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A Comparative Study of Phosphorus Based Fertilisers' Performance: An Assessment on Plant Growth Parameters and Soil Health using Lolium Perenne

Ray, Anna

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I

**A Comparative Study of Phosphorus Based Fertilisers' Performance: An Assessment
on Plant Growth Parameters and Soil Health using *Lolium Perenne***

Anna Ray

being a

DISSERTATION

submitted to

BANGOR UNIVERSITY

in partial fulfilment of a

MASTERS BY RESEARCH IN ENVIRONMENTAL SCIENCE

July 2021

University number 500484991

II DECLARATION

I declare that this is the result of my own investigation and that it has not been submitted or accepted in whole or part for any degree, nor is it being submitted for any other degree.

Candidate: Anna D. Ray

Signature:

III Acknowledgments

Thank you, Davey Jones, Dave Chadwick, Paulo Pavinato and Kara Marsden: I sincerely appreciate all the opportunities granted to me over the past few years.

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Thank you, Dad and Laws...

...And to those in the aether.

IV Abstract

The availability of the essential nutrient phosphorus is imperative for the maintenance of crop production and henceforth, food security worldwide. With the threat of reaching peak phosphorus, as well as inefficient and environmentally harmful effects of traditional fertiliser use, alternatives are essential. This investigation utilised pot experiments, ^{14}C radiolabelling, and root analysis methods to assess the performance of a novel, granular fertiliser, known as struvite ($\text{MgNH}_4\text{PO}_4\cdot\text{H}_2\text{O}$), in comparison with traditional fertilisers, and find the most effective application method. The study found traditional P-based fertilisers to be potentially toxic to microorganisms, with cumulative respiration rate from the applied ^{14}C – glucose ranging from 30% - 90% in comparison with emissions from the novel, granular struvite, ranging from just under 25 – 30%, indicating severe stress response with constrained growth at higher concentrations of traditional fertiliser compared with the granular struvite. However, struvite alone proved less productive in terms of overall plant growth parameters in pot experiments, with monoammonium phosphate (MAP) (plus arbuscular mycorrhizae fungi (AMF)) yielding an almost 10-fold shoot biomass increase from struvite alone. Crushed struvite significantly increased P uptake, suggesting that previous literature where struvite was crushed and thoroughly mixed, is not representative of struvite effectiveness when applied as a commercially used product in the unaltered granular form (240 SGN). Struvite, when applied in a mix of 20:80 MAP mix, resulted in significantly greater mean P uptake than the addition of MAP alone, 7.75 mg pot^{-1} to 4.81 mg pot^{-1} , respectively. An 80:20 struvite:MAP mix may provide greater resilience to environmental instability, such as droughts and nutrient depletion within soils, with a mean below ground biomass being 67.5% higher than in MAP alone. In all fertiliser's treatments with induced P deficiency, root growth was more greatly affected by the addition of P to the lower soil layers (6 cm), with 10.6 - 62% greater biomass in the upper soil layers in comparison with the control (no P). The depth of fertiliser placement significantly impacted root growth, with differences between root biomass observed at the 6 cm placement, where mean root biomass was 49% greater overall compared with surface placement. There was ca. 30% greater root biomass when fertiliser was placed at 3cm depth. In comparison with the control, enhancement of root growth by fertiliser addition could only be detected with ICL (crystalised) struvite placement at 6cm. Overall, struvite may be commercially viable, in terms of shoot growth, when used with traditional P fertilisers. However, the method of application (surface or sub-surface placement) must be considered, and further experimentation is suggested to determine effectiveness of granulated struvite products under field conditions.

V Table of Contents

Title Page.....	I
Declaration.....	II
Acknowledgments.....	III
Abstract.....	IV
Table of Contents.....	V
List of Tables	VI
List of Figures	VII
List of Abbreviations.....	VIII
List of Phosphorus Fertiliser Abbreviations	VIII

Chapter 1 Introduction and Literature Review

1.1 Introduction	2
1.1.1 Global Agriculture.....	4
1.1.2 Global P Reserves	4
1.1.3 Geographical Distribution and Geopolitical Instability	
1.2 P Plant-Soil Dynamics.....	6
1.2.1 Mobility and Bioavailability of P in Soil Systems	6
1.2.2 Bioavailability and Plant Exudates.....	8
1.2.3 Phosphatase Enzymes (P _o Pool)	9
1.2.4 P Deficiency and Increases in Root Biomass	9
1.2.5 Nutrient Patches and Root Proliferation	10
1.2.6 AMF Associations	11
1.2.7 Bacteria.....	11
1.2.8 P Movement in Agricultural Soils	12
1.3 Novel Fertiliser: Struvite Formation and Dynamics	13
1.3.1 Recycling P from Waste Streams	13

1.3.2 Struvite: Factors Effecting Formation	14
1.3.3 Struvite: Heavy Metals and Pathogens.....	22
1.3.4 Struvite: Run-off, Leaching and Solubility	22
1.3.5 Struvite Dissolution and Form	27
1.3.6 Ammonium and Magnesium in Struvite	27
1.3.7 Plant-uptake of Struvite: Agronomic Efficiency	28
1.3.8 P-use efficiency in Turfgrass	29
1.4 Potential Limitations with Struvite Application.....	51
1.4.1 Performance in Calcareous soils.....	51
1.4.2 Micronutrients Deficiencies and Organic Matter	51
1.4.3 Plant growing seasons and early soluble fertiliser addition	52
1.4.4 Uncertainties in Scientific Studies	53
1.5 Knowledge gaps.....	53
1.5.1 Microbial life and AMF.....	53
1.5.2 Nutrient Patches and Root Proliferation	54
1.5.3 P Dissolution and Solubility	54
1.5.4 Micronutrient deficiency.....	55
1.6 Summary	55
1.7 Aims and Hypothesis	56
Chapter 2 Microbial Activity: Phosphorus Fertiliser Applications With Isotopic Radiolabelling	
2.1.1 Abstract.....	58
2.1.2 Introduction	59
2.1.3 Aims and Objectives	62
2.1.4 Hypothesis	62
2.1.5 Methods	62

2.1.6 Statistical Analysis	63
2.1.7 Results and Discussion	65
(i) Traditional Versus Granular Fertiliser.....	67
(ii) Impact of pH and EC.....	68
(iii) Concentration Gradient and Implications for Soluble Fertilisers.....	69
2.1.8 Concluding Remarks.....	69

Chapter 3 Application of Differing P fertilisers Over a 10-week Period using *Lolium Perenne* and a Sand Medium

Experiment 1

3.1.1 Abstract	72
3.1.2 Introduction	72
3.1.3 Aims and Objectives	74
3.1.4 Hypothesis	74
3.1.5 Methods	74
3.1.6 Statistical Analysis	75
3.1.7 Results and Discussion	75
(i) P uptake and PRE (%).....	76
(ii) Differences between treatments	77
(iii) Performance of Granular Versus Crushed Struvite	78
(iv) Nitrogen uptake and AMF	78
3.1.8 Concluding Remarks.....	78

Experiment 2

3.2.1 Abstract	79
3.2.2 Introduction	79
3.2.3 Aims and Objectives	80
3.2.4 Hypothesis	80

3.2.5 Methods	81
3.2.6 Statistical Analysis	81
3.2.7 Results and Discussion	82
(i) Differing Ratios Promote Greater P Uptake.....	83
(ii) Differing Ratios Impact on Root Systems	84
3.2.7 Concluding Remarks	85

Chapter 4 Root Adaptation with Induced P deficiencies and Variability in P-fertiliser Placements

Experiment 1

4.1.1 Abstract	87
4.1.2 Introduction	87
4.1.3 Aims and Objectives	88
4.1.4 Hypothesis	88
4.1.5 Methods	88
4.1.6 Statistical Analysis	89
4.1.7 Results and Discussion	90
(i) Depth of Placement and Root Biomass Response.....	91
(ii) Mean Root Biomass Compared to Control	91
(iii) Ammonium Compared with other N Sources	92
(iii) Shoot Biomass	92
4.1.8 Concluding Remarks	92

Experiment 2

5.1.1 Abstract	94
5.1.2 Introduction	94
5.1.3 Aims and Objectives	95
5.1.4 Hypothesis	95

5.1.5 Methods	95
5.1.6 Statistical Analysis	96
5.1.7 Results and Discussion	96
(i) Above and Below Ground Biomass Yields	98
(ii) Roots in Higher Versus Lower Compartments.....	99
(iii) Root growth and P source	99
5.1.8 Concluding Remarks	99
Chapter 5 Conclusion and Further Research.....	
5.1.1 Summary.....	101
5.2.1 Limitations to Research.....	101
5.2.3 Further Research	102
References	103

VI List of Tables

Table 1	Nutritional composition of struvites' recovered from differing types of wastewater (Ahmed et al., 2018)	16
Table 2	Struvite recovery (%) from differing waste water streams (Source: Rahman et al., 2014b)	19
Table 3	A variation of leaching rates of P and N using differing fertiliser sources with a variety of soil types and methods adapted from (Ahmed et al., 2018)	23
Table 4	Experiments assessing the effectiveness of struvite (for use with Table 5), adapted from (Ahmed et al., 2018)	30
Table 5	Biomass yields and nutrient uptake in Table 4 experiments. Adapted from (Ahmed et al., 2018)	39
Table 6	Significant values ($p < 0.05$) values and upper-lower ranges for differing concentrations of P-fertilisers. $N = 3$	64
Table 7	Experiment 1: dry matter and shoot yields, cumulative P uptake and P recovery efficiency (%) of <i>Lolium Perenne</i> Shoots after 70 days ($n = 4$)	76
Table 8	Experiment 2: dry matter and shoot yields, cumulative P uptake and P Recovery Efficiency (%) of <i>Lolium Perenne</i> Shoots after 70 days ($n = 4$)	84
Table 9	Total shoot and root biomass for <i>Lolium Perenne</i> and maximum root mass for different P-based fertiliser treatments and sampling depths	89

VII List of Figures

Figure 1	Example of selected countries fertiliser application rates from 1880 – 2014 (kg of nutrients per hectare of arable land) (world bank and federico, 2008)	5
Figure 2	a. Remaining P rock reserves by country adapted from (Cordell and White 2011) reported by the International Fertiliser Development Centre in 2010; b. Fertiliser production per continent (Tonnes of Nutrient per Year) 1961 – 2014 (UN Food and Agricultural Organisation, 2010)	6
Figure 3	Overview of known P dynamics within the rhizosphere. This schematic provides information on plant-P interactions and the chemical, physical and biological process involved. Adapted from (Shen et al., 2011)	7
Figure 4	The interactions between differing soil types and Organic Acids (OA) (Oburger et al., 2008)	8
Figure 5	Global mass balance of P showing the losses in supply chain. Red arrows demonstrate losses and sizes indicate the relative size of P movement through the chain (adapted from Cordell et al., 2009)	13
Figure 6	The potential chemical species produced from the nucleation process used in the formation of Struvite (Tansel et al., 2018)	15
Figure 7	Schematic to illustrate basic microbial C pools and processes involved with transfers between them (Glanville et al., 2016)	61
Figure 8	A visual representation of the experimental concept underpinning the ¹⁴ C microbial experiment: the greatest concentration (100%) (left) representing the microbial activity closest to particulate surface.	63
Figure 9	¹⁴ C-labelled glucose mineralisation in 9 differing fertiliser treatments and 12 concentrations for soluble fertilisers. Values represent means ± SEM.	66
Figure 10	Fertiliser impact on soil pH (left) and EC (right). Soluble fertilisers along concentration gradient (top) and granular (bottom).	67
Figure 11	Pot Experiment 1: Cumulative P uptake (mg pot ⁻¹) = (U – U _a) for fertiliser treatments over a 10 – week period	75

Figure 12	Pot Experiment 1: Root weight (g pot^{-1}) for fertiliser treatments after 10 – week period	77
Figure 13	Pot Experiment 2: Cumulative P uptake (mg pot^{-1}) = $(U - U_a)$ for fertiliser treatments over a 10 – week period	82
Figure 14	Pot Experiment 2: A boxplot showing overall root biomass for fertiliser treatments over a 10 – week period	83
Figure 15	A bar chart to show mean P and N recovery efficiency (%) ($\text{P Uptake} - \text{Control} / \text{Total P applied} * 100$)	83
Figure 16	The effect of P -based fertiliser in comparison with the control on root biomass of <i>Lolium Perenne</i> at particular sampling depths	90
Figure 17	The effect of P fertiliser treatments on total root and shoot biomass <i>Lolium Perenne</i> and highlighting the highest root weight among sampling depths	90
Figure 18	Experimental design demonstrating the differing compartments of roots (R1 and R2) with roots and wick	96
Figure 19	The effect of P-based fertilisers on mean shoot and root dry weight (mg pot^{-1}) of <i>Lolium Perenne</i> under micronutrient excess, causing P-deficit conditions	97
Figure 19	A boxplot demonstrating the root and shoot biomass growth by the upper (P fertiliser) and lower (no P fertiliser) compartments of test tube for differing P-fertiliser treatments	98

VIII List of Abbreviations

Al	Aluminium
AMF	Arbuscular Mycorrhizae Fungi
CaCl ₂	Calcium Chloride
C ₆ H ₈ O ₇	Citrate
ECW	Environmental Centre Wales
Fe	Iron
FOA	Food and Agricultural Organisation
HCl	Hydrochloric Acid
NaHCO ₃	Sodium Bicarbonate
OA	Organic Acid
OM	Organic Matter
P	Phosphorus
P _i	Inorganic Phosphorus
P _o	Organic Phosphorus
P – Rock	Phosphate Rock
SOM	Soil Organic Matter
UK	United Kingdom
UN	United Nations

VIII List of P Fertilisers Abbreviations

Diammonium phosphate	DAP	$(\text{NH}_4)_2\text{HPO}_4$
Diammonium phosphate Granular	DAPG	
Diammonium phosphate Granular Crushed	DAPC	
Dipotassium phosphate	DKP	K_2HPO_4
Disodium hydrogen phosphite	DSP	$\text{HNa}_2\text{O}_3\text{P} \cdot 5\text{H}_2\text{O}$
Monoammonium phosphate	MAP	$\text{NH}_4\text{H}_2\text{PO}_4$
Monopotassium phosphate	MKP	KH_2PO_4
Struvite Granular (SGN 240)	SG	$\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$
Struvite Granular Crushed	SGC	
Struvite (ICL Crystalline)	IS	
Triple super phosphate (powdered, traditional)	TSP	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$
Granular	TG	
Crushed	TSPC	

Chapter 1

Literature Review

1.1 Introduction

A plant's capacity to uptake the limiting nutrient phosphorus (P) is imperative in maintaining crop health and productivity, ensuring maximum plant root growth for environmentally resistant crops. Therefore, the application of bioavailable P in the form of inorganic phosphate (Pi)/synthetic fertiliser from phosphate rock (PR) has become a key factor in the development of agricultural practices responsible for ensuring both the expansion and maintenance of global food supply (Rubæk et al., 2012). However, the potential for depleted stores and unstable, inaccessible reserves of PR as well as the current inefficiencies inherent in the agro-economic P-cycle, have prompted the need for further investigations into the recovery of P from a variety of waste streams to provide sustainable alternative to conventional P sources. phosphate rock (PR) is considered a non-renewable resource and although other sources of fertiliser exist, crop yields are largely dependent upon PR supplies for fertilisation (Young et al., 2005). Past and current agricultural practices (using conventional fertilisers such as PR; TSP; MAP; DAP or animal-based manures) contribute to the inefficient management of P fertiliser resources with the indiscriminate and excessive application of P containing compounds to crops that extend far beyond the capacity for plant uptake (Damon et al., 2014; Lwin et al., 2017; Sharpley et al., 2013). The water-soluble nature of conventional nutrient applications has led to severe environmental degradation in the form of widespread eutrophication (Dodds et al., 2009; Kataki et al., 2016). The potential for adverse effects of P use on the physical, chemical and biological parameters of soil health is also a major concern worldwide (Gross et al., 2017). For example, the overuse of P fertilisers may result in nutrient imbalances (Long et al., 2018), soil acidification (Zhang et al. 2020), and potentially, the depletion of ectomycorrhizal associations (Guignard et al., 2017) negatively impacting the soil's capacity to support plant life through depleted aggregation and henceforth, inadequate water filtration (Fu et al., 2019). Higher yields through greater crop productivity are dependent on the balance of inter-dependant relationships between plant and soil. Phosphorus immobilised within the soil may provide a crucial supply of future nutrient supply (Barros et al., 2005; Macdonald et al., 2012; Nobile et al., 2018; Pinto et al., 2013; Zhu et al., 2018), while external sources of P-rich substances such as animal manures and biosolids (sewage sludge) represent a high content of P that can be environmentally friendly if recycled at appropriate rates and times (ADHB, 2019). Struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$), is a by-product of sewage sludge that has been manipulated by controlled precipitation to provide fertiliser granules known as "slow-release fertilisers" (Rahman et al., 2014b). The product can be used for agriculture fertilisation while simultaneously minimising the polluting effect of P-rich human effluent in watercourses (Baur, 2009; Spitler et al., 2005), such as eutrophication (nutrient enrichment)

of water bodies (Daniel et al., 1998). Practices such as this, that manage P resources in a manner that complies with circular economy principles, inclusive of practical solutions to “close the nutrient loops” with the most economically efficient and environmentally sustainable alternatives are key to sustainability of P resources (Withers et al., 2019; 2020). Fears regarding “Peak Phosphate” have thrown into question the long-term sustainability of our current food systems (Cordell & White, 2011; Withers, 2019; 2020) but technological innovations aligned with appropriate agricultural management practices enhance the our capacity to provide food for future populations.

1.1.1 Global Agriculture

1.1.2 Global P Reserves

Global Food Security is largely dependent upon P fertilisers and as global population numbers rise and affluence increases, fertiliser demand will also grow (Schoumans et al., 2015) (Figure 1). However, the forecast estimates on P availability vary, as do predictive models, making definitive answers on reserve depletion timing difficult to assess (Cordell et al., 2013; Van Kauwenbergh, 2010; Wellmer, 2017). The UN's most recent population projection is 9.8 billion by 2050 (U.N., 2017) while crop demand has been set to increase to 100 – 110% (from 2005 levels) (Tilman et al., 2011). Overall, the FOA have predicted food demand to increase by around 70% by 2050 (FAO, 2009), increasing P fertiliser demand. A more recent study has predicted that, based upon a 'business as usual' scenario, PR reserves will have been depleted within the next 70 - 140 years (Li et al., 2017). This prediction agrees somewhat with previous studies, dating depletion at 70 - 100 years (Chowdhury et al., 2017). However, the U.S. Geological Survey (USGS) data suggest that PR reserves will continue to supply a further 370 years (Cooper et al., 2011; Wellmer, 2017) agreeing with the International Fertiliser Development Centre (IFDC) estimate (Van Kauwenbergh, 2010). The former reports have been criticised for insufficient modelling techniques, not taking in to account important information such as the P available for recovery, while large cooperate fertiliser businesses are not legally bound to release information on quantity, making global quantification and henceforth predicting future trends inaccurate (Cordell & White, 2015; Wellmer, 2017). Projections on future PR supply are varied based on a complex multiplicity of factors pertaining to: shifting consumption patterns (Figure 1); differing modelling techniques; variations in population growth forecasts; inadequate data-sets from a secretive industry. Hence forth, PR availability estimates remain inconclusive, with contrasting outcomes in numerous studies due to a fluctuating evidence-base (Survey, 2018).

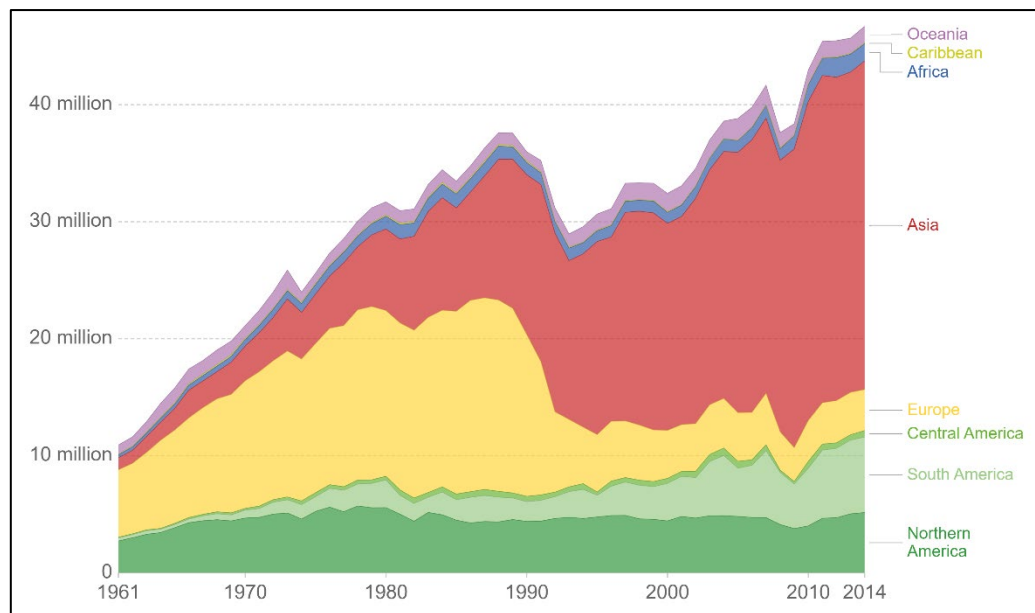


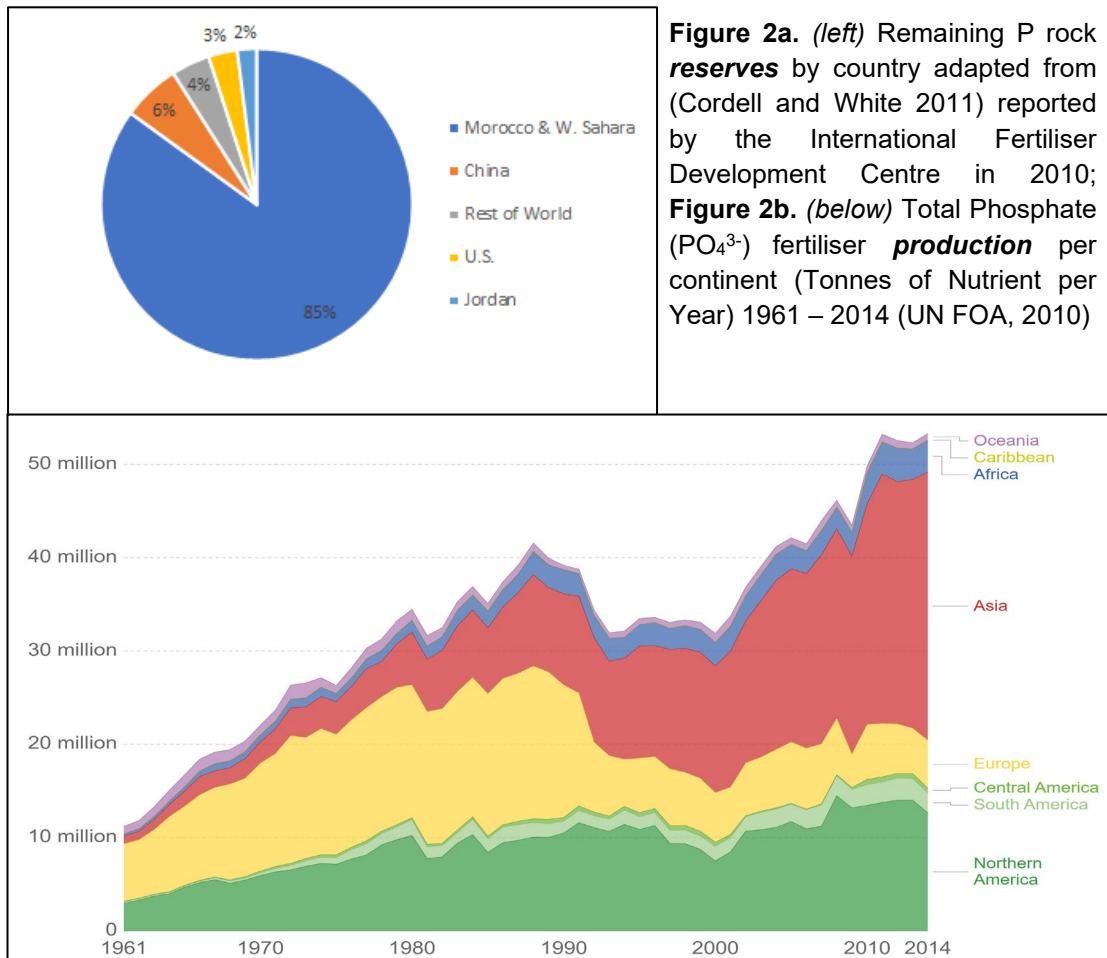
Figure 1. Example of continent's phosphate (PO_4^{3-}) fertiliser consumption rates from 1961 – 2014 (Tonnes of Nutrient per Year) (World Bank and Federico, 2008)

1.1.3

Geographical Distribution and Geopolitical Instability

The majority of P reserves are owned by few countries, and therefore worldwide food supply, as supported by agricultural intensification, is dependent on their stability, exhibiting significant risk for Global Food Security (Chowdhury et al., 2017). Morocco holds 75% of estimated PR reserves (Figure 2a), with China and the US being other key providers (Cordell et al., 2015) (Figure 2b). Political turmoil still remains within the Western Sahara regions occupied by Morocco, where vast amounts of the reserves are present (de Ridder et al., 2012). The 2011 Arab Spring uprising is said to have played a role in the increase of global phosphate prices by as much as 23.6%, resulting from a drop in exports from Tunisia by 56.6% (Matta et al., 2016). Awareness of economic instability surfaced when in 2008, P prices rose by 800%, increasing food prices world-wide (Cordell et al., 2015; Cordell & White, 2011; Mew, 2016). The spike in P fertiliser price was caused by several factors, such as changes to oil prices; spikes in dairy and meat consumption; increase in biofuel production, and importantly brought into question the long-term sustainability of PR reserves. Price volatility of PR can be partly attributed to uneven geographical distribution of the resource, encouraging tensions between competitors, contributing to geopolitical disputes and limiting alternative supply chains (Cordell et al., 2015). The functioning of global food is highly dependent on PR and its status as a new renewable resource denotes insecurity that subsequently encourages market instability (Cordell et al., 2009). As a vital component of mass food production, the P industry is an

integral part of the global economic market, with less than five countries controlling the supply chain.



