agronomic application rates, and determine the impact on plant DMY and TP_{uptake}.

Until now there have been limited studies conducted to assess how addition of treatments contributed to root and shoot DMY and TP_{uptake}, most focussed on aboveground biomass, and neglect the important effect on roots. A few (Chen *et al*, 2002; Pederson *et al*, 2002) investigated root uptake of P, and Waldrip *et al*,(2011) investigated the influence of poultry manure on root TP_{uptake} and DMY. This study has provided information to show how treatments contribute P to the soil, its influence on P available by diffusive supply, and the subsequent influence on root and shoot DMY and TP_{uptake}.

It was hypothesised that $C:P_{treatment}$ will be a better proxy for P availability than $TP_{treatment}$. Based on regression analysis $C:P_{treatment}$ was the most important mechanism determining C_{DGT} (R^2=0.72). Subsequently C_{DGT} provided a good correlation (R^2=0.8 and 0.72) for DMY and TP_{uptake} respectively. Whilst it is already established that $C:P_{treatment}$ controls release from organic amendment to soil (Gagnon and Simmard, 1999) information was lacking on its subsequent influence on root and shoot DMY and TP_{uptake}. This study bridges this gap, showing a good relationship between soil P available by diffusive supply following treatment additions, and its influence on root and shoot DMY and TP_{uptake}.

The good correlation between C_{DGT} and plant factors, suggests that the technique can be used to predict DMY and TP_{uptake} based on applications of each treatment. This can be

achieved with the use of regression equations, such as those generated in Section 6.3.3, however it would be advisable to conduct experiments using a larger number and wider range of application rates, in order to produce a reliable model to base treatment applications.

It was hypothesised that C_{DGT} will be a more accurate indicator of ryegrass root and shoot DMY and TP_{uptake} than Olsen P and Soil solution P. C_{DGT} R^2 values were (R^2=0.8 and 0.72) for combined root /shoot, DMY and TP_{uptake} respectively. This was a stronger correlation than Olsen P (R^2=0.71 and 0.52) and Soil solution (R^2=0.75 and 0.75). This suggests DGT provides the strongest indication of the relationship between each P test and DMY/TP_{uptake}. This is important as DGT has been described previously as a P test which behaves similar to plant roots. This shows that DGT measured P is related to root DMY and TP_{uptake}. A more accurate P test can help improve understanding of plant available P following treatment addition.

Previous authors have identified a similar trend; Mason *et al,* (2010) found that DGT was a greater predictor of wheat yield than Colwell and resin P in a field experiment. Menzies *et al,* (2005) found that DGT was a greater predictor of tomato yield than Colwell and Bray extractable P in a growth chamber experiment. It was expected in both of these studies that a greater correlation between DMY response and DGT was an indication of the accuracy of the technique. However Mcbeath *et al,* (2007) showed in a glasshouse experiment that DGT had a good correlation with TP_{uptake} and DMY. Correlations were greater than Bray P, but not necessarily greater than resin or Colwell P. No previous experiment investigating C_{DGT} P has been related to root biomass or root P uptake. Furthermore to the author's knowledge DGT has not been related to root uptake for any other element.

In this study total biomass (combined DMY) was partitioned relatively evenly between roots (44%) and shoots (56%). This is relatively comparable to a study by Waldrip *et al,* (2011) who found root biomass (57%) and aboveground biomass (43%). Results of this study show that the majority of the shoot TP_{uptake} (72%) is greater than the root TP_{uptake} (28%). This pattern differs from the study by Waldrip *et al,* (2011) who found similar root and shoot uptake values (~50%). However the duration of this study (180 days), was longer than Waldrip *et al,* (2011) (112 days).

The TP_{plant} of the roots for the control was significantly greater than the treatments, however this trend was not identified in the TP_{plant} of shoots. When there is sufficient P in the soil for plant uptake, P absorbed by the plant roots is transported in the xylem to the younger leaves. There is also significant retranslocation of P in the phloem from older leaves to the growing shoots and from the shoots to the roots. However when P is deficient the supply from roots to shoots by the xylem is restricted, P is then mobilised from stored P in old leaves and retranslocated to younger ones and to roots for further growth (Schachtman *et al*, 1998). It is anticipated that this mechanism is responsible for the higher P concentration of plant roots in the control than treated for experiment.

Other studies have found increase in ryegrass TP_{uptake} by ~108 -333% by adding broiler litter at between ~4.5 and 36 kg ha^{-1} /yr^{-1} (Read *et al*, 2007), and between 27 and 141% for meat and bone meal and dairy manure at 25 and 100 mg P kg^{-1} respectively (Ylivainio *et al,* 2008). Studies for DMY found 180% increase for the aforementioned study by (Read *et al*, 2007) and a maximum increase of 350% for Antille, (2011) when adding different biosolids up to 300 kg N ha^{-1}.

An improved understanding of the distribution of P between the treatment, the soil and the plant is desirable as it can be used to improve management of resources and reduce waste and environmental damage. This can be achieved by using the more accurate knowledge of P transformations to apply treatments more efficiently, based on the needs of the crop, thus reducing potential for waste or undersupplying P. Obtaining information about root and shoot DMY and TP_{upake} provides more value than measuring only shoot biomass, as it gives a comprehensive picture of how the P taken up by the plant is distributed amongst the whole plant.

(2) Comparison between organic amendments and SP

Work in this section refers to objective 4. The intention was to investigate how organic amendments perform compared to SP in the aforementioned soils.

It was hypothesised that SP will be responsible for greater P release than organic amendments, and in turn show greater DMY and TP_{uptake}.Soils which received FYM, GW and SLRY have a greater DMY than those receiving SP for root and shoot DMY. This trend was unexpected, considering the greater release of P into soils receiving SP

over FYM, GW and SLRY. It is suggested that increased DMY despite lower C_{DGT} resulted from improved overall soil fertility, from the range of benefits to soil physical, chemical and biological properties associated with the FYM, GW and SLRY.

Results demonstrate that addition of organic amendments not only acts as a source of nutrients, but also supply additional benefits to the soil which can improve plant growth. This occurs through improvement to overall soil health. Furthermore plant available P is not the most important factor limiting ryegrass growth and root development when sufficient N is applied for optimal growth. Addition of organic amendments improves soil physical, chemical and biological health which is important at determining yield and root development.

Knowledge of improved yield on a nutrient deficient soil from addition of organic amendments as a P source compared to inorganic P means that agronomic output could potentially be enhanced with reduced reliance on inorganic P sources, with future focus on improving the efficiency of P utilisation from organic amendments.

Overall a greater understanding of P release following application of organic amendments to soil, and its subsequent effect on TP_{uptake} and DMY has been established. This can potentially contribute to strategies to predict plant yield, based on organic amendment characteristics, which would improve knowledge of best application strategies required to obtain maximum efficiency from amendment application. This could be achieved through use of the aforementioned regression equations (Section 6.3.3).

8.5 Combining Olsen P and DGT

An approach combining information from Olsen P and C_{DGT} can be of value over consideration of only one method alone, as it can provide a measure of the P available by diffusive supply, as well as P which is potentially available for resupply following uptake.

Simple linear regression analysis was conducted between all Olsen P values and all C_{DGT} values from both Gleadthorpe and Kincraigie experiments (Figure 8-1). Using the regression equation created, (Equation 8-1) it is possible to determine how much P is available by diffusive supply at a given Olsen P. At the lower range of soil P there is 14

μg l^{-1} available by diffusive supply, with a potential resupply from the Olsen P pool of 3.6 mg kg^{-1}. At the upper range there is 548 μg l^{-1} available by diffusive supply with a potential resupply of 65 mg kg^{-1} from the Olsen P pool. This was derived from soil with Olsen P values from ~3.5 to ~70 mg P kg^{-1}.

DGT shows a more accurate representation of soil P available to the plant following treatment incorporation than Olsen P (**Chapter 7**); however this information only provides a time averaged flux at a point in time, and does not indicate P available for resupply following uptake. Use of Olsen P in conjunction with DGT provides information about both the time averaged flux and the pool of P potentially available to draw upon, therefore providing a more substantial understanding of P availability following treatment addition.

This approach has not been used previously; other studies which have compared the results of DGT analysis with that of extraction solutions have typically found extraction solutions give a poor indication of DMY and TP_{uptake} (Menzies *et al*, 2005; McBeath *et al*, 2007; Mason *et al*, 2010; Tandy *et al*, 2012; Six *et al*, 2012a /b). However these experiments have been conducted in Australia, Denmark, Sweden and on tropical soils. This study was conducted on British soils, where the Olsen P technique has been found to provide a reliable indication of readily extractable P (Humphreys *et al*, 2001).

