Short-term phosphorus dynamics and wheat productivity under integrated fertiliser management

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Submitted in accordance with the requirements for the degree of

Doctor of Philosophy

The University of Leeds

School of Biology

September 2019

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

Acknowledgements

With deepest thanks to Professor Les Firbank and Professor Steven Banwart for their guidance, encouragement, time and knowledge. Thanks also to Professor Pippa Chapman for her help shaping this project and for pointing me towards useful resources.

I would like to thank my peers for providing me with practical support and reassurance. Thanks especially to Hannah Davis, Lauren Smith, Sofia Biffi, Dr Conor Gould, Dr Helen Gray, Dr James Rouse, Dr Katie McDermott, Dr Mary Friel, Dr Mehrnaz Montazeri and Dr Steven Laird.

My greatest thanks go to the technical team in the School of Geography, University of Leeds, especially Rachel Gasior and David Ashely, who helped more than was required. Thanks also to Martin Lappage for help with field work and to Frank Dodds and Lance Penketh for accommodating me in the greenhouse.

To my parents, Trish and David, for believing in me, reminding me you are proud of me and only pushing me when it really mattered. Thanks for giving me perspective and balance. Also to my brothers, Thom and Michael, for being great friends and keeping me grounded.

And finally, thanks to Brendan who has endured many of the challenges I’ve faced with ease and kindness. Thanks for making life fun and exciting. I can think of no better person to go through this process with.

Abstract

Modern phosphorus (P) fertiliser production depends on phosphate rock reserves which could be exhausted by 2040. Substituting inorganic P with organic fertilisers may reduce farmers' requirements for rock-derived phosphate while encouraging the use of waste products. Research shows that integrating the use of organic and inorganic fertilisers increases P phytoavailability compared to the application of inorganic P alone. Field studies typically report results of one sampling occasion conducted after years of experimentation, with no intermittent monitoring. The aim of this thesis was therefore to determine how substitution of inorganic P with organic alternatives affects short-term P cycling and wheat productivity. Results were obtained from intact soils cores maintained in a greenhouse sown with winter wheat and fertilised with different ratios of organic-to-inorganic P. There was no statistically significant effect of substituting inorganic P with pig slurry (PS) or digested cake (DC) on available P concentration during stem elongation or anthesis compared to the application of inorganic P alone. The results suggest that increases in phytoavailable P reported in previous studies following integrated fertiliser management (IFM) may not develop immediately but through gradual accumulation of the soil reserve. The time it takes for these differences to emerge could depend on the organic amendment; repeated measures analysis showed that the PS substitution treatment provided a more sustained supply of phytoavailable P than the DC substitution treatments. Grain yield and P leaching losses were statistically similar between treatments. The thesis therefore shows that in the short term, inorganic P use could be reduced through the incorporation of organic amendments to soil without compromising yield, P phytoavailability or P leaching losses. P application rates should be matched between treatments and respond to changes in P

phytoavailability between seasons to better understand the effect of IFM on P cycling and yield over time.

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Abbreviations

$(NH_4)_6Mo_7O_{24}\bullet 4H_2O$	Ammonium paramolybdate
[N]	Nitrogen concentration
[P]	Phosphorus concentration
ACP	Acid phosphatase
AD	Anaerobic digestion
Al	Aluminium
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
B	Boron
C	Carbon
Ca	Calcium
$CaCl_2$-P	Calcium chloride extractable P
$Ca(H_2PO_4)_2$	Monocalcium phosphate
Ca-ex	Exchangeable calcium
CET	Cumulative evapotranspiration
CH_4N_2O	Urea
CI	Confidence interval
CMC	Cattle manure compost
CO_2	Carbon dioxide
Cu	Copper
DAS	Days after sowing
DC	Digested cake
diH2O	Deionised water
DOM	Dissolved organic matter

FA	Fulvic acid
Fe	Iron
GHG	Greenhouse gas
GLM	Generalised linear model
GWP	Global warming potential
H1	Harvest 1
H2	Harvest 2
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HA	Humic acid
HB	Haber-Bosch
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate
HMR	Hierarchical multiple regression
HWP	Hot water extractable P
IFM	Integrated fertiliser management
IP_6	Inositol hexaphosphate
K	Potassium
K_2O	Potassium oxide
KCl	Potassium chloride
$KSbO \bullet C_4H_4O_6$	Potassium antimony tartrate
LOA	Low molecular weight organic acid
LOI	Loss on ignition
MgO	Magnesium oxide
MWD	Mean weight diameter
N	Nitrogen

N_2	Dinitrogen gas
N_2O	Nitrous oxide
Na	Sodium
Na_2O	Sodium oxide
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
NH_3	Ammonia
NH_4^+	Ammonium
NH_4-N	Ammonium-N
NH_4NO_3	Ammonium nitrate
Ni	Inorganic nitrogen
NO_2^-	Nitrite
NO_2-N	Nitrite-N
NO_3^-	Nitrate
NO_3-N	Nitrate-N
NO_x	Nitrogen oxides
NVZ	Nitrate vulnerable zone
O_2	Oxygen
OA	Organic acid
OD	Oven-dried
OH-	Hydroxide
OM	Organic matter
P	Phosphorus
P_2O_5	Phosphorus pentoxide
Pi	Inorganic phosphorus
PK	Potash-phosphate