1.2 P Plant-Soil Dynamics

1.2.1 Mobility and Bioavailability of P in Soil Systems

Soil pedogenesis (climatic factors; biology present; parent materials; slope; time) and the underlying chemical processes determining the formation of P complexes such as precipitation or dissolution of P-bound minerals; soil colloidal surface sorption or desorption of P and organic matter hydrolysis (Figure 3) will be the primary factors influencing the quantity of P available for plant uptake within the soil profile (Vance et al., 2003). For example, highly weathered, acidic soils with larger quantities of Al/Fe oxides exhibit greater P-fixation (resulting in occluded P) through adsorption of P molecules (Fink et al., 2016). In calcareous (and neutral) soil types P can also sorb to clay minerals. However, precipitation reactions tend to dominate P immobilisation under these conditions (Shen et al., 2011). Phosphate sorbs on to

charged soil surfaces, either electrostatically or through covalent bonding, which determines the strength of sorption (Lindsay 1979). Over time, or with a change in environmental conditions, the soil's sorption capacity for P changes with factors such as pH (acidity or alkalinity); contending anions (such as organic acids) (Figure 4); the presence of basic ions and the concentration of P being heavily influential (Stutter et al., 2015). Plants uptake P in mineralised forms (orthophosphates: H_2PO_4^- , HPO_4^{2-}), which naturally occur in the soil at very low concentrations (0.1–10 μM ; (Hinsinger, 2001a)) due to the aforementioned chemical processes. The available P moves into a plant at the soil-root interface whereby a concentration gradient is then created, stimulating further diffusion of P (Barber et al., 1963). Due to the scarcity of naturally occurring, bioavailable mineralised P within the soil profile, plants have adapted mechanism to enhance the availability of P compounds, thereby ensuring greater crop health, productivity and plant growth (Merbach et al., 2010; Torres et al., 2004).

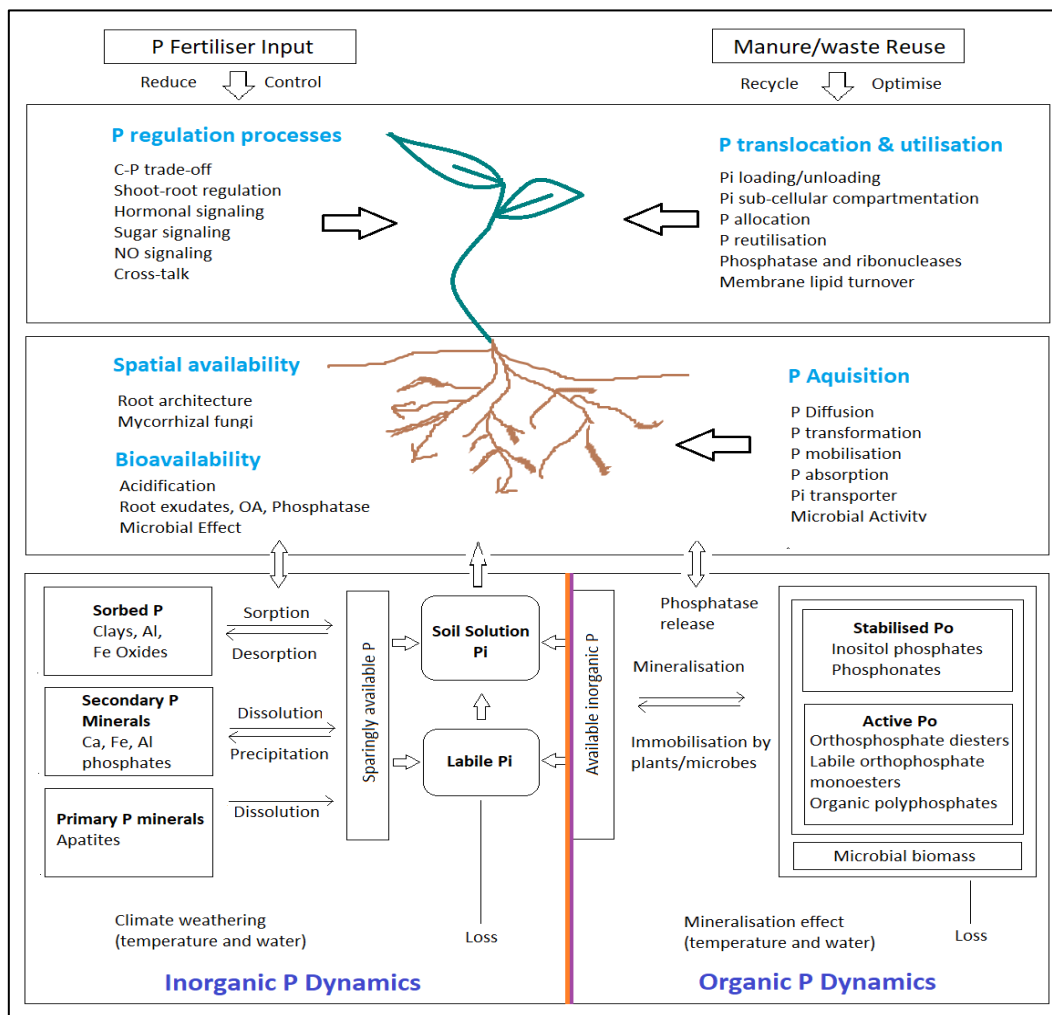


Figure 3: Overview of known P dynamics within the rhizosphere. This schematic provides information on plant-P interactions and the chemical, physical and biological process involved. Adapted from (Shen et al., 2011)

1.2.1 Bioavailability and Plant Exudates

Phosphorus is characterised by low solubility and mobility therefore, in times of P deficiency, some plants can actively modify the rhizosphere to ensure sufficient nutrient acquisition for nutrient uptake and plant growth e.g. via OM addition; ligand substitution and organic acid complexation (Hoffland, 1992; Oburger et al., 2008) (Figure 3); enzymatic interactions (plant and soil microbes) (DeLuca et al., 2015) and soil acidification through proton excretion to assist in P acquisition (Wang et al., 2008). Numerous studies have shown P deficient plants release two to three times the amount of protons compared with organic acids on an equimolar basis (Sas et al., 2001; Vance, Uhde-Stone et al., 2003) making HCl an important substance for P release. These acidifying substances have a solubilising effect on P compounds and have been shown to have a strong impact on Fe-P complexes (Randall et al., n.d.; Santos et al., 2017; Shi et al., 2017; Ström & Strom, 1997). The substances interact with P species, as well as the soil colloidal surfaces, in a variety of ways, making available the differing fractions of P-pools by directly altering soil environments in times of nutrient scarcity. This is achieved through chemical interference with soil clay surface minerals (including Fe and Al hydroxides) and the P species, while concurrently, acidification through root-induced mechanisms can result in a pH reduction within the rhizosphere by around 2 - 3 units compared to the surrounding soil, greatly enhancing the dissolution of P compounds (Shen et al., 2011; Oburger et al., 2008; Santos, Silva et al., 2017; Sinclair et al., 2017). Up to half of P compounds present in the soil solution are in organic forms, requiring mineralisation prior to plant uptake (Horst et al., 2001).

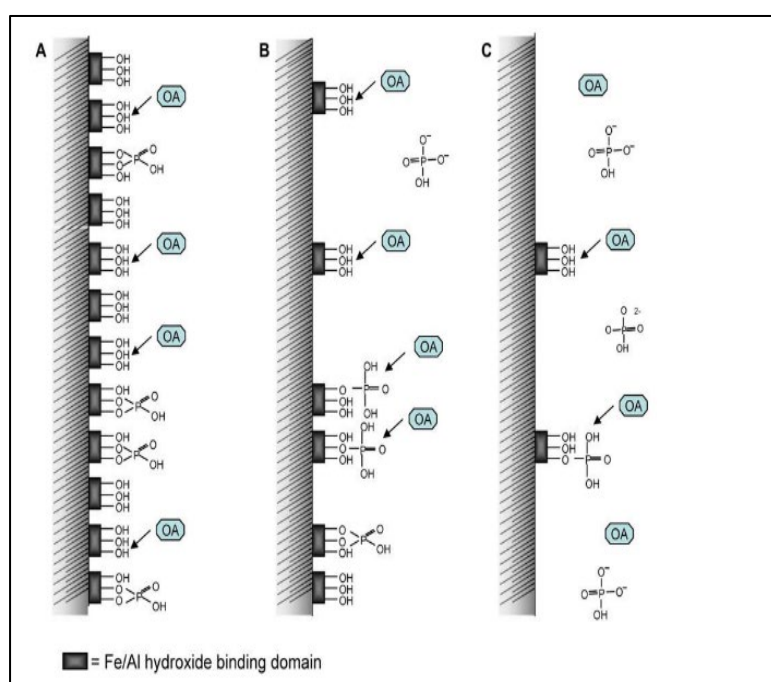


Figure 4 : The interactions between differing soil types and Organic Acids (OA). (A) shows a soil with many free binding sites (Andosol – LP) and small potential for P-release. (B) shows a soil type with moderate amounts of Fe/Al sites (Podzol), where OAs may bind to thereby saturating the surface, releasing P. With few binding sites and a large amount of P in solution, soil (C) (Cambisol) demonstrates little potential for the release of P (Oburger et al., 2008)

1.2.3 Phosphatase Enzymes (P_o Pool)

Enzymes can be released by roots and microorganisms which can facilitate the breakdown of labile forms of P_o held in soil organic matter (SOM), e.g. phytase, acid and alkaline phosphatases (DeLuca et al., 2015). These enzymes can be present in exudates discharged through microbial activity and plant roots and associated mycorrhizas (Schilling et al., 2011). Organic acids and P enzymes have been shown by many studies to be released simultaneously in species such as *Proteaceae* (Grierson, 1992; Dinkelaker et al., 1995; Lambers et al., 2002). They work by hydrolysing P from organic compounds enabling the acquisition and utilisation of P_i by plants (Torres et al., 2004). Upon P deficiency, P_o hydrolysis is typically stimulated, whereas when labile P is present, phosphatase activity is repressed (Smeck, 1985). The P_o pool in soil is highly complex containing numerous different P containing substrates which can often be highly recalcitrant (e.g. humic bound P) (Cross & Schlesinger, 1995). As such, the availability of organic P can be somewhat dependent on the soil C: P_o ratio (Mcgill & Cole, 1981). The quantity and composition of OM will dictate the dynamics of P_o (Stewart & Tiessen, 1987), while the soil properties and root soil interactions will also dictate the availability and uptake (Shen et al., 2011).

1.2.4 P Deficiency and Increases in Root Biomass

Plants may counteract P deficiency by either down-regulating productivity to conserve P resources, or employ mechanisms involving the up-regulation of specific functions to encourage P acquisition through plant root physiological and morphological adaptations (as discussed here). For example, when P is in excess supply, plant roots will extend towards the source and proliferate (Byrne et al., 2011; Hill et al., 2006; Sheil et al., 2016). However, the adaptations heavily depend upon the species, likely due to traits evolved over time in a variety of soil conditions (Chapin et al., 1987; Lambers et al., 2008). Hill et al. (2006) conducted a comparative study of 10 temperate pasture species to assess differences in root morphologies in times of P and N deficiencies vs. adequate supply. In 9 of the 10 species, decreases in root mass were resultant from decreases in P supply and the diameters of all the roots systems were from 9-32% lower in the control (no P addition). However, the root mass fraction (RMF) (fraction of root dry matter as a proportion of total plant dry matter) increased significantly for all species within the experiment when P addition was decreased. The *Vulpia* ssp. was the only species that remained without significant change in all P conditions, with the highest root length and fine root mass amongst all test species throughout. Grasses have been demonstrated to have different adaptations in accordance with the conditions from which they come (fertile or infertile soils) (Boot & Mensink, 1990). Generally, carbon is often allocated to

below ground biomass (i.e. roots), consequentially, greater RMF is observed as well as increase lateral root formations (Gilroy & Jones, 2000; Lynch & Brown, 2001; Williamson et al., 2001). Total root length is a key feature for plants to access and acquire nutrients in times of low availability, especially P, due to its low mobility in the soil (Baan et al., 1980; 1982). Root systems with higher surface area to volume ratios will be more adept for exploring greater areas of soil for nutrient mining (Bonser et al., 1996; Lynch & Brown, 2001). Plants may be down-regulating growth due to P deficiencies. However, they are adapting to achieve the greatest P acquisition from their roots.

1.2.5 Nutrient Patches and Root Proliferation

It is commonly observed that localised application of P and NH_4^+ to soil causes significant root proliferation (Drew, 1975; Hodge, 2004). For example, in a 2 year field experiment Jing et al. (2010) found total root length of maize in calcareous soil to increase from 23 – 30% in plots with localised P and NH_4^+ treatments in comparison with broadcast treatments. This may be due to increasing soil acidity within the application zone, by as much as 3 pH units, thereby increasing P availability. He et al. (2003) found plant roots to preferentially grow, as much as 70-75%, in the high P compartment of a split plot treatment. The study also demonstrated that high P treatment on one half of the compartment, with no P addition in the other, caused much greater root length in the high - P sections, where roots grew towards the P. In the treatment where fertiliser was homogenously applied to both compartments, root length was lower overall, compared with the prior, suggesting that root proliferation is signalled by low P. Ma et al. (2013) found the length and density of first order roots increased by as much as 50% in maize seedlings with the localised application of P combined with ammonium (in the forms of di-ammonium phosphate, DAP, and ammonium sulphate + P). These studies are supported by numerous others, expressing that the application of P via banding or induced P deficit in one section of the soil in comparison with another to be of crucial important root proliferation (Jackson and Caldwell, 1989; Lie et al., 2013; Liao et al., 2001). Ammonium and P work synergistically, enhancing the P available for plant uptake, which could potentially be the result of increasing rhizosphere acidity. Plant roots mine for nutrients, and upon discovery proliferate if P – uptake is lacking elsewhere in the rooting zones. Complimentary nutrients such as NH_4^+ and P (among others) ensure healthy plant growth, while plant root proliferation is increased by the appropriate rate and placement of applied nutrients. Intrinsic morphological adaptations to alter nutrient supply are also impacted by biological symbiosis such as root associations with arbuscular mycorrhizal fungi (AMF).

1.2.6 AMF Associations

A variety of plants also employ their adaptations to attract beneficial organisms, stimulating the germination and growth of arbuscular mycorrhizal fungi (AMF) (Gerdemann, 1968; Harrison, 2005; Nagahashi et al., 1996) that assist in the extension of plant roots via symbiotic associations contributing to the translocation of P compounds from further afield for plant uptake (Carvalhais et al., 2011). AMF associations are particularly well-documented, with approximately 74-80 % of vascular plant species having AMF associations (Bolduc, 2011; Shen et al., 2011). Although the effects are not present in all AMF and plants species, studies such as Jansa et al. (2005) have shown increased net uptake of P due root-fungi interactions. Other studies have demonstrated certain turf species to have increased growth rate with AMF inoculation, while the impact varies in accordance with the type of seasonal grass root systems with inherently different root morphologies (Hayman 1983; Petrovic 1984). The extent of P fertilisation already present in the soil will also impact the extent to which associations are formed: with increasing quantities of AMF naturally occurring in organically grown crops (Scullion et al., 1998). Higher levels of OM stimulate greater AMF abundance while concentrated P concentrations, such as found in intensively managed crops, will lessen the requirement for P-mining. Inherently present in the life cycle of plants is decay, by which C is released into the soil through root shedding, attracting and stimulating growth of beneficial symbionts. These symbionts also degrade, contributing additional OM, enhancing soil stability and the continuation of the cycle. The presence of AMF species improves a plant's resilience to environmental changes, such as drought (Wu et al., 2013), through both direct and indirect mechanisms including; bidirectional nutrient transfer; enhanced aggregate stability and increasing water acquisition (Begum et al., 2019; Yooyongwech et al., 2016). The extent to beneficial P accumulations traits vary among species of both AMF and plants along with soil conditions (Chen et al., 2002; Hill et al., 2006; Panneerselvam et al., 2012; Scullion et al., 1998). The addition of C to the soil via AMF associations attracts microorganisms, a beneficial attribute for plant growth and development.

1.2.7 Bacteria

Plants exudates provide nutrition for soil microbial communities that in turn, play a key role in the cycling of nutrients, and the biochemical transformation of P containing compounds contributing to their mobilisation and hence, bioavailability for plant uptake (Buscot & Varma, 2019). Modern chemical fertilisers (along with pesticides, herbicides and fungicides) have the potential to negatively impact microbial communities over time, affecting P –cycling in edaphic systems. Though the quantity of microorganisms present in the soil is heavily dependent on

the stage of soil development, microbial P is said to account from 2-10% of P held in the soil, although this figure can reach up to 50% (Oberson et al., 2005). Chen et al. (2002) demonstrated the water-soluble organic Carbon (WSOC) to be significantly higher around the rooting zone of both *Radiata* pine and *Lolium perenne*, concurrent with higher levels of biological activity (microorganisms, AMF). Using *Lolium perenne* as a test subjects, Meharg & Killham (1995) inoculated the roots with a range of microorganisms, consistently resulting in an increase in root exudates (3-34%). Mehrvarz et al. (2008) found the addition of a P solubilising bacteria (*Pseudomonas putida*) to barley seeds increased plant biomass levels, producing the maximum biological yield, sufficient to compete with the outcomes of the chemical fertiliser. Enzymes may also be produced by bacteria that make available the P bound in organic forms (Schilling et al., 2011) while P solubilising bacteria acidify the soil through exudates (e.g. citrate, malate, oxalate) (Buscot & Varma, 2019; Jones and Oburger, 2011). Bacteria are key to P – cycling through microbial turnover (Seeling & Zasoski, 1993) and metabolic processes (Richardson et al., 2009), contributing to the mineralisation of organic P; the release of P through death and decay; exudation of enzymes (e.g. phosphatase) enabling further P mobilisation (Buscot & Varma, 2019). Bacteria are also key in the short-term immobilisation of P and therefore not only make P available to plants, but also compete with plants for P, maintaining a balance of available and unavailable fractions through the microbial turnover. Interactions between plants and microorganisms are complex and often multi-dimensional though essential in maintaining crop health and resistance through optimal nutrient uptake.

1.2.8 P Movement in Agricultural Soils

Due to the chemical nature of P-fertiliser compounds in the soil, large quantities of P have accumulated in the soil, inaccessible to plants, representing a considerable loss of the resource (Macdonald et al., 2012; Merten & Minella, 2013). Soil properties; fertiliser types; agricultural intensification practices; as well as the climatic factors may encourage the loss of surface P too, resulting in the contamination of fluvial and edaphic systems (Schoumans et al., 2015; Withers et al., 2001). A breadth of studies spanning the UK demonstrate the erosive potential of agricultural soils and there is little debate on the extent to which environmental damage is caused by P containing particulate pollution from soil erosion and/or overland water flow (Brett et al., 2016; European Water Directive, 2011; Sharpley et al., 2013; Shore et al., 2017; Wironen et al., 2018). An estimation of UK soils P-saturation concluded that soils of the UK contained around 4 - 10 million t of legacy-P (Withers et al., 2014). A separate, more recent study used long-term data linking P dynamics through both hydrological and terrestrial

systems predicting the nutrient content of soil under two centuries of agricultural conditions in the UK showed an increase from 2.6 – 18.1 kg P ha⁻¹ yr⁻¹ in arable crop-land (Muhammed et al., 2018). Further studies have demonstrated similar results with P accruing in the soil at rates increasing at around 0.6 kt P yr⁻¹ (Worrall et al., 2016). As dictated by soil type and surrounding conditions, labile P can be transported into the environment as bioavailable fluvial run-off or in particulate form or, immobile P can accumulate. Understanding P_o and P_i dynamics for fertiliser use gives the potential to alter current P-use to create sustainable and efficient agro economic systems, minimising waste through leachate loses and maximising P-use efficiency in crops.

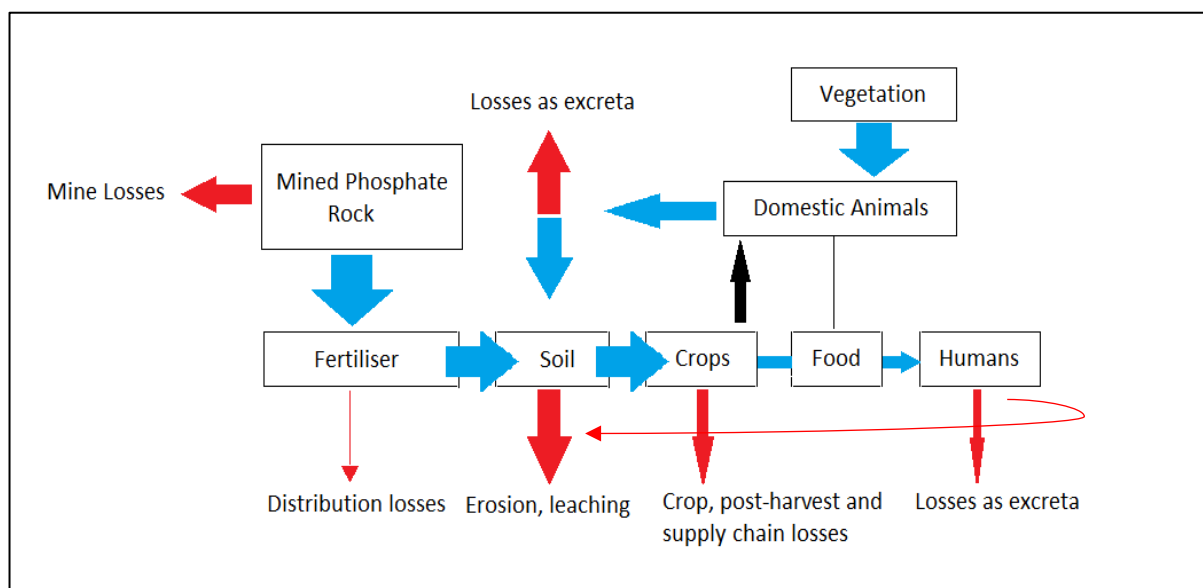


Figure 5. Global Mass Balance of phosphorus showing the losses in supply chain. Red arrows demonstrate losses and sizes indicate the relative size of P movement through the chain (adapted from Cordell et al., 2009)

1.3 Novel Fertiliser: Struvite Formation and Dynamics

1.3.1 Recycling P from Waste Streams

To promote sustainability in the current model for agro-food production, waste and inefficiencies in the P cycle must be rectified through reclamation of P from a multiplicity of waste streams to promote environmentally sound practices and the continuous supply of P fertiliser (Figure 5) (Schröder et al., 2011; Wither et al., 2020). Within Europe, 50-60% of PR could be replaced by P sources if efficiently recycled from a combination of sewage sludge,

household and industrial organic wastes and meat and bone (Table 1) (Klinglmair et al., 2015; Zoboli et al., 2016; Antikainen et al., 2005; van Dijk et al., 2016; Zoboli et al., 2016). Although many P-rich waste streams exist, the main source of P can be found in municipal sewage streams (wastewater and biosolid fractions) (van Dijk et al., 2016), representing over 50% of P available from off-farm resources (Ott and Rechtberger, 2012; van Dijk et al., 2016; Zoboli et al., 2016). Around 10% of the P used for fertiliser, additives and animal feed comes post-human consumption, leaving 90% to be attained from other sources (namely PR) (Sattari et al., 2012a, Scholz et al., 2014b). Reliance on imported PR reserves would be minimised with the use of recycled products, especially sewage sludge, while environmental issues associated with P-rich waste streams would lessen. Attaining and using P from waste streams would assist in closing the nutrient loop promoting the sustainability of agri-food cycles and ongoing food security.

1.3.2 Struvite: Factors Effecting Formation

Struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) is mineral crystal occurring as a precipitate in P enriched waste streams. To provide a commercially viable product, struvite crystallisation can be developed through nucleation, the manipulation of chemical parameters to increase crystal birth followed by the enhancement of crystal growth (Rahman et al., 2014b). The elemental composition of the granule will differ in accordance with the waste stream from which it was generated, although theoretically stated at 12.6% P; 5.7% N; 9.9% Mg (Ahmed et al., 2018) (Table 1). A multiplicity of factors influence the crystal formation including: pH; temperature; molar ratios; ionic species and their concentrations; aeration rate and type of precipitation reactor (Table 2) (Parsons & Doyle, 2002; Md Mukhlesur Rahman et al., 2014b), where varied speciation may be resultant (Figure 6). Struvite occurs as pure white powder, when chemical equilibria are met, and sold as a product in a hard-shelled, granular form of differing sizes (Munch and Barr, 2001), demonstrated as an effective slow-release fertiliser for a variety of agricultural, horticultural, turf and ornamental crops (Munch and Barr, 2001; Nelson et al., 2003; Gonzalez Poncer and Garcialopez, 2007; Yetilmezsoy and Zengin, 2009). Struvite can also express equivalent hardness to conventional fertilisers such as MAP and DAP (Bouropoulos and Koutsoukos, 2000). For example, Latifian et al. (2012) found starch and Bentonite, as a protective cover, increased crush strength (<2.5 kgf) above the recommended limit for fertiliser hardness demonstrating another benefit for agricultural use. Various studies have shown struvite to be as effective as conventional fertilisers in terms of crop production and P-use efficiency (Ahmed et al., 2018) whilst proven to have lesser leaching potential when compared with more soluble fertilisers (both chemical and organic) (Rahman et al. (2011),

Latifian et al. (2012); Ryu, Lim, Kim, et al. (2012); Ryu and Lee (2016)), lower heavy metal content (Moller et al., 2018) and lesser risk of pathogenic infections (Bischel et al., 2016). However, the form of the fertiliser applied (powdered vs. granular or method of application (Table 5) throughout the experiments discussed makes comparative analysis challenging (Bonvin et al., 2015; Degryse et al., 2017; Bastida et al., 2019), while differences in struvite's purity, due to disparity in the elemental composition of different waste streams, complicates matters further. Many questions remain unanswered as to the effectiveness of Struvite, such as plant root mechanisms employed to extract P. Struvite has been shown to have the potential to reduce the environmental costs associated with conventional fertilisers, (Talboys et al., 2012) aiding in reducing nutrient enrichment of fluvial systems associated with waste streams, while also lessening the reliance on the non-renewable forms of P fertiliser (Cabeza et al., 2011; Huygens & Saveyn, 2018; Theregowda et al., 2019; P.J.A. Withers et al., 2001).