This suggests that if the correct extraction procedure is used, based on the soil it is measuring, information from DGT and extraction combined can improve understanding of processes determining P availability between treatments soil and plants. This improved understanding can subsequently be used to improve management of treatment application, so that the treatment is supplied to provide optimum P for the plant's needs, while reducing loss (waste).

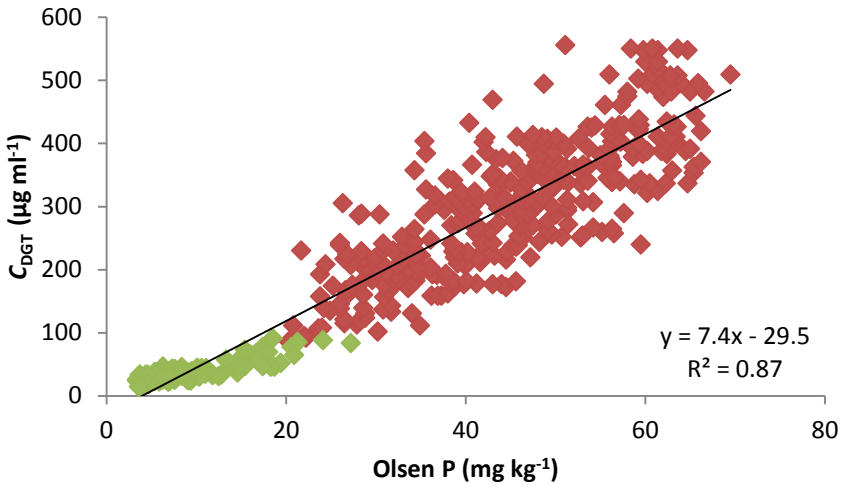


Figure 8-1: Linear regression analysis between C_{DGT} and Olsen P for all Kincraigie (green) and Gleadthorpe (red) pot experiment measurements. R^2 represents the correlation coefficient.

$$C_{\mathrm{DGT}}\ (\mu\mathrm{g\,l^{-1}}) = 7.4 * \mathrm{Olsen\ P\ (mg\,kg^{-1})} + 29.5 \qquad \textbf{Equation 8-1}$$

It is evident that in Gleadthorpe soils, which have a P index sufficient for plant growth, the addition of organic amendments results in immobilisation of the P added with the respective organic amendment. However in Kincraigie soils, P addition in the same organic amendments results in mineralisation of P added in organic amendments.

This highlights that addition of organic amendments can deliver significant benefits to soil which is deficient in P in the short term, resuling in large increases in DMY and TP_{uptake}. In addition, the repeated addition of organic amendments for at least 5 years results in a significant build-up in soil P, and soil fertility, subsequently resulting in P release which takes place slowly over time, whilst still improving DMY and TP_{uptake} associated with improved fertility from organic matter incorporation.

This is beneficial as deficient soils require the P release to take place more rapidly to meet the demands of the plant. Whereas slow release of applied P is beneficial in a soil which has sufficient P to meet the crop demands over time reducing waste associated with rapid P relaese.

8.6 Soil kinetic parameters

This work is linked to objective 5, which was to determine the effects of the different treatments on soil P kinetic parameters using the DGT Induced Fluxes in Soils (DIFS) model.

Use of the DIFS model made it possible to determine rates of P transfer from the solid phase to solution at a timescale relevant to that which occurs in the rhizosphere and generates information on the role of this kinetic control. Prior to this, there had been no study investigating the use of DIFS in soils to simulate P kinetics in response to uptake by the DGT device.

It was hypothesised that the addition of treatments to soil will result in a reduction in response time (T_c) of the soil phase to resupply soil solution for Gleadthorpe and Kincraigie soils. This study has shown that addition of treatments to soil can result in a significant reduction in response time (T_c) of the soil phase (Olsen P) to resupply soil solution (from 4- 2hours and from 97-40 mins) for Gleadthorpe and Kincraigie respectively.

It was also hypothesised that Addition of treatments will result in increased dissociation rate constant k_{-1} values overall. k_{-1}, is a purely kinetic term which unlike T_c, excludes the concentration effect. Addition of treatments does not significantly influence k_{-1} values overall. Therefore increased resupply (T_c) of P was expected to be due to increases in P supply, as dissociation rate constant (k_{-1}) did not change following treatment addition.

Using the DIFS model to simulate values for T_c and k_{-1}, adds an extra dimension to the understanding of interactions between treatments, soil and plants, by providing information on the potential resupply time between P measured by Olsen P and the soil solution, following uptake by DGT. As previous work demonstrated that DGT accurately represents uptake by plants (Section 6.6), this can provide useful information on the time taken for solid phase P to resupply soil solution following plant uptake.

This knowledge is useful in terms of utilising treatment application more efficiently. When soil P index sufficiently high to meet plant demand, it is not expected that the rate of resupply from solid phase to solution will affect plants grown on these soils (such as

those soils in Gleadthorpe experiments (index 3 and 4)). However in Kincraigie soils, where the P index is 0 before treatment addition, it is expected that rates of transfer from solid phase to solution may limit the supply of P to plants, resulting in reduced yield.

This knowledge can be used to manage treatment additions based on not only the portion of P which will be available by diffusive supply, and the portion potentially available on the solid phase, but with the time for resupply between these two phases in mind, so that rate of supply is not limiting plant growth. Although this work does not suggest organic amendments provides an improvement in resupply times over SP, more work is required to elucidate this relationship.

It is difficult to make a direct comparison to other studies as the DIFS model estimates kinetics of P response to depletion to the DGT device specifically. There have been no previous studies of this type for P, and all previous work has involved simulations of heavy metal dynamics in soils and sediments.

8.7 Limitations

The DGT technique is used in conditions representing 100% MWHC, whereas in reality soil moisture in the field is highly variable. Therefore when up scaling from laboratory and glasshouse studies to the field scale, consideration of soil moisture influences will be important.

In Gleadthorpe studies, experiments were conducted with soils, which had received different treatment application histories, and therefore had different soil physical, chemical and biological properties. Therefore the effect of comparing the response of available P between treatments is difficult as the conditions of the soils in which they were applied to were different

In both Gleadthorpe and Kincraigie studies, care should be taken in interpreting the relationship between incubation and pot studies as there are major differences in experimental set between them. First is difference in sampling dates between pot and incubation studies. The first soil sample was taken on day 30 for pot experiments and carried on for at least 180 days, whereas incubation studies focused on shorter term sampling (day 1, 7, 14, 30, 60 and 90). Incubation study results suggest that by day 30

much of the available P from treatments had already undergone transformations with the soil organic and inorganic constituents, reducing the availability of P.

The time of grass establishment (October, 2010 and November, 2011) meant that soil temperatures differed between pot and incubation experiments. Incubation temperatures were a constant 25°C, whereas in the glasshouse temperatures ranged from a minimum of 10°C and rarely exceeded 15°C, which would be expected to have been responsible for reduced MBP production in the glasshouse compared to incubation studies, as a result of less favourable conditions for microbial growth. Therefore although there is value in determining patterns of MBP production in incubation studies, results cannot be directly compared to mechanisms influencing P availability in pot experiments. This is particularly important for Kincraigie MBP experiments.

Pot experiments received application of urea as an N fertiliser to promote ryegrass growth, but incubation experiments received no additional N fertiliser. Addition of N to soil results in significant P transformations, from changes in soil pH (Shen *et al*, 2011). Plant roots also play a significant role in altering soil P dynamics. The rhizosphere is an important zone for interactions between the plant, soil and microorganisms. Plant roots influence the rhizosphere greatly through a range of physiological activities, particularly exudation of organic compounds (mucilage, organic acids, phosphatases and specific signalling substances), which are key drivers of rhizosphere processes (Shen *et al*, 2011). In addition plant roots are also responsible for P losses through uptake and transportation to aboveground biomass. In addition, pots were subjected to variable environmental influences such as sunlight, evapotranspiration and temperature fluctuations, which influence soil P dynamics in a way which incubation experiments, with a constant temperature and water regime.

There have been no studies using the DIFS model for soil P dynamics. This presents obstacles for relating the findings to the wider context of soil kinetic parameters. As is common in modeling, assumptions have to be made for some input parameters. Methods for measuring soil distribution coefficient K_d have varied between authors, with each method containing its own advantages and disadvantages, and its own set of assumptions for calculation from experimental data. The method outlined in this study is a simplistic representation, which assumes P measured by $NaHCO_3$ (Olsen P) is an

accurate representation of solid phase P. However it conforms to methodologies used in previous DIFS investigations (Zhang *et al*, 2006). Furthermore assumptions are made for input of soil diffusion coefficient D_s. It is possible to measure this directly; however (Baudreau, 1996) found that a good estimation was obtained when knowledge of soil porosity was known.