$P_{Olsen's}$	Olsen's phosphorus
$P_{organic}$	Organic phosphorus
PS	Pig slurry
PVC	Polyvinyl chloride
P_{water}	Water-soluble phosphorus
RAN	Readily available nitrogen
RPD	Relative percentage difference
S	Sulphur
SEM	Standard error of the mean
SO_2	Sulphur dioxide
SOC	Soil organic carbon
SOM	Soil organic matter
SRP	Soluble reactive phosphorus
SSA	sub-Saharan Africa
TDW	Total dry weight
TN	Total nitrogen
TP	Total phosphorus
TSP	Triple superphosphate
UAN	Urea ammonium nitrate
UK	United Kingdom
WFPS	Water-filled pore space
WWTP	Waste water treatment plant
Zn	Zinc

Chapter 1 Introduction

1.1 Overview

Cereals provide 50% of daily calorific intake in developing countries but their production is of global importance; wheat alone provides 19% of global dietary energy (FAO, 2017a; Ray et al., 2013). Cereal production will need to increase by 45.4% between 2005 and 2050 to meet the demands of a growing global population with shifting dietary habits (Alexandratos & Bruinsma, 2012; Keyzer et al., 2005; Pelletier & Tyedmers, 2010). At the same time, competition for agricultural land between food and fuel is increasing and reserves of the finite resources used in the modern food production system are declining (FAO, 2017a; Smil, 2000).

Inorganic fertilisers were one of the key components that led to rapid yield increases achieved during the Green Revolution (Tilman et al., 2002, 2001). However, their use is associated with aquatic, terrestrial and atmospheric pollution and their production depends on finite resources (Bobbink et al., 1988; Bobbink & Willems, 1987; Correll, 1998; Miyazako et al., 2015; Smil, 2000; Tipping et al., 2014; Vitousek et al., 1997). Organic amendments introduce nutrients to soil without relying on inorganic fertilisers. When derived from waste products such as municipal waste water, the use of organic fertilisers also encourages closed-loop nutrient cycling by diverting nutrients away from waterbodies and back into agriculture. This is particularly beneficial for phosphorus (P) based inorganic fertilisers sourced from rock reserves because it has been predicted that peak P production could occur as early as 2030 (Cordell et al., 2009).

The substitution of inorganic fertilisers with organic alternatives is promoted as a means to reduce farmers' requirements for inorganic fertilisers while minimising the environmental impact of agriculture (Defra, 2010; Nemecek et al., 2011; Shuqin & Fang, 2018). Long-term studies have shown that the integrated use of organic and inorganic fertilisers can maintain or increase yields compared to the application of inorganic fertilisers alone (Bedada et al., 2014; Ge et al., 2010; Pincus et al., 2016; Xie et al., 2016). This practice also increases the concentration of available P in soil after years of repeated applications, compared to the application of inorganic P only (Ahmed et al., 2019; Hu et al., 2018; Xin et al., 2017). However, the effect of integrated fertiliser management on short-term soil phosphorus dynamics and wheat productivity is not well understood. This thesis asks how substitution of inorganic P with organic alternatives affects P phytoavailability, P leaching losses and productivity during the life-cycle of wheat, compared to the application of inorganic fertilisers alone. Conducting short-term studies with matched P application rates between treatments is important scientifically, because incubation studies show differences between inorganic and combined treatments in the very short-term (Reddy et al., 2005), and in the broader agricultural setting because phosphorus is taking over as the limiting nutrient for fertiliser application rates across Europe (Sigurnjak et al., 2017).

1.2 Phosphorus and its place in agriculture

1.2.1 Phosphorus fertiliser production and agricultural use

P use in agriculture has a long and important history. Over time, the dominant source of P used for fertiliser has shifted from human and animal waste to phosphate rock (Cordell et al., 2009). Consumption of P rock rose dramatically during the mid- to late 20th century supporting rapid increases in yield (Cordell et al., 2009). Supplies of rock P are declining in terms of size and quality (Smil, 2000), with some authors predicting that peak phosphorus production will occur within 15 years (Cordell et al., 2009). More recent estimates predict that all remaining reserves could be exhausted by 2040 (Blackwell et al., 2019). It is evident that this resource must be used more efficiently or be re-captured in order to prevent the collapse of a farming system that is “addicted” to P (Cordell et al., 2009). The need for alternative P sources is particularly pertinent for countries, such as those in northern Europe, which have no P rock reserves and must rely on imports for P fertilisers (Elser & Bennett, 2011).