Figure 6. The potential species to be produced from the nucleation process (Tansel et al., 2018)

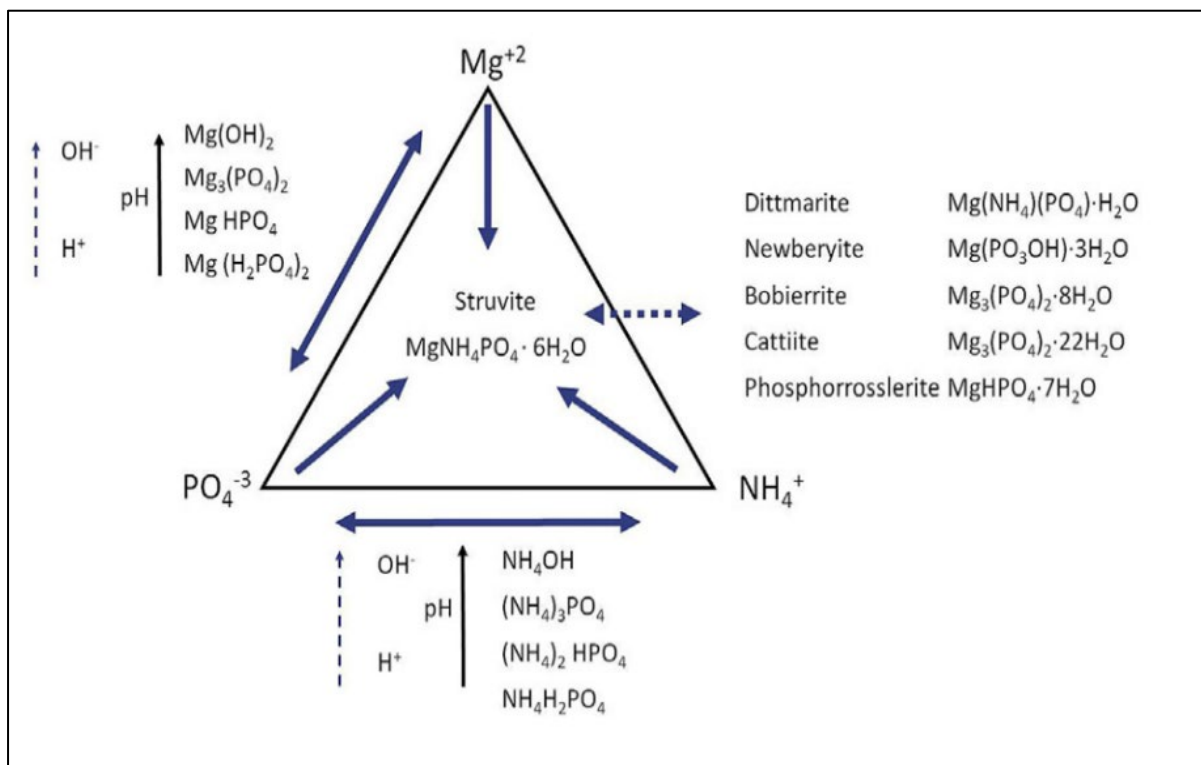


Table 1. Nutritional composition of struvites' recovered from differing types of wastewater (percentage dry weight) (Ahmed et al., 2018)

Source	P (%)*	N (%)	Mg (%)	K (wt%)	Ca (wt%)	Fe (wt%)	Na (wt%)	Reference
Theoretical Struvite Composition	12.6	5.7	9.9	-	-	-	-	Latifian et al. (2012)
Anaerobically digested effluent	13.1	5.6	9.6	-	-	-	-	Latifian et al. (2012)
Anaerobic Digester supernatant	103.9 (g / kg)	44.4 (g / kg)	131 (g / kg)	0.77 (g / kg)	12 (g / kg)	-	-	Plaza et al. (2007)
Anaerobic effluent yeast industry	10.8	3.5	7.97	1.21	2.18	-	-	Uysal et al. (2014)
Black water	11.9	5.39	10.68	0.41	1.37	0.74	0.87	Gell et al. (2011)
Dairy industry wastewater	11.13	3.3	8.64	0.73	-	-	-	Uysal and Kuru (2015)

Dairy wastewater	28 (as P ₂ O ₅)	-	4.2	0.3	17.9	-	-	Massey et al. (2009)
Digested sludge	61.1 (g / kg)	28 (g / kg)	78.4 (g / kg)	2.2 (g / kg)	27.9 (g / kg)	-	-	Kern et al. (2008)
Landfill leachate	16	4.9	8.6	-	-	-	-	Li and Zhao (2003)
Semiconductor wastewater	12.7	13.2	8.2	4.6	2.5	4.8	1.3	Ryu, Lim, Kang, et al. (2012)
Swine manure	7.8-12.4	1.2-3.71	22.5-26.2	1.37-1.79	3.19-6.79	-	-	Achat et al. (2014)
Swine manure	5.6	6.3	-	-	4.2	-	-	Ackerman et al. (2013)
Swine wastewater	15.6	14.8	10.2	2	ND	-	-	Ryu and Lee (2016)
Urine	11.66	5.26	13.97	0.24	0.96	0.48	0.51	Gell et al. (2011)
Urine	11.8	5.4	9.5	0.1	0.7	-	0.3	Liu et al. (2016)

Urine	71-190 (mg/g)	29.3-60 (mg/g)	97-645 (mg/g)	2.1-15.1 (mg/g)	6-58 (mg/g)	-	2.1-15.5 (mg/g)	Antonini et al. (2012)
Wastewater	9.6-11.8	-	5.5-8.6	-	0.5-8.6	0.4-5.5	-	Cabeza et al. (2011)

*elemental composition

Table 2. Struvite recovery (%) from differing waste water streams (Source: Rahman et al., 2014b)

Source of Waste/wastewater	Additional Chemicals	PO ₄ :Mg Molar Ratio	pH	Reactor Type	NH ₄ Removal (%)	PO ₄ Removal (%)	Crystal Types	Reference
Swine wastewater	MgCl ₂	1: 1.2	8.0-9.0	Continuous flow reactor	31	93	Irregular shaped, coarse	Rahman et al. (2011)
Swine wastewater	MgCl ₂	1: 0. 8	7.82-8.92	Batch Test CFR	65	67	Irregular shaped, coarse	Liu et al. (2011a)
Synthetic wastewater	MgCl ₂	2:1	8.5-10.5	Pilot scale continuous flow reactor	56	81	Elongated Crystals (50-100um)	Le Corre et al. (2007)
Anaerobic digester sidestream	Mg(OH) ₂ slurry	1:1.3	8.0-9.0	Pilot scale MAP reactor	-	94	Trapezoidal shape Crystals	Tunay et al. (1997)
Swine manure slurry	MgO, Mg(Cl) ₂ .6H ₂ O	1.1.6	7.0-11	Bench scale reactor	-	91	Irregular shaped, white crystal	Burns and Moody (2002)

Sewage wastewater	By-product of MgO production	1.6:1	8.28-8.41	Full scale sewage treatment plant	30	90	-	Quintana et al. (2008) Zhang
Landfill leachate	MgCl ₂ , MgO, Na ₂ HPO ₄	1: 1.1	8.4-9.0	Batch reactor	-	85.4	Coarse, irregular crystals (15-40um)	Zhang et al. (2009)
MAP containing slurry	MgCl ₂ KH ₂ PO ₄	1:1	8.0-9.0	Batch reactor	40	87	Cube granules	Cho et al. (2009)
Poultry manure wastewater	MgCl ₂ MgSO ₄ , MgO, KH ₂ PO ₄	1:1	9.0	Batch reactor	-	85.4	-	Yetilmezsoy and Zengin (2009)
Slurry-type swine wastewater	MgO, H ₃ PO ₄	1:1.5	8.0-11.0	Anaerobic Digester	20	99	Irregular white crystals	Kim et al. (2004)
Synthetic wastewater	NaCl ₂ , (NH ₄) ₂ HPO ₄ , NH ₄ Cl	1:1.2-1:2.4	7.5-8.7	Bench scale reactor	42	90	2.5-3.5 mm	Adnan et al. (2003)

Digested swine wastewater	Bittern	1.0:0.6	8.0-11.0	Batch reactor	23-29	90	Irregular mixed crystals	Ye et al. (2011) Ichihashi
Swine wastewater	N, P and Mg in wastewater		8.0-9.0	Batch reactor connected with MFC	-	79-82	Rod-shaped crystals (18-55um)	Ichihashi and Hirooka (2012)

1.3.2 Struvite: Heavy Metals and Pathogens

Heavy metals (HM) and pathogenic input associated with the application of mineral P and fertiliser generated through livestock manures could be minimised by applying the Struvite precipitation process to a variation of waste streams. For example, Liu et al (2011) found the Struvite precipitation process to reduce Na^+ , K^+ , Zn^+ Cu^{2+} and Ca^{2+} content in swine manure by 61%, 48%, 43%, 37% and 60% respectively, producing a products with HM content much lower than legal limits. While a study focusing on performance optimisation of Struvite recovery from layer manure demonstrated trace HM content and a reduction of all coliforms but 70% (Muhmood et al., 2018). Numerous other studies agree with these findings, with significantly lower quantities of HM and pathogens reported in Struvite when compared with Rock Phosphate-based fertiliser and animal-derived manures (Moller et al., 2018). The Struvite recovery process is an efficient means by which waste streams can be transformed to a higher level of purity, minimising the negative environmental and social consequences associated with fertiliser production and applications. Struvite also possesses qualities as a “slow-release fertiliser”, providing additional environmental benefit of the product.

1.3.3 Struvite: Run-off, Leaching and Solubility

Struvite's low solubility in water (Rahman et al., 2014; Talboys et al., 2016) decreases the potential for nutrient run-off in comparison with more soluble fertilisers (Bastida et al., 2019), thereby reducing the potential for fluvial nutrient enrichment (Muster et al., 2013; Latifian et al., 2012; Kataki et al., 2016). However, the extent to nutrient release is heavily dependent on the form and conditions (e.g. acidic, alkali soil types; powdered or granular form) in which a fertiliser is applied (Table 3). Around 50% of P in Struvite in granular form is soluble in citric acid, whereas only 1-2% will dissolve in water (Cabeza et al., 2011; Johnston and Richards, 2003; Kern et al., 2008) and 100% of Struvite is soluble in organic acids (Antonini et al., 2012; Cabeza et al., 2011; Kern et al., 2008; Massey et al., 2009). Everaert et al. (2018) demonstrated greater leaching for MAP applied fertiliser than for granular Struvite in a rainfall runoff experiment. Rahman et al. (2011) reported P leaching to be slow in both Struvite and the DAP treated soils, although N-leaching was considerably less with the MAP crystals. Earlier studies into Struvite properties demonstrated powdered Struvite could leach over 95% of N within 40 days whereas larger granules would release enough nutrients to fertilise crops for over a year in experimental conditions (Lunt et al., 1964). The solubility of Struvite is increased when applied in powdered form due to the increased surface area and therefore, the higher potential for reactivity within the soil profile.

Table 3. A variation of leaching rates of P and N using differing fertiliser sources with a variety of soil types and methods adapted from (Ahmed et al., 2018)

Leaching solution	Soil type	Fertiliser application rates	Leaching method	Duration	P Source	Source	P leaching rates	N leaching rates	Reference
Distilled water	Acidic	30-40 kg / ha	Column	42 days	Struvite	Swine wastewater	0.03-0.37%	1.93-2.05%	Rahman et al. (2011)
					FSP (Fused Superphosphate)	Commercial	0.23-0.25%	7.82-6.47%	
Distilled water	Water bottle	1g/100ml DW	Dialysis bag	105 days	Struvite	Anaerobic Digestion effluent/ synthetic	8.4-26.7%	9.6-23.2%	Latifian et al. (2012)
					NPK	Commercial	72%	57%	
Tap water	Sandy loam	200 mg N / kg soil	Column	48 hr	Struvite	Semiconductor wastewater	-	32.9%	Ryu, Lim, Kim, et al. (2012)
					Complex Fertiliser	Commercial	-	17.6%	

Tap water	Sandy loam	100 mg N / kg soil	Column	40 hr	Struvite	Swine wastewater	-	9.8 mg N / L in 2 hr	Ryu and Lee (2016)
					Complex Fertiliser	Commercial	-	12.0 mg N / L in 2 hr	
Effluent/distilled water	Joel soil (pH 5.2)	80 mg / kg soil 10 kg / ha	Column	-	Effluent containing Struvite	Swine wastewater	62% TP	-	Weaver and Ritchie (1994)
					Superphosphate	Commercial	92% TP	-	
Effluent/distilled water	Coolup (pH 5.5)	60 mg / kg soil 80 kg / ha	Column	-	Effluent containing Struvite	Swine wastewater	46% TP	-	Weaver and Ritchie (1994)
					Superphosphate	Commercial	76% TP	-	
Effluent/distilled water	Balkuling (pH 6.2)	80 mg / kg soil 640 kg / ha	Column	-	Effluent containing Struvite	Swine wastewater	2% TP	-	Weaver and Ritchie (1994)
					Superphosphate	Commercial	7% TP	-	

Water	Sandy loam	100 mg / column	Column (with normal and strong aeration)	200 days	Struvite + K	Synthetic	75.4% (2.3% in 1 day)	73% (3.4% in 1 day)	Rothbaum and Rohde (1976)
					Ammophoska (NPK)	Commercial	86.34% (75.6% in 1 day)	100% (100% in 1 day)	
					Potassium dihydrogen phosphate	Commercial	81.1% (62% in 1 day)	-	
					Ammonium sulphate	Commercial	-	94.3 % (88.5% in 1 day)	
Water	Sandy loam	100 mg / column	Column	85 days	Struvite + K	Synthetic	33.6% (2.5% in 3 days)	-	Rothbaum and Rohde (1976)
					Superphosphate	Commercial	93.7% (82% in 3 days)	-	
Tap water (4 meq./L salts)	Yolo loam	5.4 g struvite /	Column	100 days	Powdered Struvite	Mixed in soil	-	95%	Lunt et al., 1964
						On soil surface	-	53%	

	(0.1% krilium treated)	column (375 mg N)			Granular Struvite	Mixed in soil	-	64%	
						On soil surface	-	28%	

1.3.4 Struvite Dissolution and Form

The granular form of struvite largely determines P solubility and therefore, availability for plant uptake throughout the growing season. A study comparing differing Struvite forms with conventional MAP fertiliser demonstrated that when ground and mixed thoroughly within the soil profile, both fertilisers exhibited similar agronomic effectiveness (Degryse et al., 2017). However, in granular form, the rate of dissolution was dependant on soil pH properties, with figures varying in 0.03 mg d^{-1} to 0.43 mg d^{-1} in alkaline and acidic soils over a period of 6 weeks (Degryse et al., 2017). Talboys et al. (2016) also showed Struvite to be “slow release” in granular form (2.4mm) with initial dissolution being 9% in 6 days whereas 26% had dissolved by the time of harvesting (90 days): release of P increased with decreasing pH. Using 4D X-ray tomography, a 3.6 mm Struvite granule dissolved by 30% within a 14 week incubation period in the presence of plant roots (Ahmed et al., 2016). The surface area of any substance is likely to determine its reactivity with the surrounding compounds, impacting dissolutions rates (Briese et al., 2017). Many of the studies regarding struvite have assessed its effectiveness on plant growth when applied in powdered form, mixed extensively throughout the soil negating from the primary use of the fertiliser as “slow release” (Achat et al. 2014b; Bonvin et al. 2015).

1.3.5 Ammonium and Magnesium in Struvite

The addition of NH_4^+ and Mg^{2+} has been shown to provide synergistic benefits to P uptake in plants, offering an alternative explanation as to why Struvite may be a more effective fertiliser when compared with conventional types (Hauer-Jákli & Tränkner, 2019; Yao et al., 2011). In Maize, the uptake of P and S, as along with micronutrients, was shown to increase when NH_4^+ was added to plants in a low NO_3^- hydroponic growth solution (George et al., 2016). Other studies report similar findings with regards to increase in P and S uptake with NH_4^+ addition (Flores et al., 2001; Kirkby, 1968). When NH_4^+ and PR were added in combination, greater yields of maize were observed in comparison with the other treatment containing PR and NO_3^- based fertilisers (Aziz et al., 2006). González-Ponce et al. (2009) attributed the higher yields expressed by Struvite treated crops to the increase in Mg content while equally (Antonini et al., 2012) showed much greater Mg uptake in Struvite plots. Mg is essential for photosynthesis and henceforth, plant growth, being an element that makes up the Chlorophyll molecule (Mengel and Kirkby, 2004) therefore, the additional input for the observation of increased yields witnessed upon struvite application. The nitrification of NH_4^+ has been shown to decrease soil pH in a variety of crops (Anthonp et al., 1987; Bekele & Hofner, 1993; Thomson et al., 1993), resulting in higher levels of acidity pertaining to greater mobility of P In soil (Vogel

et al., 2018), being one possible explanation. When a nitrification inhibitor was added with struvite granules to a pot experiment, significant decreases in dry matter yield were seen (Terman & Taylor, 1965). Some studies, have report the leaching of nutrients, including P, to be largely governed by rates of nitrification process within the soil profile (N. Ahmed et al., 2018) demonstrating the significance of the process in terms of nutrient mobility and therefore, availability for plant uptake. Struvite does not leach readily and the rate of the aforementioned processes are directly related to the size of the struvite granule used in the experiment (S. Ahmed et al., 2016; Talboys et al., 2016).

1.3.6 Plant-uptake of Struvite: Agronomic Efficiency

Struvite has been shown to supply sufficient nutrients for plant growth, as well as good P-use efficiency in a variety of contexts, although it is evident from numerous studies that the time struvite has to dissolve in the soil profile greatly effects results (Tables 4 & 5). A pot experiment showed *Lolium Perenne* exhibited substantial increases in dry matter (DM) yield of 244% in an 82 day experiment and no difference in 51 days upon addition of urine derived Struvite fertilisers (Tables 4 & 5) (Antonini et al., 2012) in comparison to the control. Yetilmezsoy & Sapci-Zengin (2009) demonstrated DM yield increases of 54%, 76% and 60% when tested on *Portulaca oleracea*, *Lepidum sativum* and *Lolium perenne* in sandy soils and even greater yields in garden soils (Tables 4 & 5). In acidic and neutral soils Cabeza et al. (2011) showed struvite to be as effective as triple superphosphate (TSP) in terms of crop yield. These, and other similar studies, have shown struvite to be as effective, if not more, than conventional fertilisers (Ahmed et al., 2016). Using a meta-analysis, Huygens and Saveyn (2018) was able to demonstrate the P-use efficiency of struvite (among other recoverable fertilisers) under a variety of soil types/conditions across Europe. Dry matter yield and P – use efficiency of struvite was recorded at 0.99 and 1.05, respectively, where values >1 showed P fertilisers to be more effective than PR-based synthetic fertiliser. These data accounted for differences in soil pH; texture; feedstock; plant groups; form of fertiliser application; assessment time and experimental design and setting (Huygens & Saveyn, 2018). However, as mentioned previously, the effectiveness of struvite can be dependent on multiple interrelated factors relating to solubility within the soil rhizosphere and the plant to which the fertiliser has been applied.

1.3.8 P-use efficiency in Turfgrass

As a slow-release fertiliser, Struvite is considered more desirable for use with turf grass rather than a more soluble, conventional types of mineral P fertiliser (Rahman et al., 2011), because a slower rate of nutrient release and lower leaching rate would enable plants to access nutrients throughout plant-growing season without over-loading. Looking at P availability from different precipitated phosphates, Johnston and Richards (2003) found struvites' to be as effective as monocalcium phosphates in ryegrass. Two studies from differing authors, both comparing urine-derived Struvite with conventional fertilisers, evaluated struvite to be at least as effective in terms providing sufficient P supply to plants as conventional fertilisers (Antonini et al., 2012; Bonvin et al., 2015) (Table 4 and 5). Equally, Johnston and Richards (2003), found ryegrass yields to be similar to mineral fertilisers, while Petrovic and Barlow (2011) measured extensive benefit of P on turfgrass in comparison with DAP with significant root biomass increases with Kentucky Bluegrass species. 70-80 % of standard perennial forages root mass occurs in the top 1 m at 0-20 cm depth, while root length is largely determine by subsoil physical conditions (Wedderburn et al., 2010), but is generally characterised as dense and fibrous with thin lateral extensions and prominent AMF associations, which are similar to other species of turfgrass Perennial Ryegrass (*Lolium Perenne*) is found in temperate regions for use as standard fodder (Humphreys, 2005), whilst also being used for games pitches, lawns and landscaping (Casler and Duncan et al., 2003). Of total root biomass for grasses such as these, 70–90% of total root biomass of perennial forages to a 1 m depth occurs at the 0–20 cm layer (Steen, 1989). Bollinder et al. (2002). If struvite were applied in turfgrass species, such as *Lolium Perenne*, it may be of considerable economic importance and ecological value.

Table 4. Experiments assessing the effectiveness of struvite (for use with Table 5), adapted from (Ahmed et al., 2018)

No	Crop/plant	Struvite source	Type of experiment	Time (days)	Soil type/ growth medium	Soil pH	Chemical Fertiliser Type	Form (granular VS powdered) and application process*
1	Broad bean plant	Industrial effluents	Pot	45	Sandy clay	8.0	NPK	The struvite fertilizer was applied at a rate of 2% prior to sowing by mixing it with soil.
2	Canola	Swine Manure	Pot	46	Sandy loam	7.7	MAP/polymer coated MAP	The solids had been dried and finely ground and contained 5.6% elemental P and 6.3% total nitrogen (TN) along with approximately 50% organic matter.