8.8 Future work

It is important to use the key findings from this work to establish topics of future research. The findings of this study suggests that DGT is an effective tool for measuring the contribution of the treatments to soil P, and that organic amendments represent a resource with potential to provide crops with sufficient P to improve yields. However future work could improve understanding. Further investigation can improve understanding of how contribution of organic amendments could be managed in a way to provide a predictable quantity of P to soil, at a rate optimum for plant uptake, without causing unnecessary environmental damage or waste of P. Ways of achieving this are set out below.

There is a range of laboratory scale experiments which are necessary to understand the effects organic amendments have on soil P dynamics. A limitation identified in this work was that organic amendments were only applied at two rates. A wider and more numerous range of application rates allows models to be fitted to the response of soil and plants to application of treatments. This would allow a more accurate prediction of specific soils response to applied P. It would then be possible to determine target soil DGT values, similar to those used for Olsen P and resin P, in the RB209 fertiliser manual (Defra, 2010) which result in optimum yields. Furthermore application of treatments to a wide range of soil types allows investigation of how the dynamics of transfer between treatments and soil is affected by soil type.

Another limitation which was discussed previously was that treatment characteristics varied significantly in many respects, creating an issue, where it was difficult to accurately conclude that a specific property is limiting P release to soil. This work has identified that $C:P_{treatment}$ was the characteristic which had most influence on P release to soil and hence plant uptake. However a more robust experimental structure is required to identify the true effect of $C:P_{treatment}$ on P release. Designing an experiment where the

characteristics of the treatments were as similar as possible, with the exception of the limiting factor under investigation ($C:P_{treatment}$) would allow a better understanding of the $C:P_{treatment}$ to P release to be established.

As use of the technique for measuring P in soil has been limited, there are numerous opportunities for the development of the technique on soils. This work has shown development of a suitable methodology for in situ field deployment would be valuable. It was previously established that a limitation of the method for DGT deployment was that it requires disturbance of the soil through the process of removal, drying, sieving re-wetting and stirring, which significantly alters the P chemistry. Whilst this is appropriate for the requirements of this study, it may be beneficial to have the opportunity to deploy the device in situ when experiments are conducted at field scales. This would reflect P behaviour, in a setting much more representative of conditions experienced between the plant and soil. It was previously established that a limitation of the findings is that experiments were conducted on soils under controlled conditions and thus cannot be directly linked to actual field processes. The next logical step following these controlled experiments would be to conduct similar research at the field scale.

As well as the quantity of P transferred from treatments to soil, it is also important to consider the forms of P which are transferred from treatment soil solution, and then to the plant by diffusion. This could be achieved by a combination of the use of diffusive gradients in thin films and 31P NMR. Development of a method to determine the P forms measured by DGT using 31P NMR could allow a determination of the P forms which are available by diffusion, compared with the P forms which remain in soil.

8.9 Contributions to knowledge

The research presented in this thesis makes a significant contribution to knowledge in terms of understanding the influence of different treatments both organic and inorganic on plant available P in soil. It was well documented that a lack of information exists about the availability of P in soil following addition of organic amendments, based on knowledge gaps identified. To the author's knowledge this is the first time the DGT technique has been used to measure contribution of P from organic amendments to soil. The data derived therefore presents a novel method of understanding the influence of these materials on plant available P in soil. It was also the first time an extensive

investigation was carried out to determine the influence of different treatments to soil, and subsequent influence on ryegrass roots and shoots. The outcomes therefore help to improve understanding of the linkages and interrelationships between treatments, soil and plants.

Prior to this experiment there was a lack of understanding of the dynamics of transformations between P mineralisation following treatment additions and plant available P in soil. This study showed that DGT provides a more accurate indication DMY and TP_{uptake} of plant roots and shoots, than Olsen P.

This was the first time an investigation has been conducted to determine plant availability of P following mineralisation of P from soil MBP. It was shown that $C:P_{treatment}$ influences P release following mineralisation and subsequently on $C_{DGT,}$ suggesting P supply by diffusion is influenced by the soil microbial biomass.

To the author's knowledge, there have been no published studies, which have used the DIFS model to simulate P kinetics in soils. Simulation of T_c between P measured by solid phase and soil solution, following uptake by DGT suggests, treatment addition, reduces (T_c) in Kincraigie and Gleadthorpe soils. Therefore this helps to elucidate understanding of the processes which control transport of P between soil and the DGT device, following addition of different treatments.

The results of this work have enhanced understanding of the contribution of organic amendments to plant available P forms in soil, and enhanced understanding of how behaviour of organic amendments compares to superphosphate. In addition it has confirmed that the DGT technique can provide a consistent and reliable method of analysing this. Combining Olsen P and DGT provides information about readily available P and the potential for resupply.

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APPENDICES

Appendix A Chapter 4

A.1 Statistical analysis

Table A.1-1 Repeated measures analysis of variance for Time*Soil treatment*Treatment amount for C_{DGT}.

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	19434082	1	19434082	7434.17	<0.001
Soil treatment	700964	3	233655	89.38	<0.001
Treatment amount (kg/ha)	12451	2	6225	2.38	0.108
Soil treatment*Treatment amount (kg/ha)	132124	6	22021	8.42	<0.001
Error	88881	34	2614		
TIME	11376444	5	2275289	719.16	<0.001
TIME*Soil treatment	463986	15	30932	9.77	<0.001
TIME*Treatment amount	65336	10	6534	2.06	0.030
TIME*Soil treatment*Treatment amount	216358	30	7212	2.28	<0.001
Error	537846	170	3164		

Table A.1-2 Repeated measures analysis of variance for Time*Soil treatment*Treatment amount for Olsen P.

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	604532.6	1	604532.6	42546.67	<0.001
Soil treatment	19112.0	3	6370.7	448.36	<0.001
Treatment amount (kg/ha)	299.6	2	149.8	10.54	<0.001
Soil treatment*Treatment amount (kg/ha)	891.9	6	148.7	10.46	<0.001
Error	511.5	36	14.2		
TIME	10453.4	5	2090.7	196.11	<0.001
TIME*Soil treatment	1693.0	15	112.9	10.59	<0.001
TIME*Treatment amount	271.5	10	27.1	2.55	0.006
TIME*Soil treatment*Treatment amount	1111.6	30	37.1	3.48	<0.001
Error	1918.9	180	10.7		

Table A.1-3 Repeated measures analysis of variance for Time*Soil treatment*Treatment amount for C_{DGT} %PRR

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	2.22865	1	2.22	15.23	<0.001
Soil treatment	20.95218	3	6.98	47.72	<0.001
Treatment amount (kg/ha)	0.27427	1	0.27	1.87	0.183
Soil treatment*Treatment amount (kg/ha)	1.29849	3	0.43	2.95	0.052
Error	3.51179	24	0.14		
TIME	8.87444	5	1.77	7.75	<0.001
TIME*Soil treatment	28.11831	15	1.87	8.19	<0.001
TIME*Treatment amount	0.62230	5	0.12	0.54	0.742
TIME*Soil treatment*Treatment amount	3.78162	15	0.25	1.10	0.362
Error	27.45798	120	0.22		

Table A.1-4 repeated measures analysis of variance for Time*Soil treatment*Treatment amount for Olsen P %PRR

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	64142.4	1	64142.42	69.19	<0.001
Soil treatment	127241.2	3	42413.74	45.75	<0.001
Treatment amount (kg/ha)	1678.0	1	1677.97	1.81	0.191
Soil treatment*Treatment amount (kg/ha)	9983.2	3	3327.74	3.58	0.028
Error	22248.2	24	927.01		
TIME	43324.8	5	8664.95	10.79	<0.001
TIME*Soil treatment	156260.2	15	10417.35	12.97	<0.001
TIME*Treatment amount	1212.2	5	242.44	0.30	0.910
TIME*Soil treatment*Treatment amount	10640.5	15	709.36	0.88	0.583
Error	96334.3	120	802.79		

Table A.1-5 Table of results for repeated measures analysis of variance for Time*Soil treatment*Treatment amount for pH.

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	2965.83	1	2965.837	18745169	<0.001
Soil treatment	6.32	3	2.107	13315	<0.001
Treatment amount (kg/ha)	0.00	1	0.000	1	0.252
Soil treatment*Treatment amount (kg/ha)	0.02	3	0.005	31	<0.001
Error	0.004	24	0.000		
TIME	5.61	1	5.612	23444	<0.001
TIME*Soil treatment	2.63	3	0.877	3665	<0.001
TIME*Treatment amount	0.00	1	0.000	1	0.349
TIME*Soil treatment*Treatment amount	0.01	3	0.005	20	<0.001
Error	0.01	24	0.000		

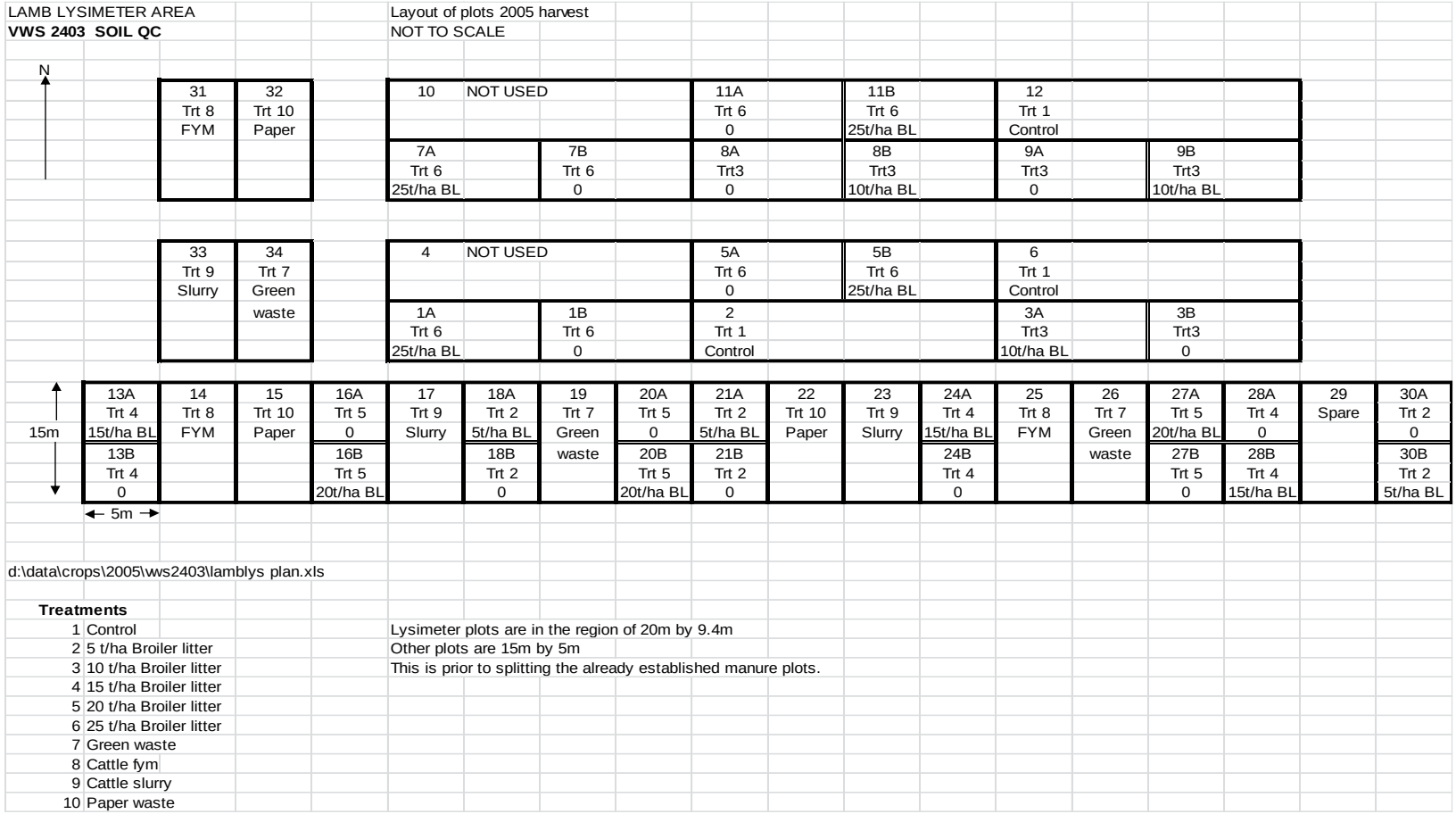
A.2 Information from ADAS-QC trials

Table A.2-1 Cattle FYM, SLRY and GW (Mean of 4 annual applications, 2004-2008, with standard error in italics). Results expressed on a fresh weight basis unless otherwise stated. Adapted from Bhogal *et al*, (2011).

	FYM		SLRY		GW	
Application rate (t/ha)	43.4		163		44	
Dry matter (%)	31.7	*2.52*	2.6	*0.82*	54.4	*0.98*
Total-N (kg/t)	8.4	*0.71*	1.75	*0.35*	6.13	*0.33*
NH4-N (kg/t)	0.17	*0.07*	0.78	*0.16*	0.02	*0.01*
NO3-N (kg/t)	0.18	*0.09*	0.01	*0.01*	0.08	*0.05*
Total-P (kg/t)	1.41	*0.19*	0.25	*0.07*	1.12	*0.06*
Total-K (kg/t)	11.6	*1.22*	1.39	*0.11*	3.43	*0.67*
Total-Mg (kg/t)	1.46	*0.25*	0.27	*0.04*	1.54	*0.07*
Total-S (kg/t)	1.84	*0.33*	0.16	*0.04*	1.01	*0.07*
Total-Na (kg/t)	1.18	*0.17*	0.15	*0.03*	0.25	*0.06*
Organic C (% dm)	41.1	*2.95*	43.3	*4.37*	14.7	*1.31*
Lignin-C (% dm)[a]	11.1	*3.39*	4.87	*1.50*	9.08	*2.85*
Cellulose-C (%dm)[a]	18.4	*5.46*	8.0	*2.90*	4.94	*1.70*
DOC (% dm)[a]	1.26	*0.52*	5.9	*1.83*	0.11	*0.04*
Aerobic stability (mg CO2/gVS/d)[a]	8.8	*2.61*	N/A	N/A	2.31	0.9
C:N ratio	16.0	*2.13*	6.1	*1.06*	13.1	1.1
pH	9.0	*0.03*	7.2	*0.11*	8.14	0.1

TP application rate (kg/ha)	61	40.8	49.3

Figure A.2-1 Diagram indicating the randomised block design of ADAS-QC experiments detailed in (Bhogal *et al*, 2011)



A.3 Olsen P analysis

Figure A.3-1 Overall difference (p<0.001) between treatments as a mean of all sampling dates for Olsen P.

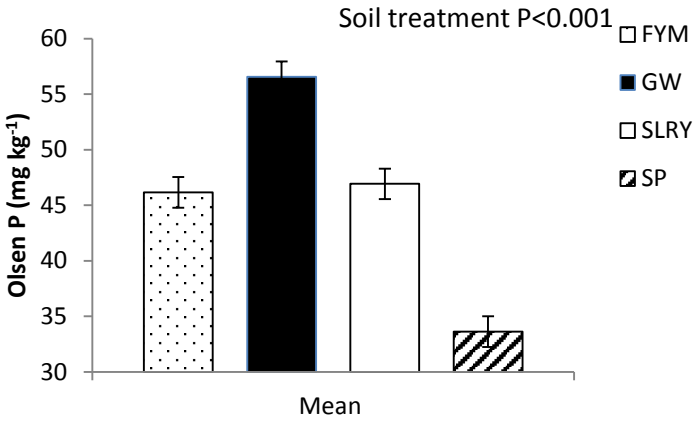


Table A.3-1 Olsen P % PRR showing the P recovered from each treatment as a mean of the two treatments (15 and 25 kg P ha^{-1}) at each sampling date

Soil treatment					STD error
	FYM	GW	SLRY	SP	
Time (Days)					
1	-61.7	-6.1	-18.5	149.6	12.3
7	-1.6	43.1	5.4	54.6	6.0
14	-25.1	-4.2	7.3	39.6	8.6
30	16.4	61.6	2.6	73.1	9.6
60	-26.3	-6.4	-5.9	18.4	15.4
90	26.7	49.3	37.7	9.3	7.4

Appendix B Chapter 5

B.1 Statistical analysis

Table B.1-1 Repeated measures analysis of variance for Time*Soil treatment*Treatment amount for C_{DGT}.