3	Chinese Cabbage	Semiconductor wastewater	Pot Pot	32	Sandy loam	5.3	Complex fertiliser	Assumed resultant precipitate
4	Chinese Chard	Landfill leachate	Pot	33	Sandy clay	7.2	Ammonium nitrate, KCl, calcium phosphate	Assumed resultant precipitate
5	Corn	Synthetic	Field	42	Silt loam	-	DAP	-
	Corn	Corn fibre processing			Silt loam, loam silt clay	5.5-6.4	Triple superphosphate	Granulated
6	Flowering Cabbage	Landfill leachate	Pot	33	Sandy clay	6.2	Ammonium nitrate, KCl, Calcium Phosphate	Assumed resultant precipitate
7	Garden Cress	Anaerobically pretreated poultry manure wastewater	Pot	-	Sand	6.5	-	Precipitate + mixing
8	Gram	Synthetic	Pot	45	Sandy loam, clay and loamy	5.3-8.2	Single superphosphate, DAP	-

							and ammonium polyphosphate	
9	Lettuce	Anaerobic digester supernatant	Pot	-	Loamy sand	-	Single superphosphate	Thoroughly mixed (2 mm sieve) Assumed resultant precipitate
	Lettuce	Semiconductor wastewater	Pot	63	Sandy loam	5.3	Complex fertiliser	
	Lettuce	Swine manure	Pot	63	Sandy loam	5.3	Complex fertiliser	
	Lettuce	Swine manure	Amber glass container	56	Loamy sand	8.1	DAP	
10	Maize	Anaerobic effluent from yeast industry	Pot	54	Light alkali nutrient poor soil	8.4	Ammonium nitrate, & potassium phosphate	-
	Maize	Dairy industry wastewater	Pot	50	Isparta soil	7.9	Ammonium nitrate, & potassium dihydrogen phosphate	Assumed resultant precipitate

	Maize	Wastewater	Pot	40	Sandy soil	5.0	Triple superphosphate	-
	Maize	Wastewater	Pot	40	Rheinsand	7.9	Triple superphosphate	-
	Maize	Swine manure	Pot	56	Sandy loam	6.0	Fused superphosphate and urea	Fertiliser and struvite were applied according to the surface area of each plot
	Maize	Black water/ human urine	Field	135	Sandy loam	4.5	Triple superphosphate & Ammonium nitrate	Granular and precipitate
	Maize	Digested sludge	Pot	53	Siliceous sand	-	-	-
	Maize	Human urine	Pot	51-82	Sandy loam	-	Mineral Phosphate Fertiliser	-

	Maize	Sewage	Pot	80	Loamy soils	5.6 & 6.8	Triple superphosphate	All test materials were thoroughly mixed with 6 kg of air-dried, 2 mm-sieved soil and 3 kg of sand in double walled plant culture pots
	Maize	Wastewater	Pot	2 years	Loamy soils	4.7 & 6.6	Triple superphosphate & PR	-
11	Oil seed, rapeseed, winter barley	Sewage	Field	-	Loamy soils	5.6 & 7.1	Triple superphosphate	-
	Potato	Synthetic	Field	-	Wick series	-	Triple superphosphate	-
12	Purslane	Anaerobically pretreated poultry manure wastewater	Pot	-	Sand	6.47	-	Precipitate + mixing

13	Rapeseed	Urine	Pot	42	Cinnamon soil	7.3	Ca superphosphate (urea and potassium sulphate supplementation)	Dried and lightly ground to match CSP particle size
	Rapeseed	Urine	Pot	42	Paddy soil			
14	Rocket, Dill, Fennel, Parsley	Anaerobically pretreated poultry manure wastewater	Pot	15	Garden soil	5.5-6.0	Ammonium nitrate, & potassium dihydrogen phosphate	-
15	Ryegrass	Synthetic Urine (¹⁵ N & ³³ P labelled)	Pot	72	Sandy loam	5.4	Ammonium nitrate, & potassium dihydrogen phosphate	-
	Ryegrass	Anaerobic digester supernatant	Pot	115	Loamy sand	5.7	Single superphosphate	thoroughly mixed, 2 mm sieve
	Ryegrass	Synthetic, sewage, corn steep, dye industry, veal manure	Pot	100	Sandy loam and sandy clay loam	6.6 & 7.1	Monocalcium phosphate	-
	Ryegrass	Anaerobically pretreated poultry	Pot	-	Garden soil/sand	7.5 & 6.5	-	Precipitate + mixing

		manure wastewater						
	Ryegrass	Human Urine	Pot	76-82	Sandy soil	-	Mineral Phosphate fertiliser	All test materials were thoroughly mixed with 6 kg of air-dried, 2 mm- sieved soil and 3 kg of sand in double walled plant culture pots
	Ryegrass	Swine manure	Pot	>60	Brunisol	6.5	Triple superphosphate	-
16	Soybean	Corn fibre processing	Field	-	Silt loam, loam, silt clay	5.5-6.4	Triple superphosphate	Granular
17	Tomato	Anaerobic effluent from yeast industry	Pot	54	Light alkaline nutrient poor soil	8.4	Ammonium nitrate, & potassium phosphate	-

18	Water Spinach	Landfill leachate	Pot	33	Sandy clay	8.2	Ammonium nitrate, KCl, calcium phosphate	Assumed resultant precipitate
19	Water convolvulus	Landfill leachate	Pot	33	Sandy clay	9.2	Ammonium nitrate, KCl, calcium phosphate	Assumed resultant precipitate
20	Wheat and Buckwheat seedlings	Wastewater	Pot	30	Sandy soil	6.0	DAP	Granular
21	Wheat	Wastewater	Pot	90	Sandy soil	6.0	Triple superphosphate	Granular
	Wheat	Dairy wastewater		-	Fine-loamy	6.5	Triple superphosphate & rock	Fertilizer treatments were mixed thoroughly Fertilizer treatments were mixed thoroughly
	Wheat	Dairy wastewater		-	Fine-loamy	7.6	Triple superphosphate & PR	-
	Wheat	Digested sludge		53	Siliceous sand	-	-	-

	Wheat	Human urine		21	Quartz (submerged in liquid)	7.4	NPK	-
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* Taken from methods section of each individual experiment. Some articles were not detailed enough to decipher exact methodology

Table 5: Application Rate (NPK), Biomass Yields and Nutrient Uptake in Table 4 experiments. Adapted from (Ahmed et al., 2018)

No	N	P	K	Growth and biomass yield	Nutrient uptake	Reference
1	20 mg / kg	50 mg / kg	40 mg / kg	54% fresh yield and 58 % DM yield increase	Higher NPL uptake in plants and beans by Struvite treatment	(El Diwani et al., 2007)
2	100 mg / kg	9.5-47.5 mg / pot	100 mg / kg	28% lower biomass yield and similar at lower application rates	18% lower P uptake at 47.5 mg P / pot although similar P uptake at 9.5 mg P / Pot	(Ackerman et al., 2013)
3	110 kg N / ha	-	-	Lower leaf length, fresh yield and dry yield	Higher P uptake but lower uptake of other nutrients (N, K, Ca & Mg)	(Ryu et al., 2012)
4	>70 mg / kg`	>60 – 80 mg / kg	>100-150 mg / kg	No difference in growth rate	172%, 105% and 2,066% increase in P, Mg, and Mo; decrease in Ca,	(Li & Zhao, 2003)

					Fe, Mn and Co uptake	
5	-	36 mg P / kg	-	Statistically similar to 100 mg DAP application in DM yield and superior to 50 mg DAP	Equivalent to 42 mg DAP in P uptake (117% uptake efficiency)	Barak and Stafford., 2006
		12- 120 kg / ha		No significant difference in biomass yield	4-21% higher P uptake depending on application rate	(Thompson, 2013)
6	>70 mg / kg	>60-80 mg / kg	>100 – 150 mg / kg	No significant difference in growth rate	76%, 105% & 1,087% increase in P, Mg and Mo, decrease in Ca, Fe, Mn and Co uptake	(Li & Zhao, 2003)
7	150 mg / l	50 mg / l	100 mg /l	18%, 25% and 115% increase in growth rate, fresh yield and dry yield	-	(Yetilmezsoy & Sapci-Zengin, 2009)

				compared with control		
8	9 mg / kg (urea	6 – 12 mg / kg	8 mg / kg KCl	Higher DM yield	Higher P uptake	(Ghosh et al., 1996)
9	55 mg / kg	4-20 mg / kg	45 mg / kg	Significantly higher yields	N, P, K, Ca and Mg uptake better than single superphosphate and increased with higher dose	(Ricardo et al., 2009)
	100 mg N / ha	-	-	High leaf length, fresh yield and dry yield	Higher uptake of N, P, K and Mg with lower Ca compared with commercial fertilisers	(Ryu et al., 2012)
	110 kg N / ha	-	-	179 %, 200 % and 275 % increase in height, fresh yield and dry yield, respectively	140% P, 43% N, 15% L and 81% Mg uptake was increased with lower Ca uptake	(Ryu & Lee, 2016)

	600 mg N / ha	300 mg P / kg	-	No significant difference in DM yield	No significant difference in N & P uptake higher Mg and Na uptake and lower Ca and K uptake	(Cerrillo et al., 2015)
10	100 mg / kg soil	308 mg / kg soil	34 mg / kg soil	No significant difference fresh yield and DM	Higher N, M and Mg uptake with 2-4 x dose	(Uysal et al., 2014)
	100 mg / kg soil	343	22	No significant difference in fresh and dry weight	Higher P uptake with no significant difference on N	(Uysal & Kuru, 2015)
	14 kg / ha	80 kg / ha	3 kg / ha	Significantly higher length, fresh yield and DM yield	Significantly higher length and biomass yield and lower P uptake	(Vaneeckhaute et al., 2016)

	14 kg / ha	80 kg / ha	3 kg / ha	No significant difference in biomass yield	No significant difference	Vaneeckhaute et al., 2016)
	-	30–40 kg / ha	57 kg / ha	22-22.4% no statistically significant biomass yield	Threefold reduction in N ₂ O emissions	(Y. H. Liu, Rahman, et al., 2011)
	80 – 100 kg / ha	30 – 200 kg / ha	-	No significant differences in yield	No significant difference in P uptake	(Gell et al., 2011)
	-	50 mg P/pot	-	-	85.9% P uptake	Kern et al., 2008
	1,530 mg / pot	24.4 mg P / kg	-	Significantly higher (216% and 244%) dry yield in experiment A (82 days); no significant difference in	Significantly higher P uptake (281%) in experiment A; no significant difference in experiment b	(Antonini et al., 2012)

				experiment B (51 days)		
	500 mg N / pot	60 mg P / kg	1000 mg K / pot	No significant difference in grain yield	Similar P uptake in pH 5.6 and slightly higher in pH 6.8	Perez et al., 2009
	-	60 mg P / kg	-	-	Comparable P uptake and performance at both soil pH	Cabeza et al., 2011
11	500 mg N / pot	60 mg P / kg	1000 mg K / pot	No significant differences in grain yield	No significant difference in P uptake for (pH 5.6) but higher P uptake for pH 7.1	Perez et al., 2009

	-	-	-	Significantly higher dry yield at initiation but lower commercial yield	Significantly higher P, N and mg uptake at initiation but lower K uptake	Hammond et al., 2007
12	150 mg / l	50 mg / l	100 mg / l	21%, 150% and 207% increase in growth rate, fresh yield and dry yield compared with control	-	(Yetilmezsoy & Sapci-Zengin, 2009)
13	200 mg / kg	200 mg / kg	100 mg / kg	No significant difference in biomass; higher yield with acid water	No significant difference in P uptake	(X. Liu et al., 2016)
	200 mg / kg	200 mg / kg	100 mg / kg	Significantly higher biomass with struvite and struvite in acid water	Significantly higher P uptake with Struvite only	(X. Liu et al., 2016)

14	150 mg / l	50 mg / l	100 mg / l	22-51%, 141-405% and 191-488 & higher growth, fresh yield and dry yield	-	(Yetilmezsoy et al., 2013)
15	23 mg N / kg	50 mg P / kg	-	Slightly higher DM yield without significant difference	Similar P and N uptake	(Bonvin et al., 2015)
	214 mg / kg	9 – 44 mg / kg	36 mg / kg	No significant difference in dry yield	Increase in struvite dose, P and Mg uptake increase; no effect on N, L and Ca	(Plaza et al., 2007)

	0.15 g N / Pot	0.087 g P / pot	0.08 g K / pot	Similar DM yield	Similar P uptake	Johnston and Richards., 2003
	150mg / l	100 mg / l	100 mg / l	156%, 257% and 402 % increase in garden soil and 54%, 76% and 60% increase in sand for growth, fresh yield and dry yield compared with control	-	Yetilmezsoy & Sapci-Zengin, 2009)
	1,530 mg / pot	24.4 mg P / kg	-	Significantly higher (131% and 134%) biomass yield in experiment A (82 days); no significant	Significantly higher P uptake (211%) in experiment A (82 days); no significant	(Antonini et al., 2012)

				difference in (76 days)	difference in 76 days	
	80 mg N / kg	50 mg P / kg	60 mg K / kg	No significant difference in DM yield	No significant difference in P uptake	(Achat et al., 2014)
16	-	12 – 120 kg / ha	-	No significant difference in biomass yield	No significant difference in P uptake	(Thompson, 2013)
17	100 mg / kg soil	308 mg / kg soil	34 mg /kg soil	Slightly lower fresh and dry yield with no significance	Higher N, P and Mg uptake in 2-4x dose but not significant	(Uysal et al., 2014)

18	>70 mg / kg	>60-80 mg / kg	>100-150 mg / kg	No significant difference in growth rate	98-186% P, 145-209% Mg, and 523-1,482% Mo increase; decrease in Ca, Fe, Mn and Co	(Li & Zhao, 2003)
19	>70 mg / kg	>60-80 mg / kg	>100-150 mg / kg	No significant difference in growth rate	102%, 115% and 779% increase in P, Mg and Mo; decrease in Ca, Fe, Mn and Co uptake	(Li & Zhao, 2003)
20	-	35 kg P / ha	-	-	70% and 15% less P uptake in wheat and buckwheat	(Talboys et al., 2016)
21	-	35 kg P / ha	-	No significant difference in grain yield	No significant difference in P uptake and recovery	(Talboys et al., 2016)

	30 mg / l, 0.027 M NH ₄ NO ₃	45-90 kg P ₂ O ₅ / pot	-	No significant difference in DM yield	No significant difference in P uptake	(Massey et al., 2009)
	-	45-90 kg P ₂ O ₅ / pot	-	Higher DM yield than control no significant difference with TSP	No significant difference but lower P uptake than control	(Massey et al., 2009)
	31 mg / l, 0.027 M NH ₄ NO ₃	50 mg P / pot	-	-	66.7% P uptake	(Kern et al., 2008)
	-	40 mg p / pot	-	Lower height and dry weight	Difficulty in nutrients uptake due to low solubility	(Ganrot et al., 2007)

1.4 Potential Limitations with Struvite Application

1.4.1 Performance in Calcareous soils

Struvite has been shown to perform well in both acidic and calcareous soils (Cabeza et al., 2011; Massey et al., 2009; Ricardo et al., 2009). However, when accounting for the greater solubility of Struvite under slightly acidic conditions, theoretically, the fertiliser should be less effective for alkaline conditions. Ackerman et al. (2013) demonstrated this in canola where the addition of MAP fertilisers produced higher biomass yields per unit of P uptake of plants in comparison with struvite on alkaline soils (pH 7.7). In contrast, studies ranging from soil pH of 7.3 – 9.2, demonstrate no significant difference between plants treated with either soluble, chemical fertilisers or struvite. For example, rapeseed showed increased growth rates at pH 7.3 while treated with struvite and Maize demonstrated no significant difference in growth rates at pH 7.9 and 8.4, between soluble fertilisers and struvite (Tables 4 and 5). The research of (Möller et al., 2018) suggests that the higher reactivity (rPeff) of struvite may be responsible for its contribution to productivity in contrasting soil types, even if the fertiliser's solubility is impacted (For examples see (Achat et al., 2014; Bonvin et al., 2015)). One aspect of plant mineral nutrition that may be negatively impacted by struvite addition is micronutrient ratios.

1.4.2 Micronutrients Deficiencies and Organic Matter

Struvite has high levels of purity, and therefore, essential micro and macronutrients required for plant growth are not necessarily recovered, potentially contributing to nutrient imbalances in crops (Bonvin et al., 2015). NH_4^+ recovery can be as little as 5% (Etter et al., 2011) and essential nutrients, such as K and S, are not recaptured at all. Plaza et al. (2007) demonstrate that the balance of elements N, P and K are essential for sufficient ryegrass performance in winter, with levels of productivity due to resilient traits in harsh weather conditions (Ihtisham et al., 2018). Even though struvite uptake and efficiency is comparable to, or even higher than conventional soluble fertilisers, lower nutrient uptake such as K, Ca, Fe and Mn could be a concern, as shown in various studies (Li and Zhao, 2003; Hammond et al., 2007; Ryu, Lim, Kang et al., 2012) (Tables 4 and 5). Marsh and Pierzynski (1998) demonstrated 70% increases in plant root growth in treatments with the highest micronutrient concentrations (Zn, K and S) within banded nutrient patches. In addition, struvite is void of OM, which can contain higher rates of bioavailable macro and micronutrients. Struvite yields greater benefit when applied with organic amendments with higher levels of OM, probably contributing to more efficient plant uptake through balancing nutrient ratios in the soil profile (Bastida et al., 2019), as well

as maintaining beneficial soil physical properties. In this study, struvite alone had 7, 3 and 11 times more P, Mn and Mg in comparison with sewage sludge, even though the content of the latter contained higher amounts of all other nutrients (Bastida et al., 2019). Micronutrients have been shown to increase grain yield from some 15-50% (Malakouti, 2008), demonstrating the importance for crop productivity. Each nutrient has a differing function that contributes to plant productivity, and often contribute to P uptake from the soil, while uptake can be dependent on the ratio in which they are occurring in the growth medium (Knecht & Göransson, 2004). Although P & N have been shown as the main limiting nutrients to plant growth, deficiencies in micronutrients may contribute to the long-term depletion of resilience in crops, unless aided with ulterior forms of micronutrient application.

1.4.3 Plant growing seasons and early soluble fertiliser addition

Due to the low water solubility of struvite, as well as the variability of plant nutrient requirements and soil conditions throughout the growing seasons a “slow release” fertiliser alone may not supply adequate nutrient to ensure sufficient plant growth in the early stages (Sheil et al., 2016). Talboys et al. (2016) assessed the impacts of struvite on plant productivity throughout the growing seasons and demonstrated early stages of plant root growth were impacted. However, crop yields appeared unaffected and the overall plant recovery was 175% greater than that of TSP. Still, (Talboys et al. (2016) later recommended struvite to be effective, although the addition of a more soluble fertiliser would be useful in early growing season. Other studies assessing the solubility of struvite show the effectiveness when compared to more soluble fertilisers, is dependent on the rate of dissolution (e.g. how the Struvite it applied) and therefore may not provide adequate supply to early seedlings (Degryse et al., 2017). Studies in wheat, potato and Chinese cabbage crops have shown lower yields and less P uptake (Ackerman et al., 2013; Ganrot et al., 2007; Hammond & White, 2008; Ryu et al., 2012; Talboys et al., 2016), which may be a consequence of the lower rates of P supply in earlier growth stages. The rate of dissolution can be dependent on a number of factors as aforementioned, while the soil temperature through seasons may also have an impact on struvite solubility, a factor difficult to control and not extensively discussed (Sheil et al., 2016). Researchers have not yet identified the exact mechanisms for dissolution and therefore, the precise manner in which struvite contributes to plant growth. In the earlier stages of growth, crops exhibit higher demand for P fertiliser, as P is essential for energy transfers within plant metabolism (Grant et al., 2001), if P from fertiliser is not released in time, this may negatively impact the growth cycle and lower the likelihood the crops will be resilient to environmental changes.

1.4.4 Uncertainties in Scientific Studies

Despite the numerous studies expressing struvite as a “slow-release fertiliser”, it has been applied in a multiplicity of forms from a variation of waste streams and with differences in P characterising/quantifying techniques, making comparative assessments difficult (Table 4). For example, Bonvin et al. (2015) discussed that with the exception of Achat et al. (2014), most other studies have not differentiated between P-uptake derived by plants from the soil, and P derived from the fertiliser. This could result in an overestimation of the impact of the P fertiliser in many studies. In terms of fertiliser application, numerous studies have assessed struvite when powdered and thoroughly mixed within soil profile: commercial grade fertiliser will likely be applied in granular form with minimal mixing (Cabeza et al., 2011; Johnston and Richards 2003). Out of over 21 experiments summarised in Tables 4 and 5, only 5 used struvite in granular form. Many studies assessing the applicability of struvite compared with other fertilisers (MAP; DAP etc) tested recovery from differing waste streams and therefore used the initial, unaltered precipitate to assess P-use and efficiency in pot experiments. The precipitate form is unlikely to be the product applied to soil and therefore not indicative of struvite granular application in real life scenarios. Evidently, further research must be carried out to assess struvite effectiveness objectively.

1.5 Knowledge gaps

1.5.1 Microbial life and AMF

While microbial communities and AMF fungi play an extensive role in the cycling of all essential nutrients and thereby soil health and crop productivity, little research has been undertaken to determine how struvite granules impact these biological communities (Bastida et al., 2019). Though the form of struvite used in the experiment is not stated, Bastida et al., (2019) have expressed its use had a positive effect on gram-positive and gram-negative PFLAs, with a significant effect on verrucomicrobia as well as various actinobacterial populations. Therefore, an assessment of specific granular sizes impacts on microbial communities may warrant further investigations. In a similar vein, the impacts of Struvite have been demonstrated to increase the abundance and variety of Arbuscular Mycorrhizae (Van Geel et al., 2016), however, it is yet to be demonstrated the impacts of differing granular sizes in laboratory conditions. The effects of AMF species on P uptake and root proliferation using struvite as a fertiliser treatment under controlled conditions are still unknown. Likewise, the effect on microbial communities has not been assessed at all. The current report aims to shed

light on the impacts of struvite on these biological parameters compared with conventional fertilisers.

1.5.2 Nutrient Patches and Root Proliferation

Nutrient patches, using conventional fertilisers have been shown to cause increase root proliferation compared with fertiliser applied homogenously (Bonser et al., 1996; Drew, 1975; He et al., 2003; Hodge, 2004; Jing et al., 2010; Ma et al., 2013): struvite as a treatment has not yet been compared with conventional fertilisers in this aspect of study. As demonstrated by Jing et al., 2010, in calcareous soils, plant roots proliferate in response to the local application of both ammonium and P while both crop growth and P uptake increase. The mechanisms underlying the process causing root proliferation are yet to be fully understood (Shen et al., 2011) though previous research suggests struvite applied in patches may yield differing results due to dissolution rates and molar ratio of elements, with the potential to alter root morphology and architecture (Bonser et al., 1996).

1.5.3 P Dissolution and Solubility

Struvite dissolution has been attributed to the presence of plant root exudates (Organic Acids; H^+ ions) increasing pH within rhizosphere zones (Oburger et al., 2008; Petersen & Böttger, 1991). However, the mechanisms of granular dissolution remain unclear and further study of the interactions between the nutrients in struvite, and surrounding chemistry within the rhizosphere is required. One of the more recent studies, Talboys et al. (2016), through both pot and laboratory experimentation, demonstrated increased dissolution of struvite with the addition of organic acids, although authors expressed further need to understand the mechanistic elements underpinning struvite dissolution. Degryse et al. (2017) showed Struvite in acidic soils to dissolve at a much higher rate than alkali, though plant root factors were not considered. Nitrifying bacteria may be the key component dictating struvite dissolution, as demonstrated by (Rothbaum & Rohde, 1976), where MAP leaching considerably decreased at 53 °C. (Bonvin et al., 2015), looking at plant-uptake of P and N in recycled P suggests further research must be conducted into the impacts of N within the struvite granules, as most literature focuses only on P. pH appears to be the most influential factor controlling a granule's solubility, although the effect of surrounding nutrients availability, as well as the nutrients present within struvite, on chemical parameters of the soil appears key in understanding struvite availability.

1.5.4 Micronutrient deficiency

Alterations in plant uptake and availability of most nutrients have synergistic and antagonistic effects, with one nutrient responsible for either increasing or decreasing requirements (in plants) for another (Ranade-Malvi, 2011). As commercial grade struvite is theoretically pure, the application may cause deficiencies in other elements. Antonini et al. (2012) has emphasised the necessity of extensive testing for micro and macronutrient deficiencies with studies such as (Ahmed et al., 2018) showing higher doses of struvite diminishing Calcium and Potassium uptake rates in plants. Due to the slow rate of dissolution associated with the fertiliser, the evidence would suggest it to have a lower impact on micronutrient imbalances earlier on in the growth stage than more soluble, conventional fertilisers. However, nutrient depletion at later stages of plant growth is a possibility (Talboys et al., 2016), therefore evaluations on soil micronutrients after granular struvite application represents a new avenue of research.

1.6 Summary

Following on from the as aforementioned perspective, little information is available with regards to the dynamics of struvite applied as granules: a mechanistic understanding underpinning plant uptake of P (as struvite) is lacking in current literature (Bastida et al., 2019; Bonvin et al., 2015; Degryse et al., 2017). Also, of the total experiments undertaken since 2003 (Tables 4 and 5) only 5 have demonstrated the impact of commercial grade, granular struvite (Ahmed et al., 2018) on plant growth, while very few have looked, in depth, at aspects such as root architecture and morphology; microbial communities and AMF associations; the potential for micronutrient deficiencies (Antonini et al., 2012; Ahmed et al., 2018); nutrient patches and plant root proliferation; mechanisms of struvite dissolution. This knowledge is essential in assessing struvite's agronomic effectiveness; improving application techniques for greater productivity and deciphering the both short and long-term environmental implications centred around soil chemical, physical and biological parameters.

Aims and Hypothesis

Aims and Objectives

1. Quantify the impact of P-based fertilisers on microbial activity
2. Distinguish the most effective P fertiliser form for crop growth, and the most effective methods of application

Hypothesis

1. Microbial activity is negatively affected by the application of P-based fertilisers and their application will result in a decline in microbial populations
2. The impact of P-based fertilisers on *Lolium Perenne* will decline with distance from the application site
3. Phosphorus uptake will be most efficient with traditional, soluble fertilisers
4. Phosphorus deficiency will result in root proliferation as plants mine for phosphorus further down the soil profile

Chapter 2

Microbial Activity: P Fertiliser Applications with Isotopic Radiolabelling

Experiment 1 P-based fertiliser impact on microbial activity with differing proximities to the soil particle surface using ^{14}C radio isotopic-labelling

2.1.1 Abstract

Differing forms of P-fertilisers vary in their effects on biological rhizosphere interactions. Microbial turnover is an indicator of the direct impact that P-fertiliser applications have on soil dynamics, both within the immediate vicinity of application, and at a greater proximity away from the soil particle surface. Observations of microbial activity provide evidence of the effects of P-based fertilisers on potential toxicity effects through observations on C-turnover. In this study, we observe the response of soil microbial communities to the application of differing concentrations and forms (granular, soluble) of P-based fertilisers to soil particulate surfaces, to gauge how higher concentrations may interfere with biological process. Using ^{14}C labelled glucose, we studied the mineralisation of C substances from agricultural soil over a 7-day period. A higher level of mineralisation to the control was found in concentrations as follows: $50 > 100 > 25 > 12.5$ (%) of maximum solubility, with no significant effect ($P > 0.05$) observed for concentrations under 12.5 %. With one exception, DKP, traditional fertilisers cumulative respiration rate from the applied ^{14}C – glucose ranged from 30% - 90 % in comparison with the novel, granular struvite ranging from just under 25 – 30 %. When comparing 50% concentrations to granular fertilisers, the following trends in mineralisation rates were observed - $\text{DAP} > \text{DKP} > \text{MAP} > \text{DSP} > \text{MKP} > \text{DAPC} > \text{DAPG} > \text{SC} > \text{SG} > \text{Control}$ - demonstrating traditional, highly soluble fertilisers are toxic to microbial populations closer to the soil particles.