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	156435.8	1	156435.8	1679.42	<0.001
Soil treatment	1889.7	3	629.9	6.76	0.002
Treatment amount	6236.5	1	6236.5	66.95	<0.001
Control/nocontrol	2826.1	1	2826.1	30.34	<0.001
Soil treatment*Treatment amount*Control/nocontrol	797.3	3	265.8	2.85	0.061
Error	2049.3	22	93.1		
TIME	3253.7	5	650.7	6.20	<0.001
TIME*Soil treatment	3620.9	15	241.4	2.30	0.007
TIME*Treatment amount	575.8	5	115.2	1.09	0.366
TIME*Control/nocontrol	197.0	5	39.4	0.37	0.864
TIME*Soil treatment*Treatment amount	1936.1	15	129.1	1.23	0.260
Error	11538.1	110	104.9		

Table B.1-2 Repeated measures analysis of variance for Time*Soil treatment*Treatment amount for Olsen P.

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	15086.17	1	15086.17	579.79	<0.001
Soil treatment	725.39	3	241.80	9.29	<0.001
Treatment amount	1528.45	1	1528.45	58.74	<0.001
Control/nocontrol	974.05	1	974.05	37.44	<0.001
Soil treatment*Treatment amount*Control/nocontrol	215.44	3	71.81	2.76	0.065
Error	598.46	23	26.02		
TIME	775.67	5	155.13	22.88	<0.001
TIME*Soil treatment	276.76	15	18.45	2.72	0.001
TIME*Treatment amount	173.09	5	34.62	5.11	<0.001
TIME*Control/nocontrol	170.62	5	34.12	5.03	<0.001
TIME*Soil treatment*Treatment amount	202.51	15	13.50	1.99	0.021
Error	779.50	115	6.78		

Table B.1-3 Repeated measures analysis of variance for Time*Soil treatment*Treatment amount for MBP.

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	4709.169	1	4709.169	160.17	<0.001
Soil treatment	6403.351	3	2134.450	72.60	<0.001
Treatment amount	550.996	1	550.996	18.74	<0.001
Control/nocontrol	149.940	1	149.940	5.10	0.034
Soil treatment*Treatment amount*Control/nocontrol	262.862	3	87.621	2.98	0.052
Error	676.199	23	29.400		
TIME	519.408	5	103.882	9.58	<0.001
TIME*Soil treatment	1695.006	15	113.000	10.42	<0.001
TIME*Treatment amount	146.628	5	29.326	2.70	0.024
TIME*Control/nocontrol	123.424	5	24.685	2.27	0.051
TIME*Soil treatment*Treatment amount	249.645	15	16.643	1.5	0.104
Error	1246.824	115	10.842		

Table B.1-4 Repeated measures analysis of variance for Time*Soil treatment*Treatment amount for C_{DGT} (%PRR).

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	0.081949	1	0.081	430.78	<0.001
Soil treatment*Treatment amount (kg ha^{-1})	0.002915	7	0.000	2.18	0.101
Error	0.002663	14	0.0002		
R1	0.006483	5	0.0012	6.96	<0.001
R1*Soil treatment*Treatment amount (kg ha^{-1})	0.008742	35	0.0003	1.34	0.141
Error	0.013025	70	0.0001		

Table B.1-5 Repeated measures analysis of variance for Time*Soil treatment*Treatment amount for Olsen P %PRR.

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	49051.09	1	49051.09	575.12	<0.001
Soil treatment*Treatment amount (kg ha^{-1})	4051.17	7	578.74	6.78	<0.001
Error	1961.63	23	85.29		
R1	8024.92	5	1604.98	57.01	<0.001
R1*Soil treatment*Treatment amount (kg ha^{-1})	5295.42	35	151.30	5.37	<0.001
Error	3237.51	115	28.15		

B.2 Kincraigie soil analysis prior to collection from field

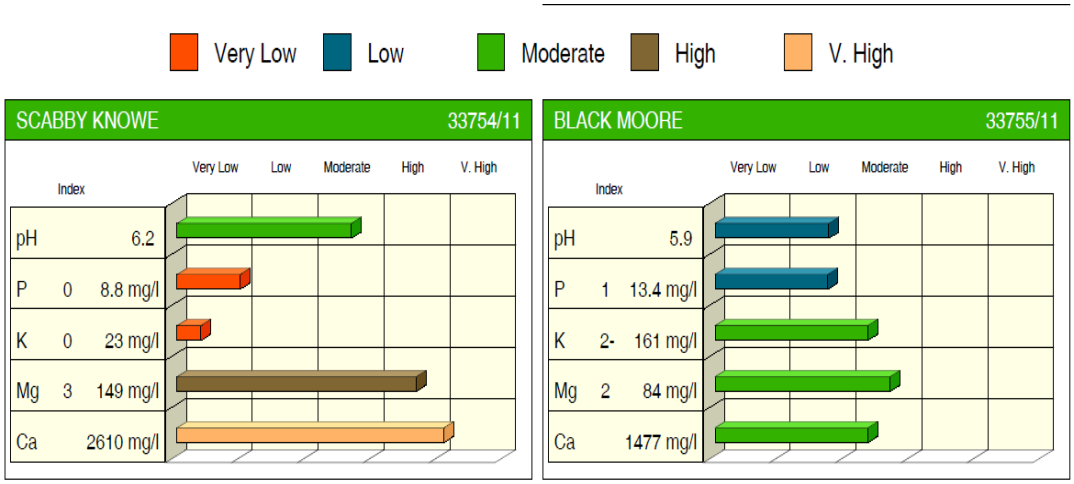


Figure B.2-1 Details of some soil chemical properties measured on Kincraigie soils prior to this experiment. Soil samples were taken from the field named scabby knowe. Scabby is taken from the fact that the site has a number of rock outcrops (Scabs in the landscape); knowe is is Scottish term for hill or mound (The relief is steep).

Appendix C Chapter 6

C.1 Statistical analysis

Table C.1-1 Repeated measures analysis of variance for Time*Soil treatment Olsen P Stage 1

	SS	Degr. of	MS	F	p
Intercept	350425	1	350425	30073	<0.001
Soil Treatment	13250	3	4417	379	<0.001
Error	513	44	12		
Time	1615	2	808	56	<0.001
Time*Soil Treatment	538	6	90	6	<0.001
Error	1268	88	14		

Table C.1-2 Repeated measures analysis of variance for Time*Soil treatment C_{DGT} Stage 1

	SS	Degr. of	MS	F	p
Intercept	15466162	1	15466162	13440	<0.001
Soil Treatment	777711	3	259237	225	<0.001
Error	50634	44	1151		
Time	186936	2	93468	72	<0.001
Time*Soil Treatment	58161	6	9694	7	<0.001
Error	114916	88	1306		

Table C.1-3 Repeated measures analysis of variance for Time*Soil treatment Soil solution P Stage 1

	SS	Degr. of	MS	F	p
Intercept	2719	1	2719	5063	<0.001
Soil Treatment	94	3	31	58	<0.001
Error	24	44	1		
Time	72	2	36	72	<0.001
Time*Soil Treatment	5	6	1	2	0.108
Error	44	88	0		

Table C.1-4 Repeated measures analysis of variance for Time*Soil treatment DMY Stage 1

	SS	Degr. of	MS	F	p
Intercept	7.96E+08	1	7.96E+08	4810	<0.001
Soil Treatment	8552735	3	2850912	17	<0.001
Error	7278760	44	165426		
Time	2E+08	2	99801931	1091	<0.001
Time*Soil Treatment	1382471	6	230412	3	0.027
Error	8046611	88	91439		

Table C.1-5 Repeated measures analysis of variance for Time*Soil treatment*Application rate Olsen P Stage 2

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	300137	1	300137	54751	<0.001
Soil Treatment	10020	3	3340	609	<0.001
Rate(kg ha^{-1})	582	2	291	53	<0.001
Soil Treatment*Rate(kg ha^{-1})	549	6	91	17	<0.001
Error	197	36	5		
TIME	1935	3	645	89	<0.001
TIME*Soil Treatment	948	9	105	15	<0.001
TIME*Rate (kg ha^{-1})	48	6	8	1	0.366
TIME*Soil Treatment*Rate (kg ha^{-1})	171	18	10	1	0.194
Error	782	108	7		

Table C.1-6 Repeated measures analysis of variance for Time*Soil treatment*Application rate C_{DGT} Stage 2

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	13300639	1	13300639	3699	<0.001
Soil Treatment	651961	3	217320	60	<0.001
Rate(kg ha^{-1})	23234	2	11617	3	<0.001
Soil Treatment*Rate(kg ha^{-1})	46990	6	7832	2	0.068
Error	129459	36	3596		
TIME	202084	3	67361	26	<0.001
TIME*Soil Treatment	74137	9	8237	3	0.002
TIME*Rate (kg ha^{-1})	44755	6	7459	3	0.012
TIME*Soil Treatment*Rate (kg ha^{-1})	40481	18	2249	1	0.613
Error	278644	108	2580		

Table C.1-7 Repeated measures analysis of variance for Time*Soil treatment*Application rate Soil solution P Stage 2

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	1836.7	1	1836.7	3510	<0.001
Soil Treatment	73.8	3	24.6	47	<0.001
Rate(kg ha^{-1})	15.7	2	7.9	15	<0.001
Soil Treatment*Rate(kg ha^{-1})	3.2	6	0.5	1	0.429
Error	18.3	35	0.5		
TIME	2.3	3	0.8	3.1	0.03
TIME*Soil Treatment	2.6	9	0.3	1.2	0.319
TIME*Rate (kg ha^{-1})	2.9	6	0.5	1.9	0.081
TIME*Soil Treatment*Rate (kg ha^{-1})	4.7	18	0.3	1.1	0.405
Error	26.2	105	0.2		

Table C.1-8 Repeated measures analysis of variance for Time*Soil treatment*Application rate DMY Stage 2

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	1.14E+09	1	13300	9748	<0.001
Soil Treatment	9372701	3	3124234	27	<0.001
Rate(kg ha^{-1})	3268946	2	1634473	14	<0.001
Soil Treatment*Rate(kg ha^{-1})	1295988	6	215998	2	0.119
Error	4227642	36	117434		
TIME	3.24E+08	3	67361	1340	<0.001
TIME*Soil Treatment	2021399	9	224600	3	0.006
TIME*Rate (kg ha^{-1})	1507090	6	251182	3	0.007
TIME*Soil Treatment*Rate (kg ha^{-1})	2413518	18	134084	2	0.058
Error	8712029	108	80667		

Table C.1-9 Repeated measures analysis of variance for Time*Soil treatment*Application rate TP_{plant} Stage 2

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	1.27E+09	1	########	34804.7	<0.001
Soil Treatment	8640770	3	2880257	79.2	<0.001
Rate(kg ha^{-1})	278120	2	139060	3.8	<0.001
Soil Treatment*Rate(kg ha^{-1})	174309	6	29051	0.8	0.577
Error	1308828	36	36356		
TIME	1400641	3	466880	13.7	<0.001
TIME*Soil Treatment	2256360	9	250707	7.4	<0.001
TIME*Rate (kg ha^{-1})	207336	6	34556	1	0.419
TIME*Soil Treatment*Rate (kg ha^{-1})	503596	18	27978	0.8	0.67
Error	3672904	108	34008		

Table C.1-10 Repeated measures analysis of variance for Time*Soil treatment*Application rate TP_{uptake} Stage 2

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	7865	1	7865	7971	<0.001
Soil Treatment	191	3	64	65	<0.001
Rate(kg ha^{-1})	38	2	19	19	<0.001
Soil Treatment*Rate(kg ha^{-1})	7	6	1	1	0.334
Error	36	36	1		
TIME	2512	3	837	1072	<0.001
TIME*Soil Treatment	45	9	5	6	<0.001
TIME*Rate (kg ha^{-1})	16	6	3	3	0.004
TIME*Soil Treatment*Rate (kg ha^{-1})	15	18	1	1	0.377
Error	84	108	1		

Table C.1-11 Repeated measures analysis of variance for Soil treatment*Application rate*Depth for C_{DGT} Stage 2

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	1617770	1	1617770	333	<0.001
Soil Treatment	82488	1	82488	17	0.001
Rate(kg ha^{-1})	52435	1	52435	11	0.007
Soil Treatment*Rate(kg ha^{-1})	3416	1	3416	1	0.418
Error	58283	12	4857		
Depth	12051	3	4017	3	0.025
Depth*Soil Treatment	1368	3	456	0	0.756
Depth*Rate (kg ha^{-1})	1345	3	448	0	0.761
Depth*Soil Treatment*Rate (kg ha^{-1})	1764	3	588	1	0.677
Error	41386	36	1150		

Table C.1-12 Repeated measures analysis of variance for Soil treatment*Application rate*Depth for Olsen P Stage 2

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	114269.4	1	114269.4	4451.556	<0.001
Soil Treatment	7588.6	1	7588.6	295.626	<0.001
Rate(kg ha^{-1})	37.1	1	37.1	1.444	0.252
Soil Treatment*Rate(kg ha^{-1})	84	1	84	3.27	0.095
Error	308	12	25.7		
TIME	314	3	104.7	4.292	0.01
TIME*Soil Treatment	58.4	3	19.5	0.798	0.503
TIME*Rate (kg ha^{-1})	134.7	3	44.9	1.841	0.157
TIME*Soil Treatment*Rate (kg ha^{-1})	117.3	3	39.1	1.603	0.205
Error	877.9	36	24.4		

C.2 Miscellaneous graphs and tables

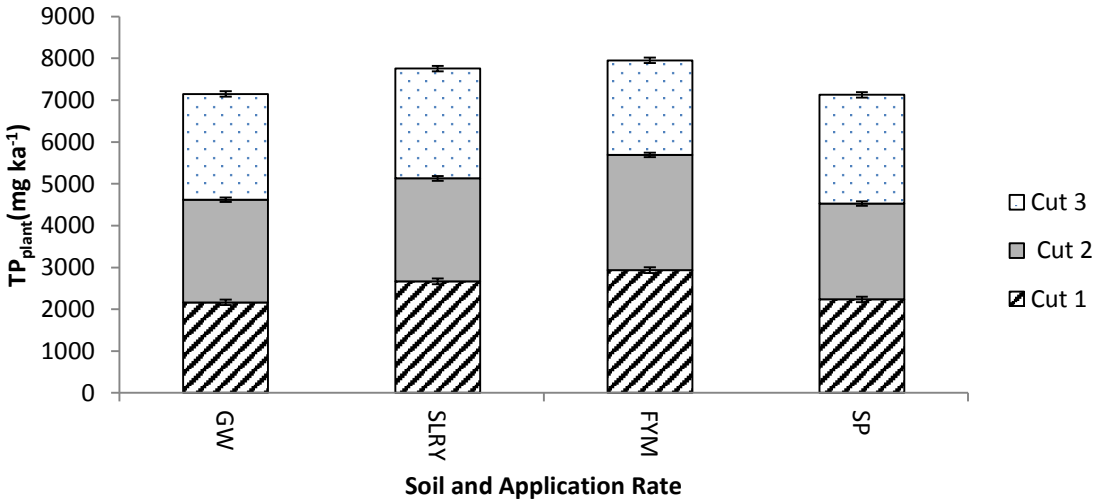


Figure C.2-1 TP_{plant} of ryegrass showing four cuts in a 6 month period for stage1 of experiment.

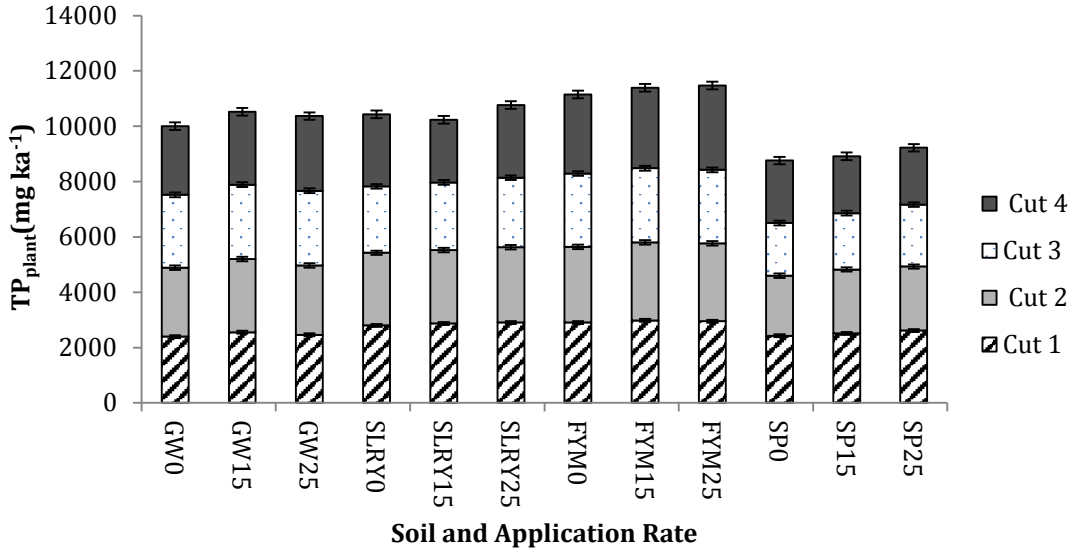


Figure C.2-2 TP_{plant} of ryegrass showing four cuts in a 12 month period for Stage 2 of experiment.