Keywords: glucose; $^{14}\text{CO}_2$; P-Use Efficiency, radio isotopes; microbial activity

2.1.2 Introduction

A multiplicity of P-based fertilisers exist (monopotassium phosphate; diammonium phosphate, monoammonium phosphate, dipotassium phosphate, struvite) and have been used globally on a wide range of crop and soil types to maximise yields and positively impact plant health and resilience to changing environmental conditions. However, their immediate impact on elemental components of edaphic systems, such as fluxes throughout Carbon pools, are only partially understood due to the plethora of complex interactions involved in soil processes. Through processes of respiration and mineralisation (Figure 7) microbes control aspects of terrestrial carbon dynamics, determining fundamental characteristics of the health and resilience of the soil system. While microbial process have significant effects on C-cycling, the plant species, soil type and organic matter constitution will also determine the type and abundance of bacterial communities present and therefore, the specific functional traits and of microbial life elucidated upon nutrient addition (Campbell et al., 2010). The direct effect of increasing nutrients supplies, and indirect impacts of pH alterations by nutrient addition, are major contributing factors to microbial activity changes (Zhang et al., 2017). As a key pool of carbon, their activities act as indicators to help decipher the soil's complexities, with variations in heterotrophic respiration indicative of perturbations (Hogberg et al., 2001) in plant-soil dynamics (Hees et al., 2005). As well as aiding soil and plant health, P-fertiliser application can also inhibit activities in the soil due to its potential toxicity to soil biota (Ruiz et al., 2020) and can differ in both long and short-term impacts to the system as a whole.

Nutrient addition to soil systems has been shown to cause a shift in microbial community composition in both short- and long-term studies, linking above ground biomass and nutrient cycling to shifts in microbial activities (Zhang et al., 2017). Using a variety of methods in a diversity of ecosystems, experiments have been able to demonstrate these shifts quantitatively. In a laboratory experiment, using high through put sequencing of marker genes, and sequences metagenomics, (Leff et al., 2015) were able to demonstrate population shifts towards specific taxonomic groups (and functional traits) throughout 25 grassland sites across the globe. Phosphorus addition expressed marginal effect on bacterial biodiversity (0.5%). Across all sites, bacterial community composition was significantly affected as certain taxonomic groups grew in dominance, while others decreased. After witnessing significant N and C losses due to long-term fertilisation, a field experiment comparing both long (25 years of annual nutrient addition) and short term (6 weeks) fertilisation impacts within arctic tundra,

showed a decrease in abundance of microbes and substantial changes in community structures (Campbell et al., 2010). Campbell et al., 2010 linked these shifts to alterations in both N and C, as well as plant species changes. The study used 16S rRNA pyrosequencing, along with physiological profiling. A study that also looked at microbial abundance and transitioning under nutrient addition (Jian Li et al., 2015) used PFLA analysis to show an increase in fungi/bacteria ratios after 3 years of P fertiliser addition in a field experiment in a secondary tropical forest in China. Above-ground, plant species type shifts and/or biodiversity losses, as well as significant losses of both N and C within the soil systems (Suding et al., 2005), are invariably subject to, whilst also having significant impacts on, microbial community structure and function. The implicit interconnectedness of each systemic component requires that a shift in nutrient availability will alter microbial activities, in turn, further effecting nutrient cycle and therefore, nutrient availability and the characteristics of above and below-ground biomass combined having positive feedback mechanisms. A crucial element of nutrient availability is soil type and its chemical composition.

Nutrient addition has an indirect effect on soil systems by altering the soil pH, impacting the chemical species found within the soil (Aciego Pietri & Brookes, 2008b) and shifting the type of microbes' present, shaping community structure, and henceforth regulating activity. In a trans-continental study, Lauber et al., 2009 uses pyrosequencing to assess the correlation and causation between pH impacts and microbial communities. Key species of bacteria; *Acetobacter*, *Actinobacteria* and *Bacteroidetes*, were demonstrated to characterise changes in pH, acceding in prominence upon condition alterations. The relationship between biomass C and soil pH has been shown to be statistically significant, while CO₂ evolution was seen to increase with pH (Aciego Pietri & Brookes, 2008a). (Cho et al., 2016) suggests that pH is not the only factor effecting biodiversity and abundance of microbes, having found that bacteria still grew at the same rate, even in suboptimal pH conditions. Organic matter mineralisation can cease upon the alteration of pH to either extremely alkaline or acidic soil (J. M. Lynch, 1995).

The application of N and P have a significant effect on nutrient cycling in ecosystems and therefore, the capacity to modify global C stocks (Jianwei Li et al., 2019). However, there is variability in reports on the impact on Carbon Use Efficiency (CUE) (the ratio of Carbon allocated to growth) a key indicator of the nutrient cycling functioning and the microbial activity inherent within c-cycling systems (Bradford & Crowther, 2013). Across 6 grassland site (Africa, USA and UK), demonstrated no significant difference in CUE upon P addition (Widdig et al., 2020). However, reductions in soil respiration were witnessed upon N addition alone (Spohn et al., 2016; Widdig et al., 2020). This evidence stands in contrast to predictive models, which suggest a change would occur with microbial inputs (Eliasson & Ågren, 2011; Manzoni et al.,

2017). Studies such as (Neufeld and Mohn, 2005; Fierer and Jackson, 2006; Jangidet al., 2008), support the evidence, also indicate the importance of mineralizable carbon as a prominent control of microbial biomass activity. Stable microbial CUE implies sufficient C storage and no net Carbon loss, suggesting healthy microbial biomass, with the capacity to maintain population stability, and potentially, further store Carbon (Kallenbach et al., 2019): an efficient system. The inconsistency of results means that effects of nutrients additions on CUE are still unclear, especially for P containing fertiliser compounds.

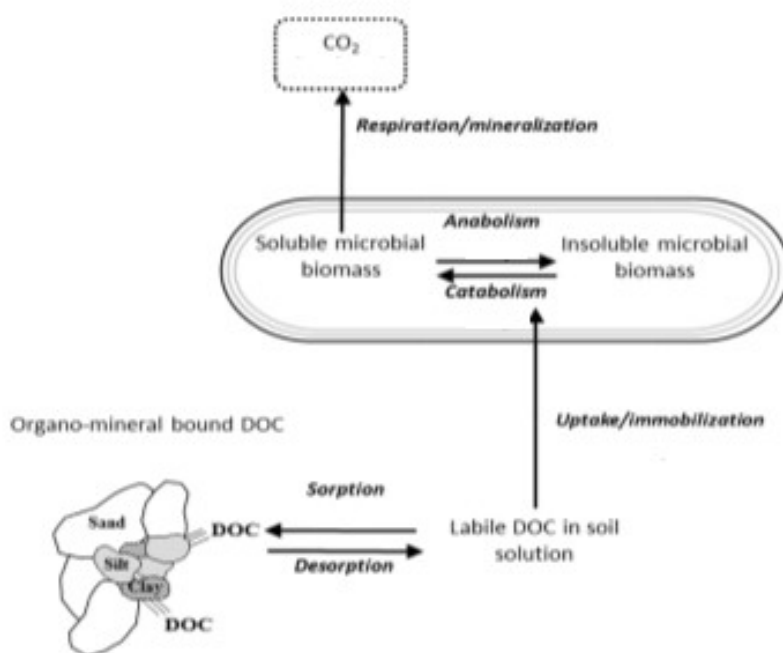


Figure 7. Used to illustrate basic microbial C pools and processes involved with transfers between them, adapted from (Glanville et al., 2016)

Using ^{14}C radio isotope labelling, this novel study aims to assess the impact of nutrient addition, and the potential for causing microbial dead zones at highest concentrations, closest to the soil particulate surface to demonstrating population losses. Understanding the effect of numerous fertiliser types on microbial C fluxes gives insight into the most efficient means of application of nutrients and promotes further understanding of the effects of commonly used P-based fertilisers on microbial populations and henceforth, the overall structure of soil systems.

2.1.3 Aims and Objectives:

1. Determine the temporal ^{14}C production post P application for a variety of P-based fertilisers
2. To show the theoretical impact of P-based fertilisers at differing proximities away from the soil particle surface.

2.1.4 Hypothesis

1. Higher Concentrations of soluble fertiliser will negatively impact the activity of microbial populations
2. Carbon Use Efficiency will decrease upon the application of traditional P-based Fertilisers
3. The application of struvite, granular fertilisers will have no effect upon microbial activity

2.1.5 Methods

Agricultural soils were collected from Henfaes Research Centre, Abergwyngregan (531140 N, 41010 W), 21.2.2020. The annual rainfall at the location is 1250 mm while soil temperature at sampling depths averages at 11 °C. The soil is classified as a Eutric Cambisol (Hill et al., 2008). 0.6 g of 2mm sieved, field moist soil (refrigerated at 4 ° c) was added to 1 mm thick and 1 cm diameter cap and placed in a sterilised 50 ml test tube. 100 µl of P-salts (KH_2PO_4 (DKP); K_2HPO_4 (MKP); $\text{NH}_4\text{H}_2\text{PO}_4$ (MAP); $\text{HNa}_2\text{O}_3\text{P} \cdot 5\text{H}_2\text{O}$ (DSP); $(\text{NH}_4)_2\text{HPO}_4$ (DAP); $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ (TSP)) were added in a dropwise fashion at the following percentages of maximum solubility 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.0975 (%) (Figure 8). Granular fertilisers ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ (DSP), $(\text{NH}_4)_2\text{HPO}_4$ (DAP), $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ (TSP)) were both placed whole in the centre of the cap, and also crushed to a powder, with the addition of 100 µl of H_2O . 100 µl of H_2O only was added to the control (N = 3). All samples were incubated at 20 ° c. After 24 hours, 10 µl of ^{14}C radiolabelled glucose added (10mM; 690 kBq ml^{-1}) to the soil, directly followed by a 1 M NaOH traps (1 ml). Each ^{14}C glucose addition had a 30 second interval. NaOH traps were changed at 1 hour; 3 hours; 6 hours; 24 hours; 72 and 7 days to give CO_2 emitted at these time intervals. On day 7, the soils were extracted using 10 ml 0.5 M K_2SO_4 . pH of soil was then tested at the differing concentrations. Glucose was used as a microbial tracer as its use is almost ubiquitous in the microbial community.

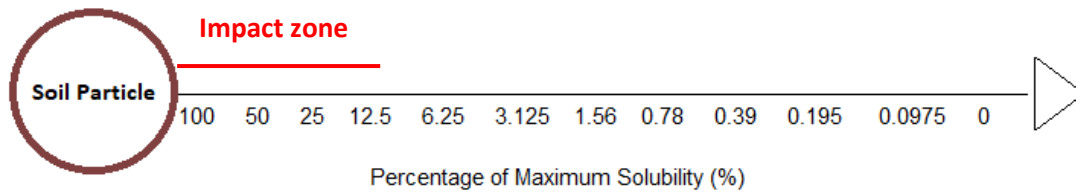


Figure 8. A visual representation of the concept. The greatest concentration (100%) representing the closest proximity to soil particle surface with concentrations diminishing

To calculate P sorption to the soil surface for soluble fertilisers, 1 g of soil was added to a vial along with 2 ml of each of the 11 fertiliser concentrations. The granules were not tested for P sorption due to the nature of their solubility. Each soluble fertiliser concentration was then radiolabelled with ^{33}P at 70,000 DPM (1.7 kBq). Soils were left to incubate on a shaker for 24 hours. After 24 hours the samples were left to stand for 5 mins when 0.5 ml of supernatant was then centrifuged for 10 minutes. 0.25 ml of the supernatant was then counted on a scintillation counter. The ^{14}C and ^{33}P content were determined using HiSafe-3 scintillation fluid (PerkinElmer Corp., Waltham, MA) and a Wallac 1404 liquid scintillation counter (PerkinElmer Corp.) with automated quench correction. The total P - max adsorption of each soil was determined by the Sorption Isotherm methodology, based upon approach by Sui & Thompson (2000).

$$Y = ax^b$$

$$Y = \text{P Adsorbed (mg P kg}^{-1}\text{)}$$

$$a = \text{K Value}$$

$$x = \text{P in solution (mg P l}^{-1}\text{)}$$

$$b = \text{Constant for the adsorption curve}$$

The colorimetric test, Molybdate Blue, was used to quantify the soil P in each fraction (Biologically - based P methodology) (Edward et al., 2015).

6 Statistical Analysis

All treatments were carried out in triplet while variance homogeneity and normality of data were tested for by visual inspection (quantile – quantile plots, gg-density) and statistical aids (Shapiro- Wilk test, Crawley, 2007) before conducting further analysis. Data were transformed (\log^{10}) and significant outliers were removed upon visual inspection. Akaike Information Criterion (AIC) was used to test for the model that best fit the data: a one-way ANOVA. When

significant, means were compared using Tukey ($p < 0.05$). All the statistical analyses were performed by R Programming.

Table 6 Significant values ($p < 0.05$) values and upper-lower ranges for differing concentrations of P-fertilisers. N = 3				
	Concentration.diff	Concentration.lwr	Concentration.upr	Concentration.p.adj
12.5-Control	0.991437	0.045225	1.937648	0.030426
25-Control	1.73522	0.789009	2.681432	1.52E-07
50-Control	2.416704	1.470493	3.362916	6.39E-11
100-Control	1.644947	0.698735	2.591159	9.60E-07
25-0.195	1.667657	0.721445	2.613868	6.10E-07
50-0.195	2.349141	1.402929	3.295352	6.40E-11
100-0.195	1.577383	0.631172	2.523595	3.58E-06
25-0.390625	1.611453	0.665242	2.557665	1.86E-06
50-0.390625	2.292938	1.346726	3.239149	6.42E-11
100-0.390625	1.52118	0.574968	2.467392	1.03E-05
25-0.78125	1.674358	0.728147	2.62057	5.33E-07
50-0.78125	2.355843	1.409631	3.302054	6.40E-11
100-0.78125	1.584085	0.637874	2.530297	3.15E-06
25-0.977	1.663093	0.659484	2.666702	4.29E-06
50-0.977	2.344577	1.340968	3.348186	6.60E-11
100-0.977	1.57282	0.569211	2.576429	2.07E-05
25-1.5625	1.465451	0.519239	2.411663	2.83E-05
50-1.5625	2.146935	1.200724	3.093147	7.47E-11
100-1.5625	1.375178	0.428966	2.321389	0.000134
25-3.125	1.498254	0.552042	2.444465	1.57E-05
50-3.125	2.179738	1.233526	3.125949	6.86E-11
100-3.125	1.40798	0.461769	2.354192	7.70E-05
25-6.25	1.239774	0.293563	2.185986	0.001144
50-6.25	1.921258	0.975047	2.86747	2.63E-09
100-6.25	1.149501	0.203289	2.095713	0.004176
50-12.5	1.425268	0.479056	2.371479	5.72E-05

2.1.7 Results and Discussion

Overall, there were significant differences in ^{14}C glucose mineralization rates ($p < 0.05$) (Table 6) between fertilizer treatments. Both the type of fertilizer and concentration were responsible for these differences ($P < 0.05$ for combined effect of fertilizer * concentration) although concentration was found to be of greater significance than the fertilizer type (Figure 8). Significant differences were not evident between treatments until 12.5% of maximum solubility where, at this point, the quantities of $^{14}\text{CO}_2$ emitted began to deviate between treatments and from the control. There was no significant difference between the means of the granular fertilizers and the control. Significant differences in C mineralization rates were seen between granular and soluble fertilizer concentrations for 12.5 %, 25 % and 50 %; 100 % respectively (Figure 9). The P sorption results were inconclusive and therefore were not considered further in this study.

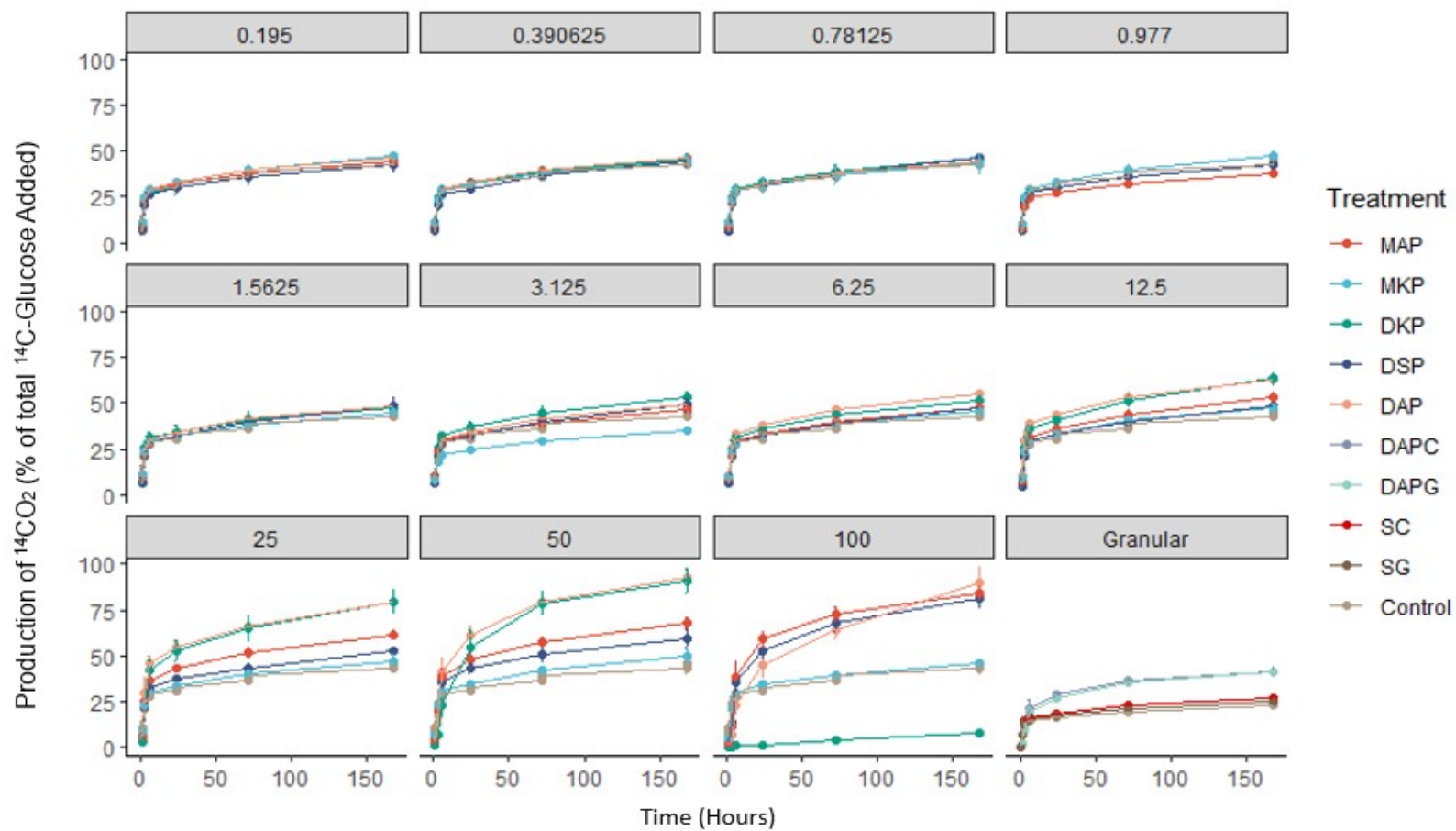


Figure 9. ^{14}C -labelled glucose mineralisation in 9 differing fertiliser treatments and 12 concentrations for soluble fertilisers. 0.0975 used as Control. Top left was the lowest concentration while bottom right showed the granular fertilisers. Y axis representing percentage of cumulative Carbon Dioxide release after Time (X axis elapsed). Values represent means \pm SE. N = 3.

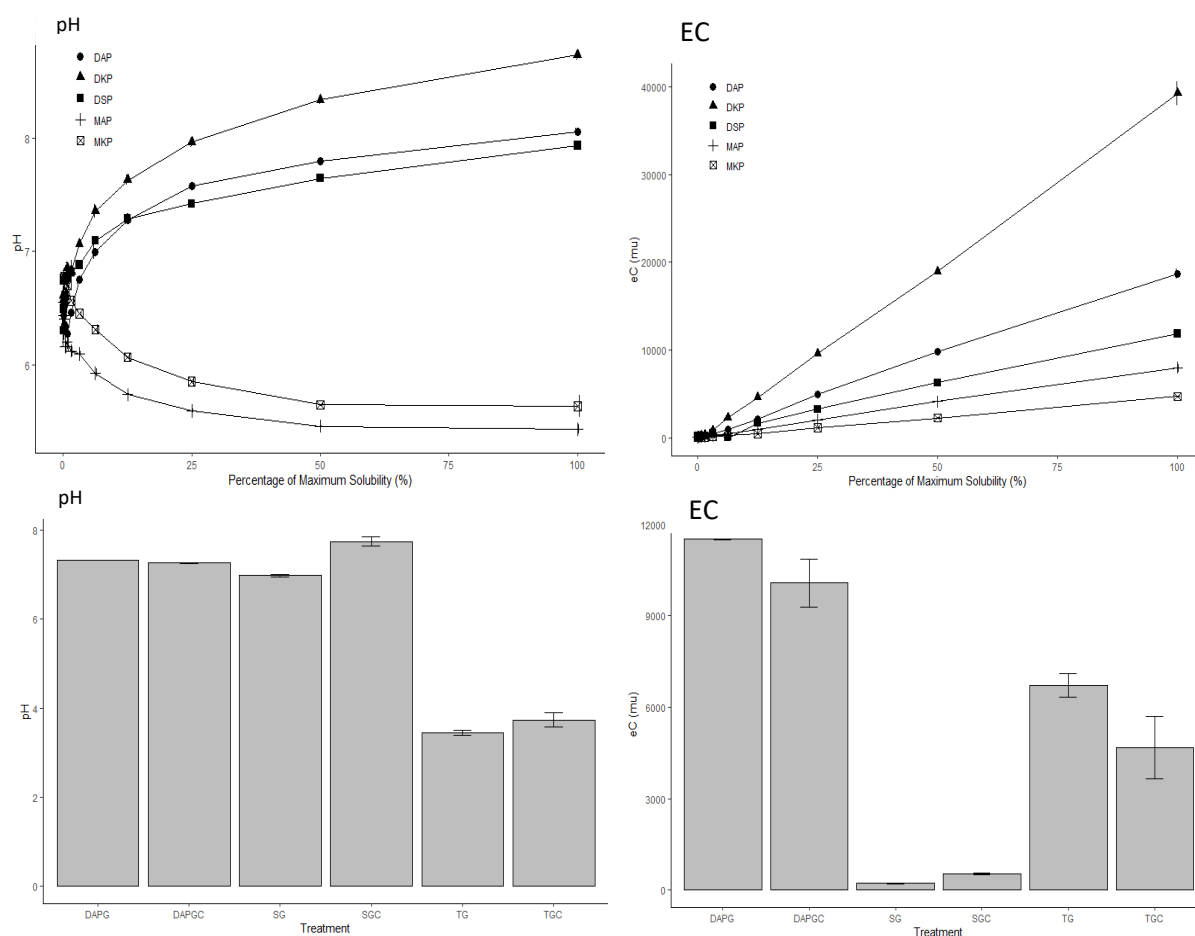


Figure 10. Fertiliser impact on soil pH (left) and EC (right). Soluble fertilisers along concentration gradient (top) and granular (bottom). Values represent means \pm SE. N = 3

(i) Traditional Versus Granular Fertiliser

Results from this study show that granular fertilisers are less toxic to microbial life when compared with elevated concentrations of traditional fertilisers of greater solubility (Figure 9 comparing 100, 50 and 25 % to Control), which is likely due to their slow-release mechanism, critically diminishing concentration of nutrient release. Effects of traditional soluble fertilisers and granular forms are shown in Figure 8, whereby overall mineralisation rate increased in accordance with the concentration of fertiliser applied once 12.5 % of maximum solubility was reached. The microbial response to concentrations above 12.5 % was distinct for all soluble treatments, with an initial rapid $^{14}\text{CO}_2$ release within the first hour, illustrating a sharp decline in microbial populations, and therefore, an instantaneously toxic effect. This was followed by a steady increase in ^{14}C production within 24 hours signifying a rapid intensification in mineralisation (Figure 9) in comparison with other concentrations, as well as with the less soluble fertilisers. The $^{14}\text{CO}_2$ was continually emitted at greater quantities for the 7-day period,

suggesting a longer-term impact. The granular fertilisers maintained a level of $^{14}\text{CO}_2$ production consistent with the control ($p > 0.05$) and therefore the effects are experimentally demonstrative as non-toxic to microbial life when these fertilisers are applied directly to soil. The rapid $^{14}\text{CO}_2$ production suggests an imbalanced environment resulting in constrained, inefficient microbial growth patterns, as the Carbon is not being stored by microorganisms to create new biomass (or SOM) but rather being depleted as $^{14}\text{CO}_2$ (Manzoni et al., 2012; Rui et al., 2016), in part, due to the compromises between alleviation of stress and attainment of resources (Malik et al., 2018). According to Ahmed et al. (2016), around 30 % of a struvite granule will dissolve in 14 weeks in slightly acidic soil. Dissolution of traditional fertilisers are almost instantaneous, with nutrients available at higher concentrations (Degryse et al., 2017). The combined effect of excess nutrients, as well as alterations of pH within the soil particulate zone, provides a conclusive theory for the cause of microbial death followed by increased respiration rates.

(ii) Impact of pH and EC

The fertilisers used in the experiments drastically effect the soil pH and EC, which may have been largely responsible for the alteration in soil microbial mineralisation. For example, DAP granules NH_4^+ with the potential to damage seedlings and have a toxic effect on plants because of nutrient leaching from the granular structure, increasing the surrounding soil pH to 7 (Nadarajan & Sukumaran, 2021). From Figure 10 we can see the EC of DAP granules is significantly greater than other treatments and pH significantly higher. Figure 8, demonstrates the CO_2 emissions to be slightly higher, expressing a negative effect on microbial life at the extreme pH. The application of soluble DAP and DSP exhibit a similar salting effect. However, DKP, with a pH above 8, appears to have depleted microbial activity completely when applied at maximum solubility, which could be potentially due to the microenvironment exceeding tipping point, permanently damaging microbial populations. The microbial carbon cycling process is directly affected by changes in pH, with losses increased due to either increased decomposition or reduced growth efficiency (Malik et al., 2018), while small alterations in pH can also drastically shift the dominant microbial species (Krause et al., 2012). Fertilisers demonstrating extremely high pH result in a similar effect on CO_2 production, potentially because the extremes may be, at least in part, responsible for imbalances in the system (Motavalli et al., 1995).