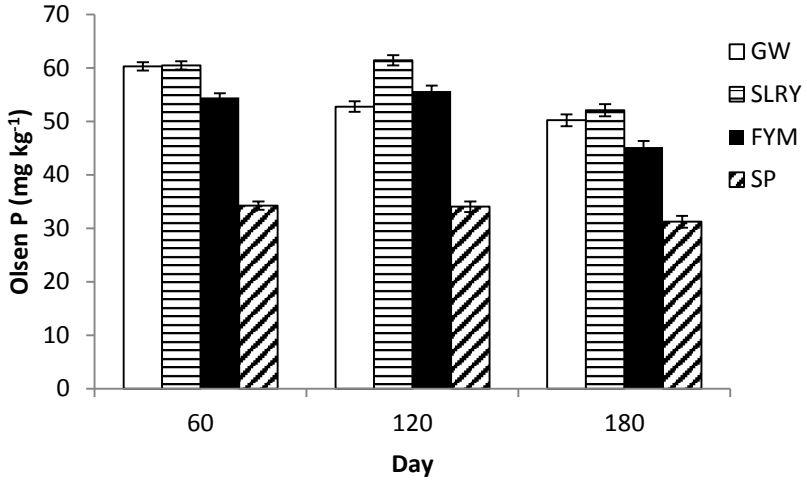


Figure C.2-3 Olsen P change with time over a 6 month period for Stage 1 of experiment

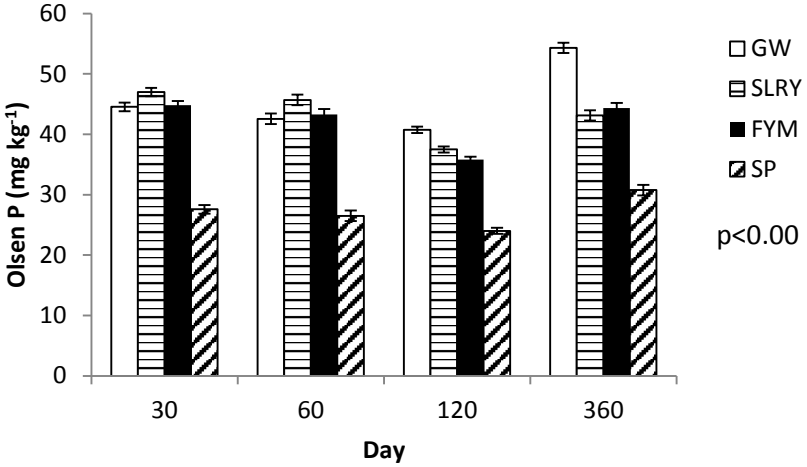


Figure C.2-4 Olsen P change with time over a 6 month period for Stage 2 of experiment

Table C.2-1 Linear regression analysis relationships between mean Olsen P application rate (kg P ha^{-1}).

Treatment	Regression equation	R^2	p-value
GW	y = 0.33x + 41.51	0.72	<0.001
SLRY	y = 0.17x + 38.85	0.83	<0.001
FYM	y = 0.13x + 40.34	0.90	0.383
SP	y = 0.05x + 26.41	0.53	0.670

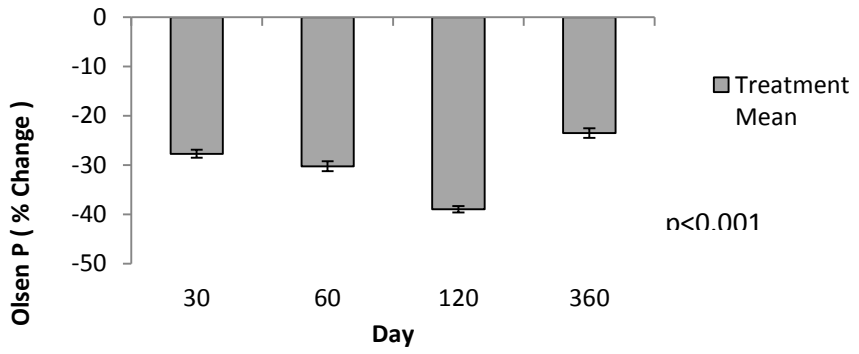


Figure C.2-5 % Change in Olsen P with time for the mean of all treatments following application of treatments

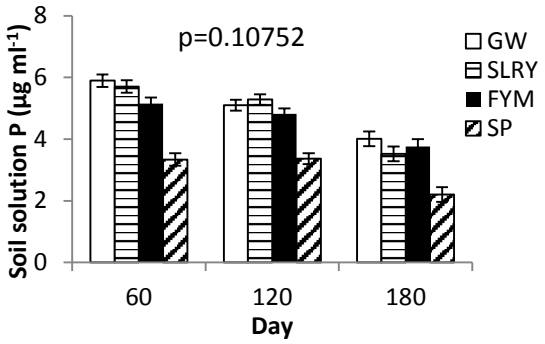


Figure C.2-6 Change in Soil solution P with time for 6 months, mean of all treatments for Stage 1

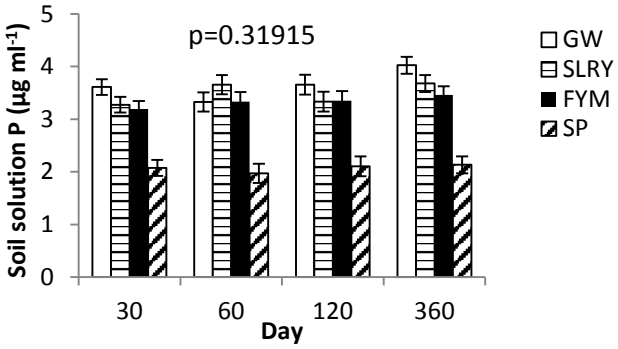


Figure C.2-7 Change in Soil solution P with time for 1 year, the mean of all treatments for Stage 2

C.3 Photograph of the Cranfield University glasshouse facility

Figure C.3-1 Photograph of Cranfield University glasshouse

Appendix D Chapter 7

D.1 Statistical analysis

Table D.1-1 Repeated measures analysis of variance for Soil treatment*Application rate*Time for C_{DGT}

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	7569.379	1	7569.379	2683.77	<0.001
Soil treatment	606.590	3	202.197	71.69	<0.001
Treatment amount	94.086	1	94.086	33.35	<0.001
Control/nocontrol	246.380	1	246.380	87.35	<0.001
Soil treatment*Treatment amount*Control/nocontrol	21.007	3	7.002	2.48	0.086
Error	64.870	23	2.820		
TIME	1131.680	3	377.227	336.79	<0.001
TIME*Soil treatment	189.908	9	21.101	18.83	<0.001
TIME*Treatment amount	35.439	3	11.813	10.54	<0.001
TIME*Control/nocontrol	52.441	3	17.480	15.60	<0.001
TIME*Soil treatment*Treatment amount	83.758	9	9.306	8.30	<0.001
Error	77.283	69	1.120		

Table D.1-2 Repeated measures analysis of variance for Soil treatment*Application rate*Time for Olsen P

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	121298.1	1	121298.1	14303.5	<0.001
Soil treatment	4899.7	3	1633.2	192.59	<0.001
Treatment amount	2532.5	1	2532.5	298.64	<0.001
Control/nocontrol	6016.8	1	6016.8	709.51	<0.001
Soil treatment*Treatment amount*Control/nocontrol	153.5	3	51.2	6.03	0.003
Error	203.5	24	8.5		
TIME	7217.0	3	2405.7	123.67	<0.001
TIME*Soil treatment	2852.9	9	317.0	16.30	<0.001
TIME*Treatment amount	1005.0	3	335.0	17.22	<0.001
TIME*Control/nocontrol	1016.0	3	338.7	17.41	<0.001
TIME*Soil treatment*Treatment amount	358.4	9	39.8	2.05	0.045
Error	1400.5	72	19.5		

Table D.1-3 Repeated measures analysis of variance for Soil treatment*Application rate*Time for Soil solution P