(iii) Concentration Gradient and Implications for Soluble Fertilisers

The concept of microbial dead zones from P fertilisers have not been proven through this experiment. However, the dilution of fertiliser has demonstrated diminishing effects on microbial activity representing mass transport away from the soil particulate surface (Figure 8). In a paper with greater experimental detail, Ruiz et al. (2020) explored the impact of N-fertilisers microbial communities through mathematical modelling of the structure within the soil. A “dead zone” is considered where microbial activity is inhibited to less than 10 % of normalised microbial activity, while 50 % is the cut-off for “inhibition zones”. Within the first hour, microbial activity was depleted, on average, to <50% for all soluble fertilisers at 100% concentration, and all except MKP for the 50% concentration. It is suggested that DKP and DAP create a microbial “Dead Zone” at these highest concentrations and at 50%, the majority of fertilisers induce “Inhibition Zones”. A small amount of the fertilisers will move by mass transport further away from the particle surface (most will be sorbed to the soil and therefore diffusion will be less than for other chemical compounds), effecting microbial populations and potentially inhibiting growth. Further research is warranted to prove this concept practically.

2.1.8 Concluding Remarks

This is the first study to compare both novel and conventional fertilisers and to assess the potentially toxic impact of P based fertilisers on microbial life. At higher concentrations, a toxic effect is evident and traditional fertilisers should be substituted where possible, with granular, slow-release forms, to ease environmental impacts and ensure soil health and resilience in the long-term.

The impact of pH and EC should be studied further within the context of this experiment, focusing on comparing CO₂ emissions from both P based and non-P-based chemical compounds at varying pH levels. Techniques such as PFLA and 16s RNA analysis would elucidate the composition of microbial species present in differing soil pHs with P-based fertiliser additions.

Longer term application more than the 7-day period explored here, would provide an explicit indication of the long-term effects on C microbial mineralisation, while field-scale study would also be recommended to elucidate the impact of use in commercial settings. Extending the “microbial dead zone” theory, as briefly explored in this paper, would involve a more detailed exploration of the temporal and spatial dynamic in micro-pore space within the soil by using techniques and modelling from work such as (Ruiz et al., 2020).

Chapter 3

Factors Influencing Phosphorus Uptake From Struvite and other P-based Fertilisers

Experiment 1. Pot experiment to determine P uptake and Root Proliferation from the Application of Differing P fertilisers

Experiment 2. A Pot experiment to quantify the most effective ratio of combined MAP to struvite application as assessed by P Uptake and plant growth parameters

Experiment 1 Pot experiment to determine P uptake and Root Proliferation from the Application of Differing P fertilisers

3.1.1 Abstract

Ever dwindling Phosphate rock reserves, as well as increasing environmental degradation from current agricultural practices, have elucidated the necessity for sustainable alternatives to traditional land management techniques within the global food industry. Fertilisers are just one of many aspects of the anthropogenically tailored nutrient cycling systems that require profound alteration. This study aimed to compare the impact of traditional P-based fertilisers (TSP, MAP) of greater solubility with granular and crystalised forms (MAP, struvite, TSP), extracted from waste-water streams, and to assess the most effective methods of application. A glasshouse pot experiment was set up with *Lolium Perenne* sown in a sand medium. P based fertilisers were added prior to sowing and sequential, biweekly cuttings were made for a 10-week period. The trend of resulting P uptake with *Lolium Perenne* was as follows Soluble monoammonium phosphate + AMF (MSA) > soluble monoammonium phosphate (MS) > granular triple super phosphate + AMF (TGA) > granular triple super phosphate (TG) > granular triple super phosphate crushed (TGC) > granular struvite crushed (SGC) > crystalised ICL struvite (IS) > granular struvite + AMF (SGA) > granular struvite (SG), whereas the greatest root biomass differed accordingly: IS > MS > SGC > SGA > C > MSA > SG > TGC > TG > TGA > TS. The application of MAP with AMF yielded a 10-fold cumulative P uptake in comparison with unaltered SG at 1.41 to 11.41 mg pot⁻¹, respectively. Crystalised ICL struvite demonstrated the highest root biomass at 3.20 g pot⁻¹, followed by MAP at 2.83 g pot⁻¹. The granular fertilisers were more effective with either crushing or the addition of AMF. Overall, the application method is key to struvite effectiveness for the fertiliser to yield comparable results to traditional P-based fertilisers such as MAP and TSP.

Keywords: struvite; cumulative P uptake; root zone; turfgrass; rhizosphere; granular fertiliser

3.1.2 Introduction

Agricultural production is largely dependent upon the application of P-fertilisers, derivatives of Phosphate rock, to provide nutrients for adequate crop yields. However, estimates predict reserves of PR will deplete within the next 50-300 years (Cordell et al., 2013; Van

Kauwenbergh, 2010; Wellmer, 2017). In addition, the process of P - fertiliser application and extraction is often inefficient, with legacy P unavailable for crop use and surface run-off of P directly associated with environmental issues such as eutrophication (Damon et al., 2014; Lwin et al., 2017; Sharpley et al., 2013). Alternatives to conventional fertilisers with the greatest cost-effective and environmentally sound application methodology must be explored to counter the threats reserves from ever-dwindling P rock supplies, expanding human populations and environmental degradation (Ahmed et al., 2016).

The form in which P is present in the soil is influenced by the chemical characteristics of the medium (Ortiz-Reyes & Anex, 2018; Wironen et al., 2018; Withers et al., 2001) and, in an agricultural capacity, the type of fertiliser type applied (Ahmed et al., 2018). These factors regulate P solubility and sorption and henceforth have major implications for P uptake (Atalay, 2005). Conventional, soluble chemical fertilisers, such as MAP ($(\text{NH}_4)_2\text{HPO}_4$), DAP ($\text{NH}_4\text{H}_2\text{PO}_4$) and TSP ($\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$) increase P availability for plant uptake (Darch et al., 2014). Phosphorus is transported across the plant cell wall (mainly in root tips) by transporters (found within a family of membrane proteins) in the form H_2PO_4^- (Smith, 2002). Some P will be sorbed to form complexes in accordance with the chemical composition of the soil profile (i.e. oxisols will form AlPO_4) (Roy et al., 2017), while some accumulates in microbial biomass (Joshi et al., 2018). However, a percentage will be present in the available form and will be taken up by the plant through the protein pump mechanism (Smith, 2002) or alternatively, for transport and could be subject to leaching and causing environmental issues (Kleinman, 2017) henceforth, the need for chemical fertilisers of lower solubility.

Novel fertilisers, such as struvite, have been applied through various studies and are shown to be as effective as conventional fertilisers. However numerous other studies demonstrate much lower quantities of P uptake (Ackerman et al., 2013; Ganrot et al., 2007; Hammond & White, 2008; Ryu et al., 2012; Talboys et al., 2016) and plant growth. The method of application has been shown to impact struvite dissolution in the soil, increasing the surface area through crushing or powdering the granule, results have been comparable to MAP and TSP (Rech et al., 2018).

The slow-release aspect of the granule, as is such when applied for commercial use, may have negative consequences for a plants early growth and degrade a plants capacity for resilient in later stages. The most efficient application method for traditional and novel fertilisers will assist in the exploration of alternative fertiliser to challenge the current status quo.

3.1.3 Aims and Objectives

1. To assess crop yield, nutrient uptake and below ground biomass for differing P-fertiliser (soluble and granular) treatments over a 10 - week period
2. To assess the impact of differing application methods on P-uptake and plant root growth.
3. To assess P uptake with AMF inoculation

3.1.4 Hypothesis

1. P uptake will differ significantly among treatments, with higher levels of uptake evident in fertilisers of greater solubility
2. Root growth will differ amongst fertiliser treatments, with significantly greater root biomass found with slow-release fertilisers
3. AMF inoculation will increase P uptake and root biomass

3.1.5 Methods

A glasshouse pot experiment was conducted from 26.8.2020 to 4.10.2020, at Henfaes Research facility, Abergwyngregen. Approximately 1500 g of sandy substrate provided by ICL fertiliser co. was added to 1.5 L (15 x 12 x 11.3 cm) pots. P-fertilisers ($\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ (TSP, both granular and soluble); $\text{NH}_4\text{H}_2\text{PO}_4$ (MAP); $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ (struvite Crystal Green® (Ostara) and ICL) (see Rech et al., 2018 for chemical properties); $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$) were then added to each pot in accordance with RB209 nutrient requirement for grass growth. P, N (added as NH_4Cl) and K (added as KCl) rates were equivalent in each pot (120 (P_2O_5); 60 (N) and 120 kg (K_2O) per hectare. Additionally, arbuscular mycorrhizae fungi (AMF) was added in granular form (Plantworks Ltd RG150) to the following treatments: MAP, TSP, struvite (struvite Crystal Green® (Ostara)) granular) and TSP (granular). The P - fertilisers and additional nutrients were then mixed in to the top 2 cm of the soil surface. All nutrient – P were added to the Control. *Lolium Perenne* was then seeded at 30 g m³ as recommended by ICL Fertiliser Co.. Pots were randomised bi- weekly and then watered with Hoagland's micronutrient solution in accordance with greenhouse humidity. Cuttings were taken when taken every 2 weeks from 2-3cm of the soils surface and oven dried at 105°C prior to weighing. After the 10-week period all plant pots were then harvested. Three soil cores were taken to determine whether the AMF inoculation was successful using (Vierheilig et al., 2005). The roots were then washed and dried in the oven at 105°C and weighed to determine below ground biomass.

3.1.6. Statistical Analysis

Variance homogeneity and normality of data were tested for each parameter by visual inspection (quantile-quantile plots) and a statistical aids (Shapiro- Wilk test) (Crawley, 2007) before conducting further analysis. Data were transformed and outliers were removed. The data were submitted to 3-way ANOVA. When significant, means were compared using Tukey ($p < 0.05$). All the statistical analyses were performed by R Programming. Phosphorus Recovery Efficiency (PRE) (%) was determined using the following calculation:

$$*PRE = \frac{P \text{ (g/pot)} - \text{Control P (g/pot)}}{P \text{ Fertiliser added g/pot}} * 100$$

3.1.7 Results and Discussion

We found statistically significant difference ($P < 0.05$) in P uptake in accordance with fertiliser types applied (see table 7). The average root biomass was also significantly affected by the type of fertiliser applied with IS around 50% greater than the lowest figure, TG. Average P uptake differed significantly among the fertiliser applications following the trend MAP > TSP > struvite based (Figure 11).

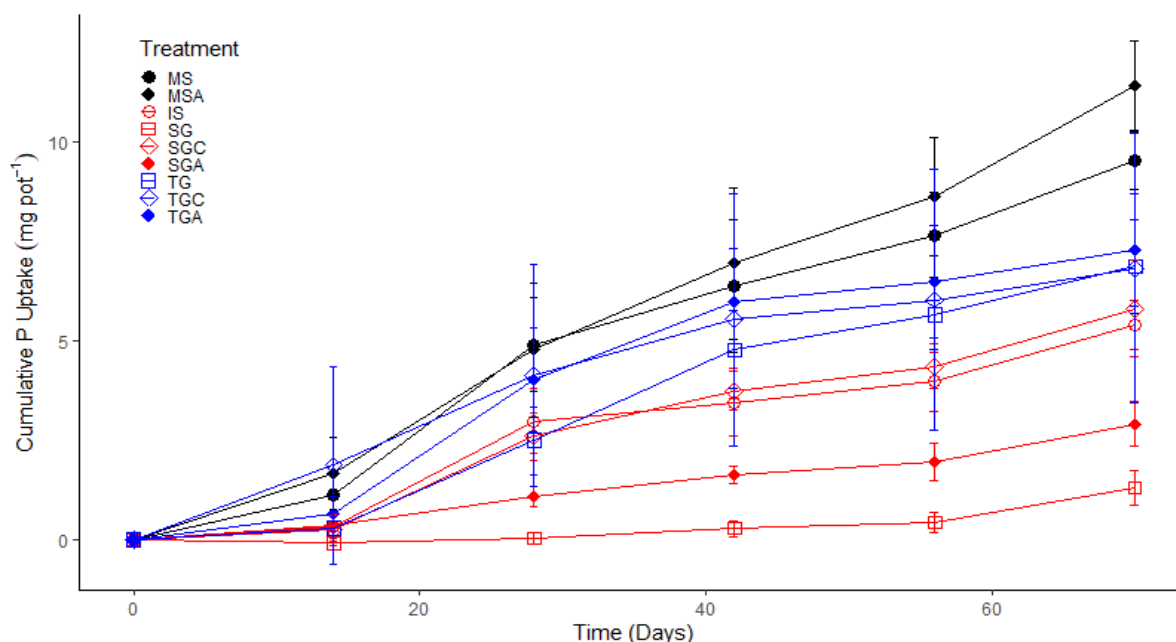


Figure 11. Cumulative Phosphorus uptake (mg pot^{-1}) = ($U - U_a$) for fertiliser treatments over a 10 – week period using solution (MS), MAP solution plus AMF (MSA), ICL co. struvite (IS struvite Crystal Green® (Ostara) (SG), struvite Crystal Green® (Ostara) crushed (SGC), struvite Crystal Green® (Ostara) plus AMF (SGA), TSP granular (G), TSP granular crushed (TGC), TSP granular plus AMF (TGA) as fertilisers. N=4. Vertical lines = ± 1 standard error.

Table 7. Dry Matter and Shoot yields, cumulative P Uptake and P Recovery Efficiency (%) of *Lolium Perenne* Shoots after 70 days (n = 4)

Treatment	Cumulative Shoot DM (g pot ⁻¹) (Treatment – Control)	Root DM (g pot ⁻¹)	Cumulative P Uptake (mg pot ⁻¹) = (U – U _a)	PRE* (%) based on cumulative P uptake
IS	1.31 ± 0.06	3.20 ± 0.20	5.41	5.85
MS	1.5 ± 0.08	2.83 ± 0.07	9.53	10.31
MSA	1.50 ± 0.07	1.70 ± 0.10	11.41	12.35
SG	0.89 ± 0.07	1.66 ± 0.06	1.41	1.52
SGA	1.19 ± 0.04	2.37 ± 0.11	2.92	3.16
SGC	1.35 ± 0.07	2.38 ± 0.11	5.80	6.28
TG	1.12 ± 0.07	1.52 ± 0.10	6.88	7.44
TGA	1.08 ± 0.05	1.84 ± 0.08	7.29	7.89
TGC	1.17 ± 0.12	1.64 ± 0.24	6.83	7.39

(i) P uptake and PRE (%)

Granular fertilisers are ineffective as standalone treatments in the place of conventional P fertilisers. Distinct trends for P uptake and P Recovery Efficiency can be seen between MAP, TSP and struvite-based fertilisers, as well as the application methods (i.e. with/without AMF, or powdered/granular/solution) (Figure 11). MS and MSA had the highest cumulative P uptake ranging from 9.53 - 11.41 mg pot⁻¹, with MS and MSA being significantly higher than most other treatments (p > 0.05) (Table 7). In contrast to this study's findings, (Rech et al., 2018) found similar total shoot P content between struvite to TSP (p < 0.01) treatments, in a pot experiment using *Lolium Perenne*, with uptake amounting to as much as 80% in struvite treatments: in this study uptake was <10 %. Studies applying struvite in granular form, such as Talboys et al. (2016) found no significant difference between struvite and TSP granules. The current study demonstrates struvite as less effective as other fertilisers due to its slow-release aspect in early growing season.

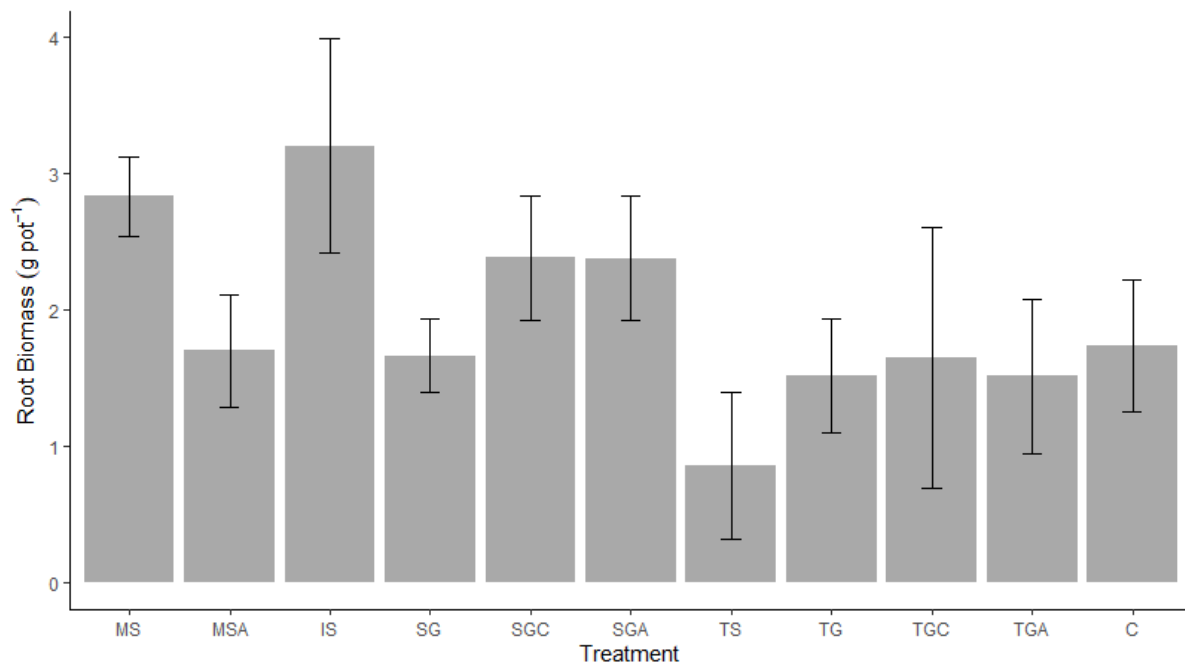


Figure 12. Root weight (g pot⁻¹) for fertiliser treatments after 10 – week period using MAP solution (MS), MAP solution plus AMF (MSA), ICL co. struvite (IS), struvite Crystal Green® (Ostara) (SG), struvite Crystal Green® (Ostara) crushed (SGC), struvite Crystal Green® (Ostara) plus AMF (SGA), TSP granular (G), TSP granular crushed (TGC), TSP granular plus AMF (TGA) as fertilisers. N=4. Vertical lines = ± 1 standard error.

(ii) Differences between treatments

The type of fertiliser, as well as the method of application determines both P uptake and below ground biomass growth (Figure 12). MS and MSA had significantly higher cumulative P uptake while the crystallised struvite had a more positive effect on root biomass, similar to that of MAP. Through both direct and indirect mechanisms including; bidirectional nutrient transfer; enhanced aggregate stability and increasing water acquisition (Begum et al., 2019; Yooyongwech et al., 2016), the application of AMF can improve a plant's resilience. The root weight of *Lolium Perenne* differed significantly among treatments whereas only ICL fertiliser and MAP differed significantly from the control. Significant root biomass increases with Kentucky Bluegrass species were found using struvite compared with DAP (Petrovic and Barlow, 2011), a contrasting result to this study. This study demonstrated lower root biomass yields for struvite application, as well as P uptake.

(vi) Performance of Granular Versus Crushed Struvite

Struvite is significantly more effective when crushed, which is likely due to the increasing surface area and henceforth, P availability. In this study, powdering struvite enabled an almost 5-fold P uptake in comparison with the granular form (Figure 10): significantly different ($p = 0.007$). However, the same effect was not observed for the TSP based granule. In general, experiments claiming struvite to be as effective as other traditional fertilisers, have applied the fertiliser crushed or powdered and mixed the substance within the soil (Achat et al. 2014b; Bonvin et al. 2015), making the dissolution rates, and therefore available P, significantly different to 3.0 mm granules (size for commercial use) (Degryse et al., 2017) by as much as 76 %. Evidently, increasing the surface area and dissolution rate of a substance will increase the availability of compounds within the substance. The additional P will be in greater quantities available for plant uptake.

(v) Nitrogen uptake and AMF

In both MS, MSA and TG, Nitrogen uptake had a significant impact on P uptake. However, in this study, due to the variability in the mean of the repetitions, we were unable to come to a firm conclusion on the impact of N on P uptake. A noticeable difference between AMF treatments was observed. However, they were not significant and AMF were undetectable in the core samples (Vierheilig et al., 2005).

3.1.8 Concluding Remarks

Struvite has been shown as a less effective fertiliser in terms of P uptake and root biomass. As a relatively novel product, research has not yet explored the most effective method of application for whole struvite granules. There are very few studies that compare struvite in its commercially used form and therefore, comparison with published data is tenuous. However, this study firmly concludes that as a product, Ostara green is not commercially viable as a standalone treatment for use in a neutral pH, sandy medium with *Lolium Perenne* but is more effective powdered and potentially, with the use of AMF. It is recommended that trials are held at a field scales for an entire growing season using *Lolium Perenne*.

Experiment 2 A Pot experiment to quantify the most effective ratio of combined MAP to struvite application as assessed by P Uptake and plant growth parameters

3.2.1 Abstract

Crop production is heavily dependent on P fertilisers, a non-renewable and non-synthesisable element for plants use. Alternative application methods to current traditional P-based fertiliser are necessary to ensure crop yields are maintained to an adequate level when transitioning to novel fertilisers types. This study observed the impact of combining differing ratios of using two differing kinds of struvite (granular and crystalline) mixed with MAP on plant P uptake and below ground biomass. A glasshouse pot experiment was carried with the application of P-based fertilisers prior to sowing *Lolium Perenne*. Sequential cuttings were made biweekly for a 10-week period. Ratios of struvite:MAP ranged from 0:100, 20:80, 40:60, 60:40, 80:20, 100:00. These P fertiliser mixtures were applied to the seedbed and *Lolium Perenne* was sown. The most effective combination was struvite to MAP, 80:20 ratio, increasing root biomass in comparison with struvite and MAP alone by 31.25 and 67.5 % respectively. For granular forms, trends for root biomass increase were as follows: 80:20 > 60:40 > 100:0, whereas trends for P uptake were 20:80 > 40:60 > 60:40. The crystallised form demonstrated differing trends, with 60:40 ratio most effective for P uptake overall but with no significant difference with 80:20 or 40:60 ratios and no change in root biomass. The findings demonstrate the optimum ratios by which MAP and struvite can be applied simultaneously, as a more soluble fertiliser can be, in part, supplemented with Struvite, to enhance the environmental sustainability of the product.

3.2.2 Introduction

An essential nutrient for plant growth and repair, the production of P based fertilisers are crucial in maintaining global food security. However, models predict P production to peak within the next 50-300 years (Cordell et al., 2013; Van Kauwenbergh, 2010; Wellmer, 2017), threatening our ability to feed an ever-growing human population (Therregowda et al., 2019). The capacity of a plant to uptake P is dependent on the form in which it is present and is therefore applied in more readily obtainable P forms: fertilisers. Although they have been shown to enhance crop growth, the increased solubility leads to leaching further down the soil profile, where P is then incorporated into insoluble forms, leading to excessive amounts of labile P (Withers et al., 2018). The process of P extraction from PR is often inefficient (Damon

et al., 2014; Lwin et al., 2017; Sharpley et al., 2013) while the environmental damage caused by P bound soil particles (via run-off with soil erosion) such as eutrophication is also of great concern (Withers et al., 2001; Withers et al., 2014). Struvite, a slow-release P based granular fertiliser of low solubility is formed from the extraction of nutrients from wastewater streams offering a product that aims to shift the linearity of the current anthropogenically designed P cycle towards a sustainable, “closed-loop” system (Möller et al., 2018).

Low solubility is a key element to struvite’s saleability as an environmentally friendly product. However, to make the fertiliser commercially viable, it must supply sufficient nutrient for early growth and has mainly been demonstrated as a comparably effective product (to conventional fertilisers) when applied in a powdered or crushed form (Ackerman et al., 2013; Ganrot et al., 2007; Hammond & White, 2008; Ryu et al., 2012; Talboys et al., 2016). Talboys et al., 2016 found a mixture of soluble DAP and struvite optimised early season P uptake, with 20:80 struvite: DAP around 0.9 mg pot⁻¹ greater uptake: significantly different. The addition of a soluble fertiliser in early growing season enables plants to establish a strong root system, enhancing its resilience to environmental change by; improving the uptake of water, and other macro and micro nutrient for and overall yield potential optimisation (Flavel et al., 2014; Talboys et al., 2016).

The reported effectiveness of the novel struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) has varied between studies, with diversity of application methods used and therefore, differing results acquired (Ahmed et al., 2016). Commercial viability and environmental safeguarding must combine in order to ensure a stable global food with a sustainable future.

3.2.3 Aims and Objectives

1. To determine the most effective soluble: granular fertiliser application ratios for ICL Co. and Ostara Green struvite fertiliser treatments.

3.2.4 Hypothesis

1. P uptake and root proliferation will increase as the ratio of soluble to granular fertiliser increases
2. P uptake and root proliferation will be great with the crystallised form of treatment when compared with struvite (granular)
3. Crystallised forms of struvite will enhance root proliferation that granular forms

Keywords: struvite, recovery efficiency, cumulative P uptake turfgrass; p-fertilisers

3.2.5 Methods

A glasshouse pot experiment was conducted from 3.11.21 to 12.1.21, at Henfaes Research facility, Abergwynnregan. Approximately 1500g of sand was added to 1.5 L (15 x 12 x 11.3 cm) pots. monoammonium phosphate was then added in combination with either struvite or ICL crystallised product, both of which are a mixture of $MgNH_4P$, in accordance with RB209 amounts stated for optimum grass growth (P, N (added as NH_4Cl) and K (added as KCl) rates were equivalent in each pot (120 (P_2O_5); 60 (N) and 120 kg (K_2O) per hectare). The ratios of MAP: $MgNH_4P$ fertilisers were applied in the following ratios (100:0, 80:20, 60:40, 40:60, 20:80, 0:100). P, N (added as NH_4Cl) and K (added as KCl) rates were equivalent to RB209 recommendations for each pot. The P - fertilisers and additional nutrients were then mixed in to the top 2 cm of the soil surface. *Lolium Perenne* was then seeded at 30 g m³ as recommended by ICL co.. Pots were randomised bi- weekly and then watered with Hoagland's micronutrient solution in accordance with greenhouse humidity. Cuttings were taken when taken every 2 weeks from 2-3cm of the soils surface and oven dried at 105 °C prior to weighing. After the 10-week period all plant pots were then harvested. The roots were then washed and dried in the oven at 105°C and weighed to determine below ground biomass. Dry matter yields were determined and P uptake in the shoots was analysed using the X-ray fluorescence spectroscopy.

3.2.6 Statistical Analysis

All treatments were carried out in triplet while variance homogeneity and normality of data were tested for each parameter by visual inspection (quantile-quantile plots) and a statistical aids (Shapiro-Wilk test) (Crawley, 2007) before conducting further analysis. Data were transformed and outliers were removed through subjective measures. The data were submitted to ANOVA. When significant, means were compared using Tukey ($p < 0.05$). All the statistical analyses were performed by R Programming. P Recovery Efficiency (PRE) (%) was determined using the following calculation:

$$*PRE = \frac{P \text{ (g/pot)} - \text{Control P (g/pot)}}{P \text{ Fertiliser added g/pot}} * 100$$

3.2.7 Results and Discussion

The results show significant differences between ratio treatments for both root biomass and cumulative P uptake ($p < 0.05$) (Figures 13 & 14) implying that specific application methods will enhance plant growth factors. We found statistically significant difference ($P < 0.05$) in average P uptake within both the struvite and ICL treatments for ratios of soluble to granular fertiliser application, with the most significant for struvite application being 20:80 (highest) and 100:0, and for ICL struvite, 20:80 (highest) and 40:60 (Figure 13). The average root biomass was also significantly affected by the type of fertiliser applied, as well as the ratios applied, with struvite demonstrating significantly greater biomass with greater significance between ratio treatments than ICL (Figure 14). Overall, P recovery efficiency (%) was higher in the ICL treatments, although the errors bars were too large to identify significant differences in the treatments (Figure 15).

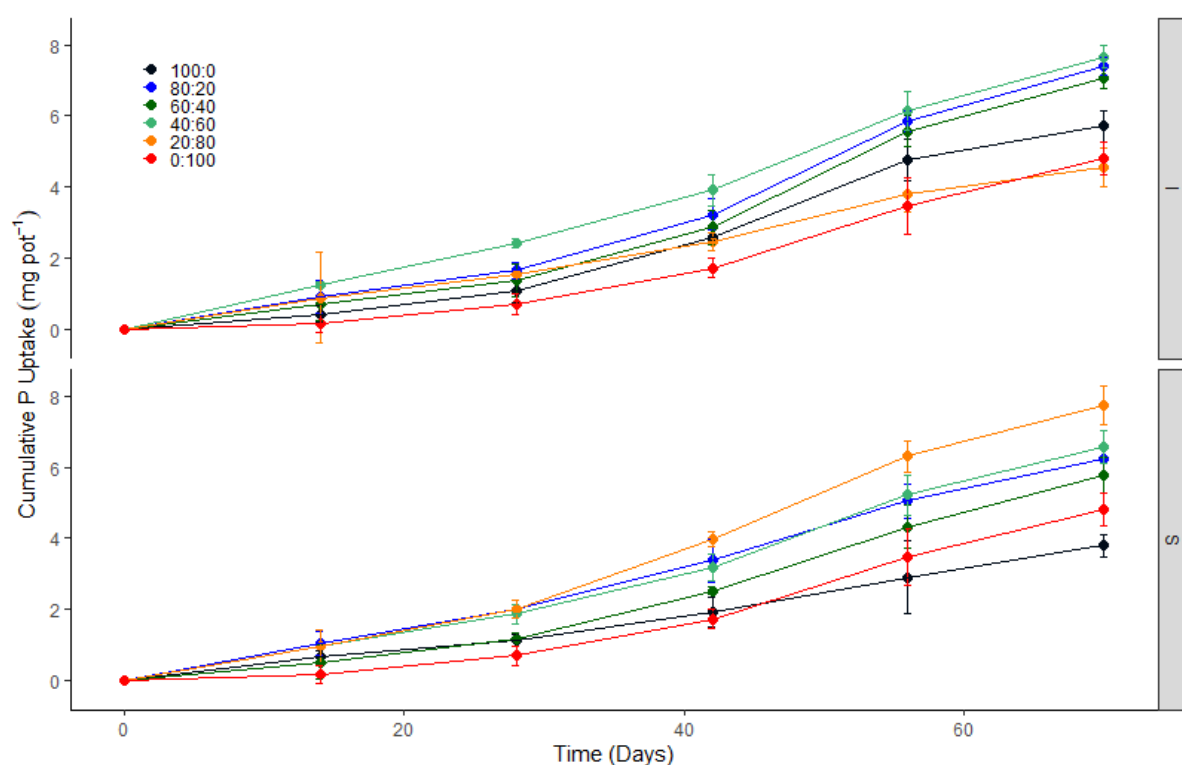


Figure 13. Cumulative phosphorus uptake (mg pot⁻¹) = ($U - U_a$) for fertiliser treatments over a 10 – week period using MAP solution, ICL co. Struvite (I, above) and struvite Crystal Green® (S, below) in combined ratio of struvite : MAP. N=3 Vertical lines = ± 1 standard error.

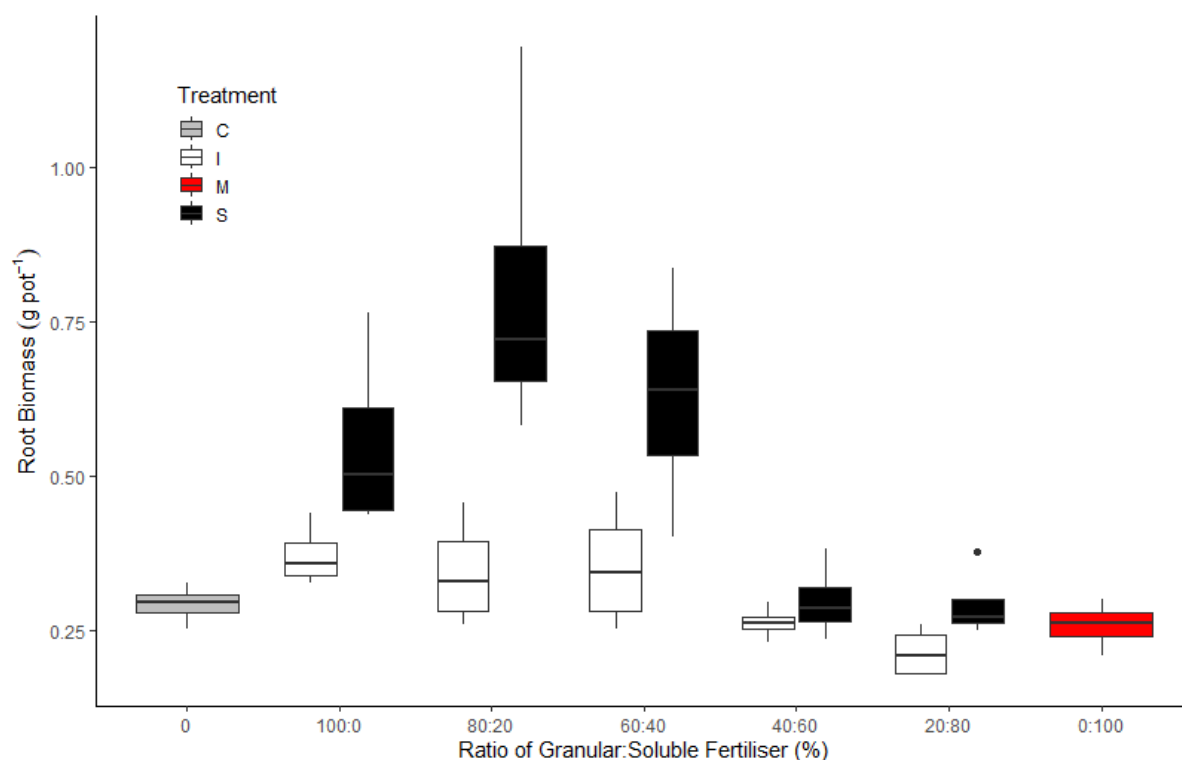


Figure 14. A boxplot showing overall root biomass for fertiliser treatments over a 10 – week period using MAP solution, ICL co. struvite (I, white) and struvite Crystal Green® (S, black) in combined ratio of struvite:MAP. Control (C) = Grey; MAP (M) = Red. N=3 Vertical lines = ± 1 standard error

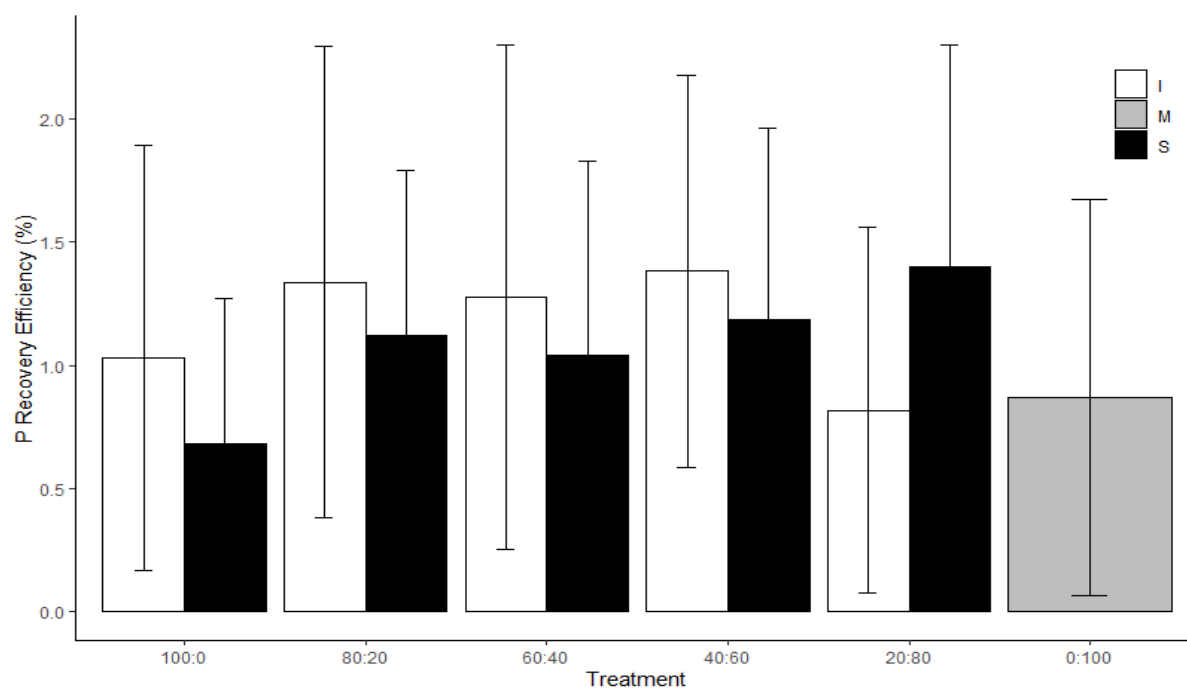


Figure 15. A bar chart to show mean P recovery efficiency (%) (P Uptake – Control / Total P applied * 100). I = ICL co. struvite, S = struvite Crystal Green®, M = MAP. N=3, Vertical lines = ± 1 standard error.

Table 8. Dry matter and shoot yields, cumulative P uptake and P recovery efficiency (%) of *Lolium Perenne* Shoots after 70 days (n = 4)

Treatment			Shoot DM (g pot ⁻¹)	Root DM (g pot ⁻¹)	Cumulative Uptake (mg pot ⁻¹) = (U – U _a)	P *PRE (%)
Crystalised Struvite	ICL	100	1.30 ± 0.01	0.37 ± 0.01	5.17	1.41
		80:20	1.28 ± 0.01	0.34 ± 0.02	7.41	1.38
		60:40	1.29 ± 0.03	0.35 ± 0.02	7.07	1.40
		20:80	0.96 ± 0.04	0.26 ± 0.006	4.54	1.04
Struvite Granular (240 GSM)	(240 GSM)	100	1.28 ± 0.03	0.55 ± 0.03	3.80	1.38
		80:20	1.48 ± 0.01	0.80 ± 0.07	6.23	1.45
		60:40	1.20 ± 0.05	0.63 ± 0.04	5.80	1.30
		20:80	1.48 ± 0.04	0.30 ± 0.01	7.75	1.60
Monoammonium Phosphate		100	1.14 ± 0.03	0.26 ± 0.01	4.81	1.23

(i) Differing Ratios Promote Greater P Uptake

The fertiliser type and ratios applied result in differing trends for P uptake in *Lolium Perenne*, likely due to the improved availability of P throughout the growing period. Talboys et al (2018) discovered that mixing 20:80 ratios of Struvite with readily available DAP resulted in yields of plant P uptake comparable to the use of DAP alone. The experiment used buckwheat, lasting for 30 days as oppose to 70 days in this experiment (Talboys et al., 2016). The highest cumulative P uptake, 7.75 mg pot⁻¹, was expressed at 20:80 ratio (day 70) of Struvite to soluble MAP, a greater quantity of P than that of MAP alone. ICL Struvite, resulted in P uptake of 7.41 mg pot⁻¹ (day 70) at 80:20 and 7.07 mg pot⁻¹ (day 70) for 60:40. Lower yields and less P uptake, may be resultant from the lower rates of P supply in earlier growth stages for Struvite without the addition of a more soluble fertiliser (Ackerman et al., 2013; Ganrot et al., 2007; Hammond & White, 2008; Ryu et al., 2012; Talboys et al., 2016). The significant increase in

cumulative P uptake may be related to an increasing availability of P in the early growth stage due to the input of the fertilisers of higher solubility (Talboys et al., 2016), while the continuous supply from the slower release struvite throughout the growing season contributed to the majority of treatments being higher in uptake than pure MAP alone.

(ii) Differing Ratios Impact on Root Systems

The low rate of dissolution of Struvite may be responsible for the increased root proliferation. Some studies have shown P leaching from Struvite from 3 times (Rothbaum and Rohde (1976)) to 10 times less than that of TSP (Latifian et al., 2012), while in citric acid, 50 % of struvite P will solubilise from granular form and only 1-2% will dissolve in water (Cabeza et al., 2011; Johnston and Richards, 2003; Kern et al., 2008). In this study, Struvite (20:80) increased mean root biomass by three times as much as the control. These results were not found in the ICL fertiliser, crystallised form, suggesting it is the form of the product, rather than the chemical composition that effects root biomass. The increase in root biomass observed in the struvite Crystal Green® (Ostara) treatment may be due to the ongoing, slow-released P supply throughout growing season, enhancing P proliferation in the direction of the P source. Plants with higher root biomass can compete more effectively for nutrients and exhibit greater resilience overall.

3.2.7 Concluding Remarks

The combination of slow-release and more soluble fertilisers, aids in the growth and resilience of *Lolium Perenne*, more so than either treatment alone. Struvite is more effective than the the Crystallised ICL Struvite formula when applied with the optimum ratio, with 20:80 to MAP fertiliser resulting in the largest P uptake and root biomass. Further study into the agronomic efficiency for the use of Struvite (Ostara and ICL) in mixed ratios for field scale use, as the practicality of applying two different forms is yet to be assessed.

Chapter 4

Root Adaptation with Induced P deficiencies and Variability in P-fertiliser Placements

Experiment 1. Banded application of struvite granules placed at lower soil depths will be more effective in increasing plant root proliferation in comparison to conventional P-based fertilisers.

Experiment 2. Phosphorus uptake with induced P deficiency with liquid solution: root adaptation of *Lolium Perenne* Roots

Experiment 1

4.1.1 Abstract

Agricultural production is largely dependent upon the application of P-fertilisers, derivatives of Phosphate rock (PR), to provide nutrients for adequate crop yields. The threat of reaching peak phosphate as well as inefficiencies of, and the environmental degradation associated with the anthropogenic cycling of P, there is a need to establish alternatives to the traditionally used fertiliser types to ensure crop health and resilience. This study aimed to assess the most effective methods of application for alternatives to traditional fertilisers. A glasshouse experiment rhizotron experiment was carried out. Sand medium was added to a 30 * 2 * 20 cm rhizotron with fertiliser placed at banded depths. *Lolium Perenne* was then sown on the surface and harvested after a 10-week period. The depth of fertiliser placement significantly impacted root growth at differing depths with differences between root biomass observed at the placement 6 cm, where mean root biomass was nearly 49.2% greater overall compared with surface placement. Root biomass yield were 30% greater when fertiliser was applied at the 3 cm band compared with fertiliser applied at the soil surface. In comparison with this study's control, enhancement of root growth by fertiliser addition could only be detected with Crystallised ICL Struvite placement at 6 cm. This study implies that a placement at a lower depth is favourable for increasing root growth and therefore, should be considered when applying both soluble and granular fertilisers.

Keywords: rhizosphere; root zone; rhizotron; glasshouse; banding; eutrophication; resources; efficiency; slow release

4.1.2 Introduction

Resource conservation and environmental safeguarding have become ever more important elements of effective land management practices within the agricultural sector. With the move towards alternative, sustainable fertilisers to conserve resources and minimise nutrient losses (Withers et al., 2018), new products are being assessed to ensure maximum efficiency for commercial practices. Selective placement and banded application of nutrient fertilisers have been shown to positively impact root and shoot growth in a variety of plant species (Jing et al., 2010; Ma et al., 2013) providing yield advantaged in products such as maize (Degryse et

al., 2017). Plant roots extend towards the P source consequently, enhance growth with P sources at further depths (Byrne et al., 2011; Hill et al., 2006; Sheil et al., 2016).

Struvite is a novel, granular fertiliser ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$), offering a recovered source of P (Rahman et al., 2011; Mu, 2012; Nelson et al., 2003; Yetilmezsoy & Sapci-Zengin, 2009). However, studies have demonstrated that, when applied in granular form, it is far less effective in terms plant growth parameters when compared with less environmentally friendly P fertiliser forms (Ahmed et al., 2019). Studies thus far have not looked at Struvite in comparison to other P-based fertilisers with differing treatment bands, nor have they looked at the impact of separating the P from other nutrients, inducing deficiencies, henceforth, the aims and objectives are highlighted below.

4.1.3 Aims and Objectives

1. To determine the most effective placement depth of fertiliser to increase root proliferation
2. To quantify plant root proliferation as affected by the form of fertiliser applied

4.1.4 Hypothesis

1. Banded application of Struvite granules placed at lower soil depths will be more effective in increasing plant root proliferation in comparison to conventional P-based fertilisers.

4.1.5 Methods:

A glasshouse rhizotron experiment was conducted at Henfaes Research Facilities from 19.1.21 to 30.3.21. Greenhouse conditions were variable, with averages of 5 ° c temperature, 85 % humidity and 74.7 % cloud cover. Rhizotron were 20 * 2 * 30 cm (L * W * H) in size. Markings were made on the rhizotron for 3 and 6 cm from the surface. A sandy substrate provided by ICL fertiliser co. was then added to the marked levels while the third treatment, at the surface of the rhizotron, was also added. Phosphorus fertilisers MAP, Granular Struvite (whole and crushed) and Crystallised ICL Struvite (IS) were carefully placed on the sand at the surface, 3 and 6 cm placements. N (NH_4Cl) and K (KCl) were then added (P, N (added as NH_4Cl) and K (added as KCl) rates were equivalent in each pot (120 (P_2O_5); 60 (N) and 120 kg (K_2O) per hectare) with (20 ml) of H_2O to ensure RB209 nutrient requirements were met.

The rest of the rhizotron were then filled to 1 mm of the top of the rhizotrons. *Lolium Perenne* was seeded at 50g / m² and were watered with Hoagland's micronutrient solution instantly, and then in accordance with greenhouse humidity. The controls were subject to the same treatments – P. After 10 weeks the plants were harvested, and the soil in rhizotrons were divided into 5 sections for sampling roots: 0.0-2.5, 2.5-5.0, 5.0-10.0, 10.0-20.0, and 20.0-30.0 cm. The roots were then washed, dried in an oven at 80 °C and weighed.

4.1.5 Statistical analysis

Variance homogeneity and normality of data were tested for each parameter by visual inspection (quantile-quantile plots) and a statistical aids (Shapiro- Wilk test (Crawley, 2007)) before conducting further analysis. Data were transformed and outliers were removed. The data did not satisfy tests for parametric analysis therefore the Kruskal–Wallis, non-parametric model was used, followed by the post-hoc pair-wise Wilcox test, p value adjustment method BH.

4.1.6 Results and Discussion

Significance ($P < 0.05$) was demonstrated between the means of treatments; Control – Soluble Monoammonium Phosphate (MS); Control – Struvite (S) in terms of effect on root biomass across sampling depths (Table 8). For the effect of fertiliser placement on root mass, significant differences were observed between 0 and 6 cm placement from surface while $p = 0.01$ for the difference between 0 and 3 cm sampling depths (Figure 16).

Table 9. Total shoot and root biomass for different treatments and sampling depths. N=3

Treatment	Total Shoot Biomass (g rhizotron ⁻¹)	Total Root Biomass (g rhizotron ⁻¹)
Control	708 ± 14.9	14.07 ± 0.02
ICL Struvite	776 ± 28.8	16.55 ± 0.08
MAP	575 ± 56.0	12.42 ± 0.07
Struvite Granular	665 ± 13.04	12.41 ± 0.13

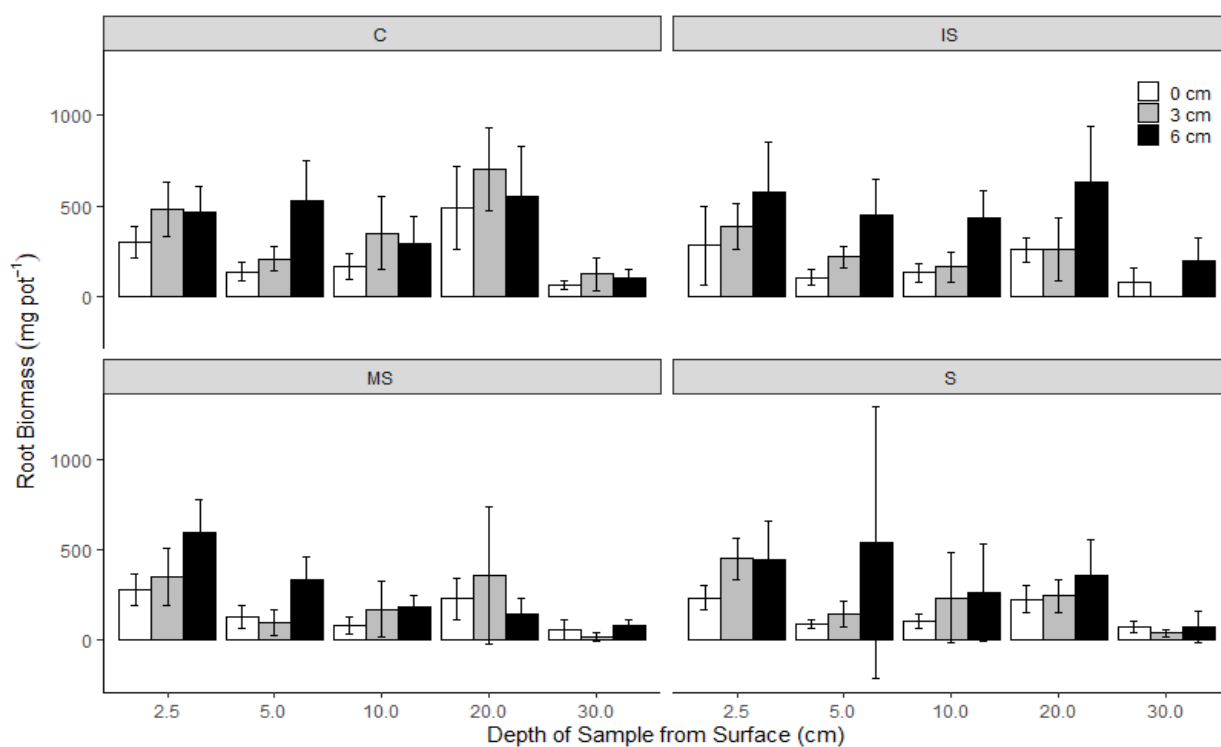


Figure 16. P - based fertiliser in comparison with the control. Root biomass at sampling depths in rhizotron where P fertilisers had been placed at different soil depths. MAP solution (MS), ICL co. struvite (IS), Ostara green struvite (SG.), Ostara green struvite crushed as fertilisers. N=3. Vertical lines = ± 1 standard error

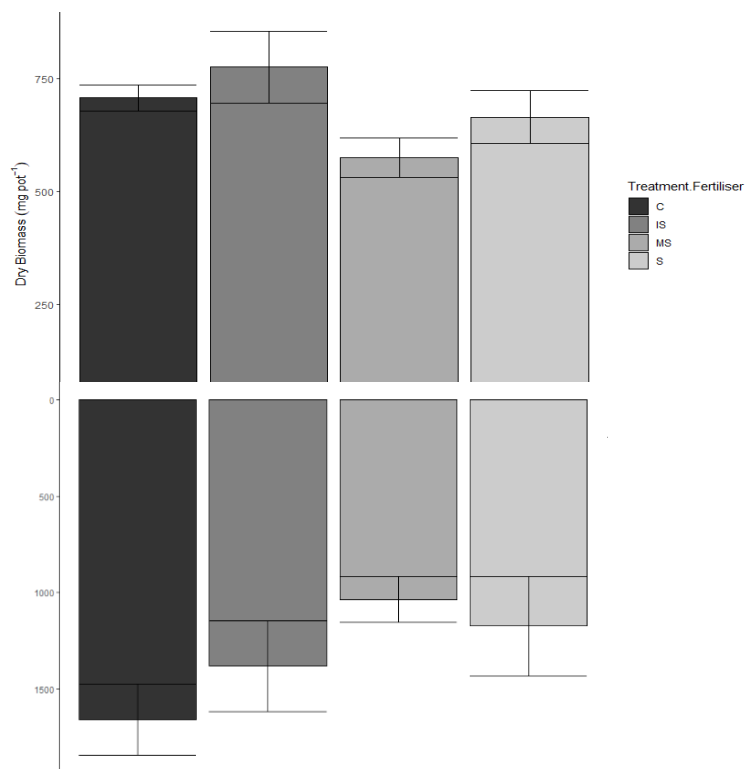


Figure 17. Showing the total root (lower) and shoot biomass (upper) (g pot⁻¹) for each treatment and the highest root weight among sampling depths.