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	10157681	1	10157681	1348.32	<0.001
Soil treatment	107384	3	35795	4.75	0.008
Treatment amount	42583	1	42583	5.65	0.024
Control/nocontrol	592872	1	592872	78.69	0.000
Soil treatment*Treatment amount*Control/nocontrol	11114	3	3705	0.49	0.690
Error	203406	27	7534		
TIME	351548	3	117183	19.31	<0.001
TIME*Soil treatment	72817	9	8091	1.33	0.232
TIME*Treatment amount	13437	3	4479	0.73	0.532
TIME*Control/nocontrol	158872	3	52957	8.72	<0.001
TIME*Soil treatment*Treatment amount	25998	9	2889	0.47	0.886
Error	491444	81	6067		

Table D.1-4 Repeated measures analysis of variance for Soil treatment*Application rate*Time for DMY

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	592.0656	1	592.0656	4231.94	<0.001
Soil treatment	53.9294	3	17.9765	128.49	<0.001
Treatment amount	2.2713	1	2.2713	16.23	<0.001
Control/nocontrol	40.4988	1	40.4988	289.47	<0.001
Soil treatment*Treatment amount*Control/nocontrol	2.7066	3	0.9022	6.44	0.001
Error	3.7774	27	0.1399		
TIME	123.6452	3	41.2151	265.33	<0.001
TIME*Soil treatment	27.8826	9	3.0981	19.94	<0.001
TIME*Treatment amount	1.4624	3	0.4875	3.13	0.029
TIME*Control/nocontrol	10.2538	3	3.4179	22.00	<0.001
TIME*Soil treatment*Treatment amount	5.7203	9	0.6356	4.09	<0.001
Error	12.5818	81	0.1553		

Table D.1-5 Repeated measures analysis of variance for Soil treatment*Application rate*Time for TP_{uptake}

Effect	SS	Degr. Of freedom	MS	F	p
Intercept	709.4414	1	709.44	3732.39	<0.001
Soil treatment	15.8479	3	5.28	27.79	<0.001
Treatment amount	5.0589	1	5.05	26.61	<0.001
Control/nocontrol	25.6849	1	25.68	135.12	<0.001
Soil treatment*Treatment amount*Control/nocontrol	0.4094	3	0.13	0.71	0.549
Error	5.1321	27	0.19		
TIME	96.2502	3	32.08	185.86	<0.001
TIME*Soil treatment	57.5153	9	6.39	37.02	<0.001
TIME*Treatment amount	0.6957	3	0.23	1.34	0.266
TIME*Control/nocontrol	15.3841	3	5.12	29.70	<0.001
TIME*Soil treatment*Treatment amount	6.7686	9	0.75	4.35	<0.001
Error	13.9819	81	0.1726		

Table D.1-6 Repeated measures analysis of variance for Soil treatment*Application rate*Depth for C_{DGT}

	SS	Degr. of	MS	F	p
Intercept	853.163275	1	853.163275	465.385	4.6E-09
Treatment	8.40	1	8.40	4.58	0.060
Cont/Nocont	27.88	1	27.88	15.20	0.003
Error	16.50	9	1.83		
Depth	2.05	2	1.03	1.54	0.241
Depth*Treatment	1.51	2	0.76	1.13	0.343
Depth*Cont/Nocont	0.97	2	0.49	0.72	0.496
Error	11.9983	18	0.6666		

Table D.1-7 Repeated measures analysis of variance for Soil treatment*Application rate*Depth for Olsen P

	SS	Degr. of	MS	F	p
Intercept	853.1633	1	853.16	465.38	<0.001
Treatment	8.4017	1	8.401	4.58	0.061
Cont/Nocont	27.8756	1	27.87	15.20	0.004
Error	16.4992	9	1.83		
Depth	2.0533	2	1.02	1.54	0.241
Depth*Treatment	1.5108	2	0.75	1.13	0.349
Depth*Cont/Nocont	0.9703	2	0.48	0.72	0.496
Error	11.9983	18	0.66		

D.2 Miscellaneous graphs and tables

Table D.2-1 Pot experiment water regime and treatment mass

Soil	Rate (kg ha^{-1})	Soil (g)	Treatment (g)	Water to make field capacity (ml)
FYM	80	1500	34.5	875
FYM	120	1500	51	912.25
GW	80	1500	34.5	790
GW	120	1500	51	827.25
SP	80	1500	1.1	775
SP	120	1500	1.6	775
SLRY	80	1500	117	525
SLRY	120	1500	199	645
CONT	0	1500	0	775

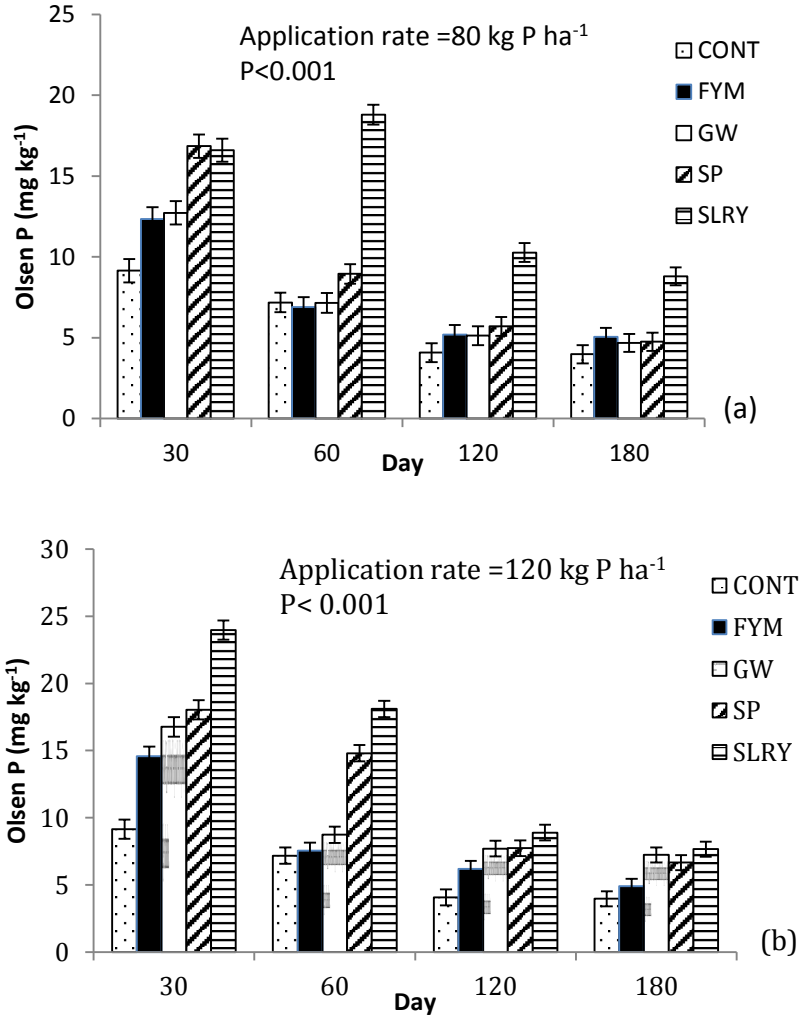


Figure D.2-1 Change in Olsen P with time at (a)80 and (b) 120kg P ha^{-1} as well as the control soil

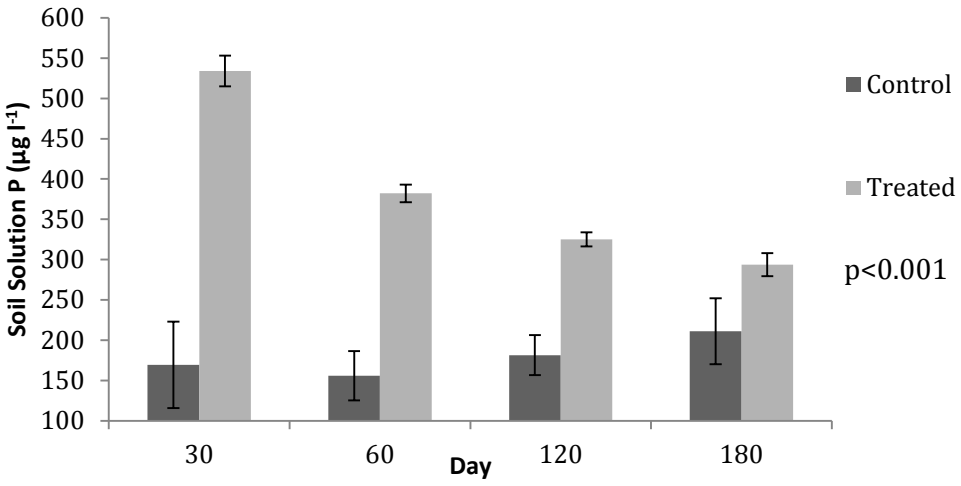


Figure D.2-2 Change in Soil solution P with time for control and treated soils