(i) Depth of Placement and Root Biomass Response

Sub-surface placement of P fertiliser treatments represents an effective way of increasing root depth and DM yield, assisting in crop resilience to instances of drought and nutrient deficiencies in the soil (Figure 16). TA placement depth of 6 cm resulted in the highest mean root biomass overall, followed by the 3cm depth and then surface application. ICL fertiliser biomass was most impacted by the 6 cm treatment placement, where the greatest increase was observed in comparison with the control, RW was almost 3- fold that of the mean root biomass at the 6 cm placement across all sampling depths. S and MAP were generally lower than the control in terms of root biomass quantities. However, IS can exceed the controls when placed at depths of 6 and 3 cm. In a study looking at alfalfa forage over a 5 -year period, (Malhi et al., 2001) demonstrated that a 5 cm depth band of P fertiliser placement increased DM yields and PUE for both annual and one-time application. While studies such as Ma et al. (2013) show a 50% increase in rooting density and length through localised application of P with ammonium. Supplying nutrients in such as manner have been demonstrated in various studies to induce preferential root growth towards zones rich in nutrients (Drew and Saker, 1975, 1978; Snapp et al., 1995; Zhang and Forde, 1998). However, some studies have found lower placement of granules to be ineffective (Kabir et al., 2010). The signalling of P deficiency may be responsible for enhanced root growth towards the P source (Jackson and Caldwell, 1989; Lie et al., 2013; Liao et al., 2001), in this experiment's case, towards the 6 cm banding. However, further investigation is warranted to decipher the disparity between the predicted outcomes and the current state of results.

(ii) Mean Root Biomass Compared to Control

For the control plots (no P), addition of P to banded areas of NH_4Cl and KCl appeared to reduce root biomass (Figure 17). Root biomass overall did not deviate significantly from the control for IS, MS and S. IS root biomass was the highest of the treatments at, 16.55 g pot⁻¹ (Table 9) followed by 12.42 and 12.41 g pot⁻¹, for MS and S, respectively. The reduction in root biomass was unexpected, as treatments receiving P have been shown to increase root biomass in general and not reduce it (Huang et al., 2018; Jing et al., 2010). A study by Marsh & Pierzynski (1998) suggested that the addition of P and N at their highest rates may have reduced root growth around the fertiliser band while Hill et al. (2006), showed decreases in root mass were resultant from decreases in P supply and the diameters of all the roots systems were from 9-32% lower in the control (no P addition). This study used the highest rates of fertiliser as proposed by RB209 for grassland swards, i.e. 80 kg P/ha, and this may have contributed to the reduction in biomass. The mechanisms underlying the process causing root

proliferation are yet to be fully understood (Shen et al., 2011) though previous research suggests Struvite applied in patches may yield differing results due to dissolution rates and molar ratio of elements, with the potential to alter root morphology and architecture (Bonser et al., 1996). However, generally, studies observe the opposite trend to the experimental data collected here.

(iii) Ammonium Compared with other N Sources

The addition of NH_4Cl and KCl to each band to the control may have contributed to the discrepancy between this study's results and reported findings due the interaction between the N and P source, and also, the increase in acidity altering nutrient availability for plant uptake. As aforementioned Marsh & Pierzynski (1998) suggested the excess N may have a counterproductive effect. Amendments of N are known to increase the activity of belowground biomass (Ghafoor et al. 2017; Poeplau et al. 2018). However, NH_4^+ and P have been shown to work synergistically, enhancing the P available for plant uptake, which could potentially be the result of increasing rhizosphere acidity. Decreases in pH are also a result of plant root modifications to the rhizosphere, with the exudation of additional organic acids increasing P - based compounds availability for plant uptake (Oburger et al., 2008)

(vi) Shoot Biomass

Average shoot biomass appears to decrease with the application of any type of P-based fertiliser which is in stark contrast to a plethora of studies. MS, at 575 g per rhizotron is the lowest of all yields, followed by Struvite at 665, control at 708 and IS at 776 (Table 8). Again, the below and above ground biomass correlate: lower root to lower shoot mass (Figure 17). There was a significant difference for shoot biomass, as IS exceeded MS by just over 14% for dry weight. In contrast with the present experimental findings, Ackerman et al. (2013) reported a lower growth rate for Struvite when compared with commercial fertilisers whereas our study demonstrated an increase. The contrasting experimental results have not been made any clearer through this study, with only observable, yet no significant differences between the treatments.

4.1.8 Concluding Remarks

The application of all P fertiliser treatments at 6 cm increases overall root growth in *Lolium Perenne* in comparison with surface and 3 cm placements. Further investigation is required to

monitor the acidity of the rhizosphere with and without ammonium to ensure effects are based on P fertilisers and not NH_4 . Field experiments are again required to assess how effective banding depths would be with Struvite in differing soil types, in an environment more representative of use on commercial scale for grass swards. As banding has successfully been applied *in situ* with other fertilisers, it would appear to be a case for applying these principles with Struvite.

Experiment 2

5.1.1 Abstract

Phosphorus (P) is an essential nutrient for plant growth. The use of P - based fertilisers for ensuring sufficient agricultural production in order to feed exponentially growing human populations has been practiced since the green revolution. However, inefficient and environmentally harmful practices, as well as the impending threat of reaching peak P production, has prompted the need to seek alternatives to conventional, soluble P-based fertilisers. This study seeks to demonstrate how *Lolium Perenne* acts under P nutrient deficiency under different P-based treatment regimes, specifically focusing on the direction of root growth. In a growth cabinet, falcon tubes were filled with a sand medium, fertiliser(s) were mixed into the top 0.5 mm and *Lolium Perenne* was sown). Struvite granules resulted in the highest root to shoot biomass ratio at 1.6 in comparison with fertilisers of greater solubility: struvite granular (SG) > struvite granular crushed (SGC) > ICL crystallised struvite (IS) > triple super phosphate (TSP) > monoammonium phosphate (MAP) > triple super phosphate granular (TG) > triple super phosphate granular crushed (TGC). In all fertiliser treatments, root growth was affected, with 10.6 - 62% greater biomass in the upper compartments of the tubes in comparison with the control. The trend was as follows: MAP > TSP > TG > TGC > IS > SG. There were significant differences amongst treatments while the more soluble fertilisers (TSP and MAP) shows the greatest differences in root biomass between top and bottom compartments at 55 and 62 %, respectively. The study demonstrates that root growth is directional and therefore, the application of P-based fertiliser must be placed appropriately for the root to grow in the direction required.

Keywords: slow release; deficiency; root zone; rhizosphere; growth rates; direction

5.1.2 Introduction

N, P and K are the 3 main essential macro-nutrients required for plants growth, henceforth, the sustainability of our current food systems. As peak P supply approaches the need to find efficient methods of fertiliser application, that ensure environmentally sound agricultural practices is imminent (Withers et al., 2018). Plant roots extend towards the P source consequently, enhance growth with P sources at further depths (Byrne et al., 2011; Hill et al., 2006; Sheil et al., 2016).

Struvite is a novel, granular fertiliser ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$), offering a recovered source of P (Rahman et al., 2011; Mu, 2012; Nelson et al., 2003; Yetilmezsoy & Sapci-Zengin, 2009). However, studies have demonstrated that, when applied in granular form, it is far less effective in terms plant growth parameters when compared with less environmentally friendly P fertiliser forms (Ahmed et al., 2019). Studies thus far have not looked at Struvite in comparison to other P-based fertilisers with differing treatment bands, nor have they looked at the impact of separating the P from other nutrients, inducing deficiencies, henceforth, the aims and objectives are highlighted below.

5.1.3 Aims and Objectives

1. To quantify the directional flow of plant root movement towards differing P-based fertiliser sources under induced P-deficiency

5.1.4 Hypothesis:

1. Inducing P deficiency will cause and increase in root proliferation in the direction of P-fertiliser sources.

5.1.5 Methods

An experiment was conducted in a growth cabinet (find growth cabinet details), with temperature at 21 °c, humidity at 80 % with controlled lighting (8 hours sunlight, 16 hours dark). The bottom of 36 50 ml falcon test tubes were removed and a 2 mm mesh attached to the base, enabling root growth while ensuring the sandy substrate was kept within the tube. A 10 cm piece of wick was then passed through the mesh. Approximately 60 g of a sandy substrate provided by ICL fertiliser co., was added to each 50 ml tube. The fertilisers were then weighed out and was mixed in to the top 2 cm of substrate in the tubes at the P equivalent to three Struvite granules: P sources were added as follows: Struvite (granular and crushed), ICL (MgNH_4P , crystalised), TSP (granular and crushed), MAP and TSP (soluble). The controls were subject to the same treatment – P. And extra 5 g of the substrate was added in a shallow layer above the fertiliser to provide a neutral layer for seedling propagation. *Lolium Perenne* was added with 8 seeds per pot, an estimated amount to ensure no overcrowding. Another set of 36 50 ml tubes were filled with a Hoaglands nutrient solution (-P up) to 30 ml to provide sufficient nutrient supply to the seedlings with the exception of P. This was to induce P-deficiency in the plant system (80 kg per hectare. The wick from the fertiliser-seed tubes was

then immersed into the tube containing Hoaglands (-P) solution. The experiment ran for 56 days, shoots were cut and roots were carefully removed from the sandy substrate and then washed, oven dried (80 and 105°C). Roots were cut in to 2 compartments, R1 being the roots within the test tube and R2, section of the root that grew below the test tube (Figure 18).

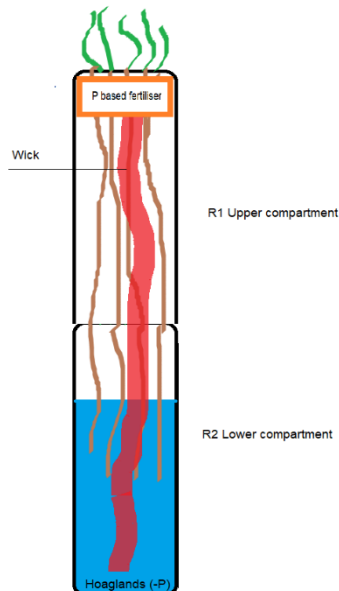


Figure 18. Experimental design showing R1, being the upper compartment of the root zone and R2 showing the lower compartment of the root zone. P-based fertiliser is placed at the top of the falcon tube and the wick (red) goes from the upper to lower, avoiding the P based fertiliser.

5.1.6 Statistical analysis

Variance homogeneity and normality of data were tested for each parameter by visual inspection (quantile-quantile plots) and a statistical aids (Shapiro- Wilk test ($p > 0.05$)) (Crawley, 2007) before conducting further analysis. Data were transformed and outliers were removed. The data were submitted to a Kruskal Wallace to test the fertilizer effect through the main parameters: Root and shoot biomass. When significant, means were compared using Tukey ($p < 0.05$). All the statistical analyses were performed by R Programming.

5.1.7 Results and Discussion

Most fertilizers demonstrated significant difference in root biomass between the upper and lower layers of the falcon tube, indicating roots growth favorably towards the P fertilizers. Root-shoot ratios also differed significantly among treatments (Figure 19).

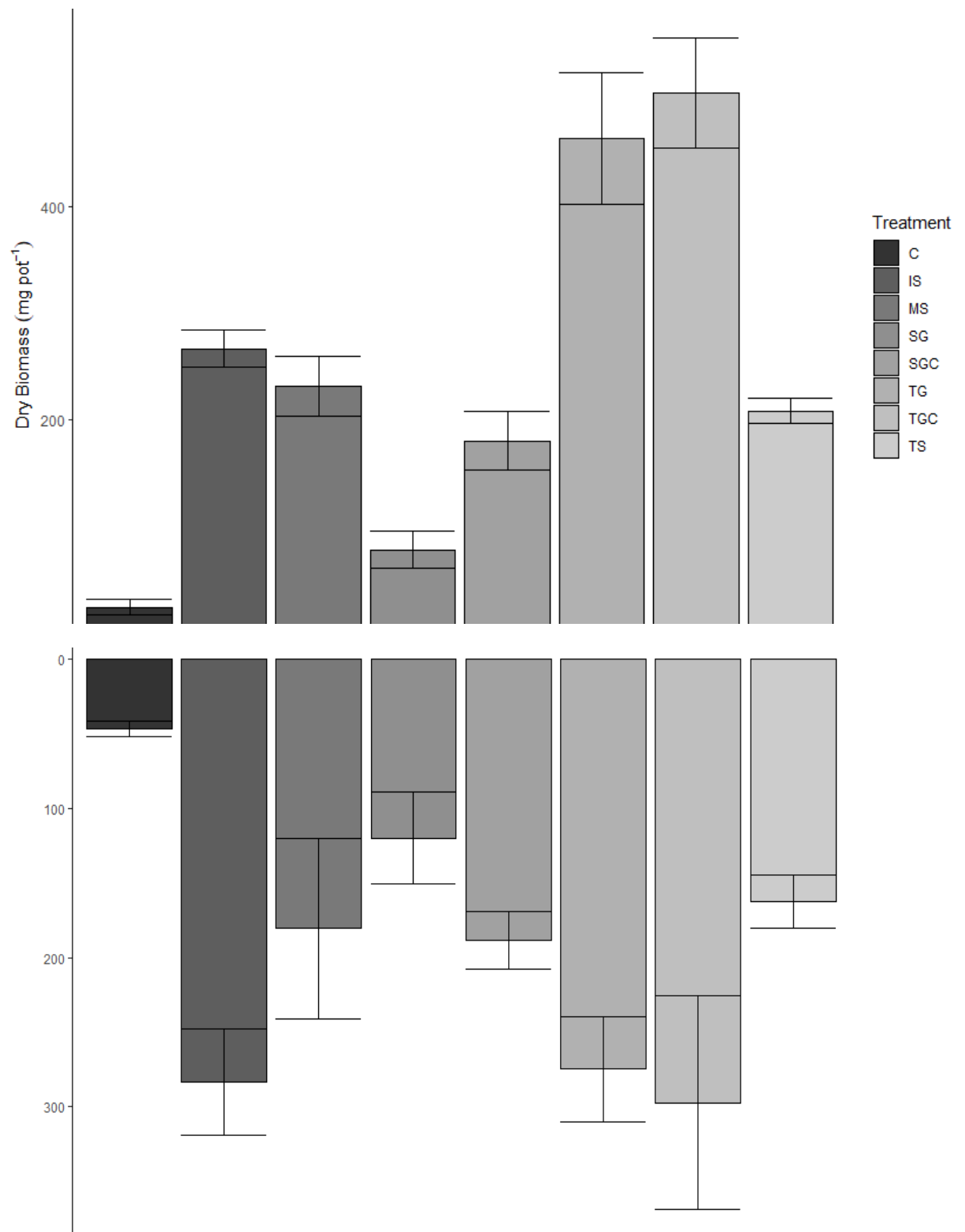


Figure 19. Shoot dry weight at top, root dry weight at bottom (mg pot⁻¹) of P-based fertilisers: MAP solution (MS), MAP solution plus AMF (MSA), ICL co. struvite (IS), Ostara Green struvite (SG), Ostara green struvite crushed (SGC), Ostara green struvite plus AMF (SGA), TSP granular (G), TSP granular crushed (TGC), TSP granular plus AMF (TGA) as fertilisers. NG=4, Vertical lines = ± 1 standard error.

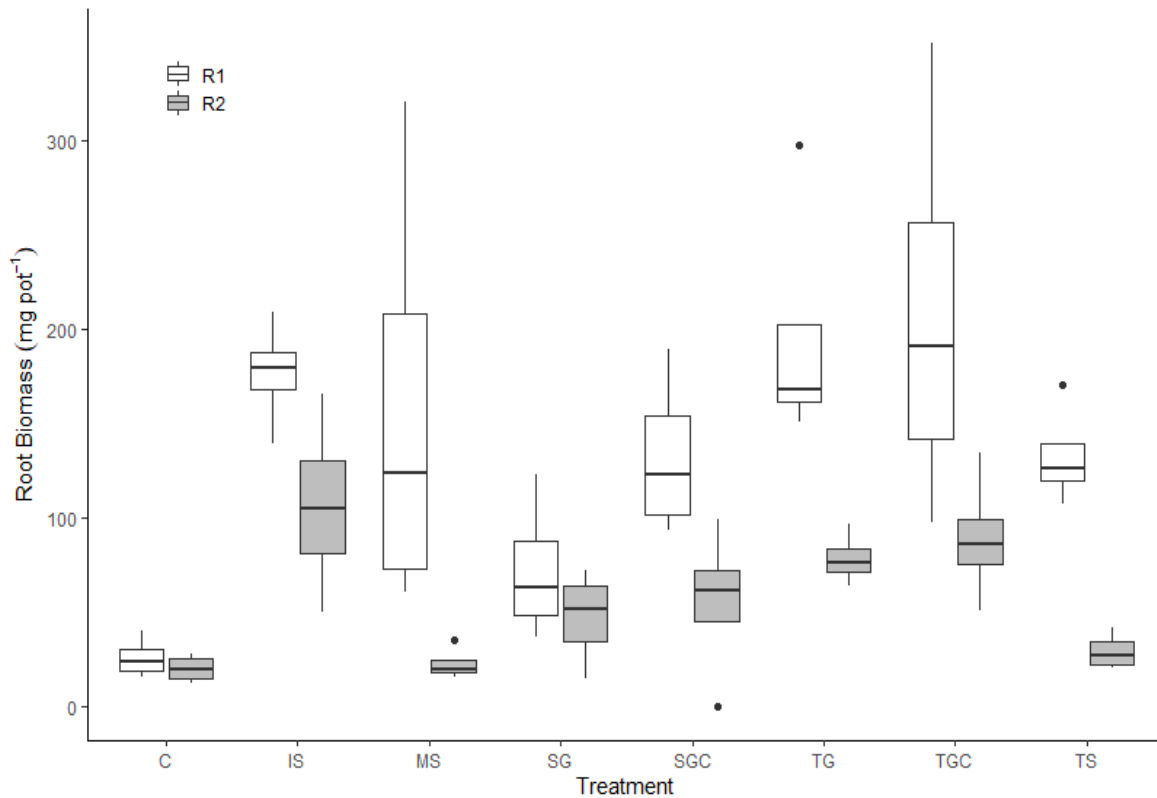


Figure 20. Root to Shoot Biomass by compartment: R1 is the upper, R2 is the lower. MAP solution (MS), ICL co. struvite (IS), Ostara green struvite (SG), Ostara Green struvite crushed (SGC), Ostara Green struvite plus AMF (SGA), TSP granular (G), TSP granular crushed (TGC) as fertilisers.

(i) Above and Below Ground Biomass Yields

Above and below ground biomass are impacted by P fertiliser treatment, with variability of the effectiveness in differing forms applied. For example, TG and TGC demonstrated the greatest above ground DM yields at 507 and 464 mg pot⁻¹ whereas the treatments with greatest root biomass, MAP and TSP, were less than half that of the granular TSP form at 232 and 208 mg pot⁻¹. There was no significant difference in overall root biomass between the above-mentioned treatments which suggests, overall, the TSP granules are improving allocation of nutrients to the shoot of the plant. The only additional nutrient in TSP is calcium, which has been shown to limit P uptake and therefore growth in various studies (Jakobsen, 1979; López-Lefebvre et al., 2001). Calcium has the potential to heighten soil pH, which may neutralise any acidifying effect of the nutrients in the solution below, potentially assisting with uptake as oppose to depleting it. The granular form allows a greater amount of nutrients release throughout the growing season due to the slower rate of dissolution, which may explain why the granular version was more effective in this case. The TSP in solution may also have

leached through the soil profile, although, Figure 19 would suggest otherwise, with a high rate of root biomass growth in the upper compartment.

(ii) Roots in Higher Versus Lower Compartments

Roots grew preferentially towards all P fertiliser treatments (Figure 20) in comparison with the control, all P fertilisers demonstrated observable differences between root weight in the higher compartment versus the lower compartments. The greatest difference between R1 and R2 in comparison with the control was 62 and 54.3 % for MAP and TSP, respectively. He et al. (2003) found plant roots to preferentially grow, as much as 70-75%, in the high P compartment of a split plot treatment, supporting the findings in this paper. Xin et al. (2000) also demonstrated P uptake as well as increase in biomass under split-root culture for wheat, while Jing et al. (2010) showed plant roots proliferate in response to the local application of both ammonium and P with DM yields and P uptake increasing. Where P is deficient, root zones in low P may allocate a portion of carbon resources to the zones with higher P portion.

(iii) Root growth and P source

The form, as well as the chemical composition of the P fertiliser applied may affect root growth towards the P source. The highest percentage of root growth towards the P source was MAP (62%) and the following trend was observed: MAP > TSP > TG > TGC > IS > SG. Roots of *Lolium Perenne* grew preferentially towards the most available P sources in the upper compartments.

5.1.9 Concluding Remarks

The P fertiliser type and form impacted the plants growth, with greater biomass found with the TG and TGC treatments. When inducing P deficiency with adequate supply of other macro- and micronutrients, the roots tended to grow towards the P, suggesting that in times of deficiency, they grow towards a P source. This agrees with several studies. Granular struvite remains a low performer, even in times where root growth is enhanced towards the fertiliser, suggesting the mechanism for dissolution is not so strongly affected by root growth (i.e. CaCl_2 root exudates) around the granule as previously suggested (Hinsinger, 2001b). Overall, this study confirms roots grow towards P – based fertilisers in times of deficiency, and that TSP is the most effective fertiliser to demonstrate this root activity.

Chapter 5

Conclusion and Further Research

5.1.1 Summary

This study aimed to evaluate the overall efficiency of struvite in comparison with conventional P-based fertilisers and assess implications for plant health and resilience to environmental change. Overall, this investigation confirms that commercial grade, granular struvite is less effective in terms of enhancing plant performance in comparison with fertilisers of greater solubility in a sandy medium with *Lolium Perenne*. However, the fertilisers' minimal impact on microbial activity suggests it may be an effective tool to enhancing aspects of environmental welfare. This study has demonstrated that struvite performance can be enhanced through methods such as banded placement (3 and 6 cm); additional of a soluble fertiliser in early growing season; the application of crystalised struvite or crushed granules (or smaller size with greater surface area); potentially AMF addition although additional research is required to confirm the final point.

5.1.2 Limitations to the research

Chapter 3 expressed theoretical impacts of fertilisers on the soil particle and would have benefited greatly from a combination of our theoretical model and practical application. The lack of ^{33}P results was also limiting: the quantities of Phosphorus that were bound to the soil for each fertiliser concentrations were indecipherable and therefore, to what extent the P concentrations and pH were responsible for microbial activity, was also unexplained.

Both pot experiments would have benefited from cuttings every 3 weeks, increasing biomass quantity and therefore, allowing for N analysis. For "Pot Experiment 1", AMF inoculation would have been tested prior to the onset of the pot experiments as there was no evidence of fungi presence in the core samples, even though the plants performed better than with no inoculant in general.

Root analysis for the Rhizotron experiments would have been performed in greater detail to further investigate the impact of struvite on root architecture. The combination of light roots and the sandy medium did not enable sufficient definition of the root profile for such analysis. The addition of NH_4^+ and KCl to the controls may have altered the experimental results and, were the experiment to be repeated, controls would have only micronutrient addition.

The variability of results throughout each chapter suggests that either the fertilisers performed differently in the variation of settings or there were errors made throughout experimentation. However, the latter may have manifested in larger error bars and therefore the reasons for the variability remain inconclusive.

5.1.3 Further research

Further investigations on pH and EC within the context of ^{14}C microbial activity that would encompass analysis on CO_2 emissions under P fertiliser application, as well as alternative fertilisers (minus P) combined with the examination of microbial species composition within a variety of soil types using molecular techniques (PFLA; 16s RNA analysis). A longer field experiment, or alternatively, a pot experiment, would provide a realistic understanding of microbial activity with plants present and a more practical example of C mineralisation and respiration. In addition, investigations into material distances using microtome techniques would elucidate the theoretical experiment of chapter 2. The “microbial dead zone” theory, as briefly explored in Chapter 3, would involve a more detailed exploration of the temporal and spatial dynamic in micro-pore space within the soil by using techniques and modelling from work such as (Ruiz et al., 2020).

Investigation of each pot experiment with multiple forms of would be of benefit in order to establish the most effective N based fertiliser for plant growth with struvite.

Additional investigations are warranted in the field to establish the viability of Struvite in a commercial setting. The practicality of the application methods such as fertiliser banding and additional soluble fertiliser must be tested in a variation of soils and environmental conditions as the slow release mechanism is a key feature in its effectiveness.

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