1.2.2 Phosphorus cycling in agricultural soils

1.2.2.1 Inputs

The soil P cycle can be separated into four major stages: (i) inputs of P; (ii) P in temporary soil P pools; (iii) transformation of P between pools; and (iv) losses of P from the soil system (Figure 1.1). P enters agricultural soils via the weathering of phosphorus minerals such as apatite, applications of mineral and organic fertilisers and atmospheric deposition in dust and soil (Chianu et al., 2012; Tipping et al., 2014). P inputs to agricultural soils are likely to be dominated by fertiliser applications although the contribution of each input varies between systems (Kruse et al., 2015). Inorganic P use on winter and spring cereals in

Britain decreases in the order potash-phosphate (PK), triple superphosphate (TSP), other straight P (Defra, 2018). Approximately 51,000 tonnes of TSP, which is derived from rock phosphate, was applied to winter cereals in Britain during 2017 (Defra, 2018).

1.2.2.2 Phosphorus in soil

There is confusion in the scientific literature surrounding phosphorus nomenclature given the various phosphorus forms that exist in soil and the numerous methods that are used to extract P from soil. For clarity, it would be simplest to discuss P chemically, by referring to specific molecules such as apatite, inositol phosphate or dicalcium phosphate. While this is perhaps the most conclusive way to discuss P, authors are often more interested in P availability to plants and microbes and vulnerability to leaching than molecular definitions. For this reason, phosphorus is often discussed in terms of pools or fractions (Figure 1.1).

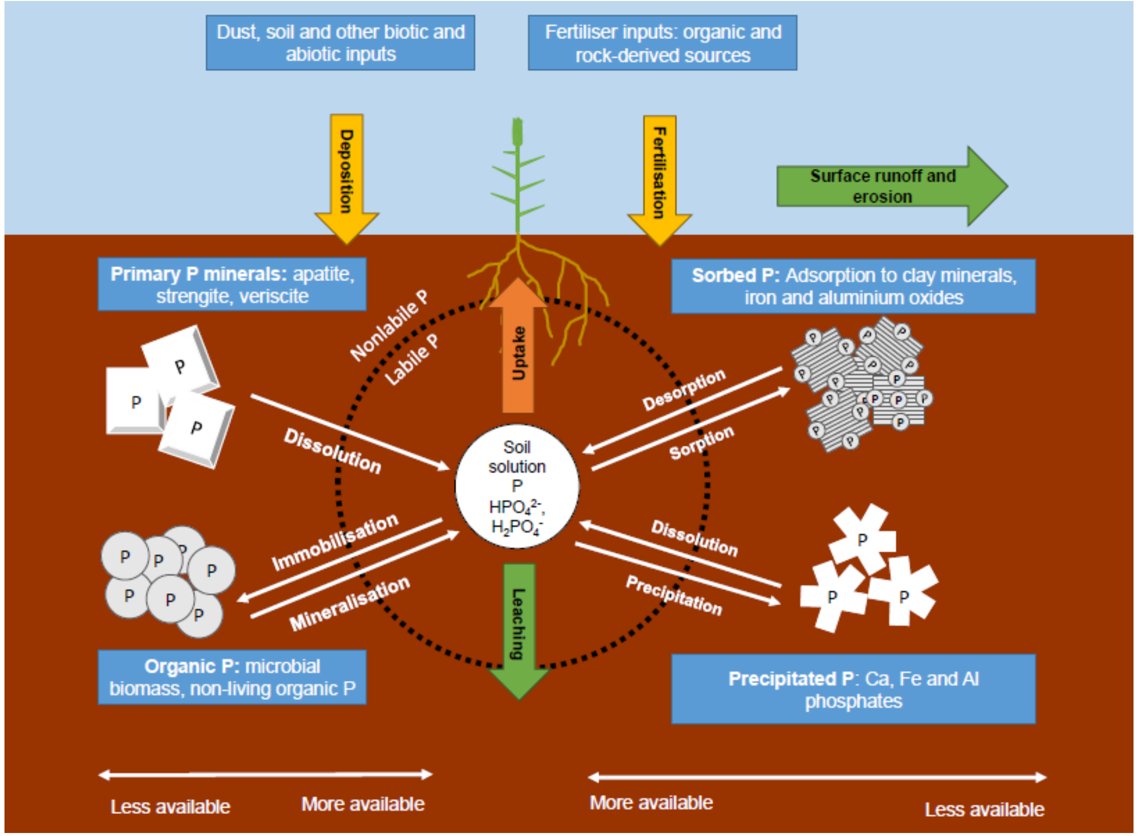

Figure 1.1: The soil phosphorus cycle. Inputs, losses and plant uptake are represented by yellow, green and orange arrows, respectively. As phosphorus (P) forms are dissolved, desorbed or mineralised they become available to plants and vulnerable to leaching. P can be chemically defined, existing in molecules such as dicalcium phosphate (precipitated P) or inositol phosphate (organic P). P forms can also be defined based on their availability to plants rather than chemical properties. For example, labile P doesn't relate to one P molecule but to forms that are directly exchangeable with soil solution. Adapted from Shen et al., (2011)

Soil P can be divided into five major pools (Figure 1.1): (i) primary P minerals; (ii) secondary P minerals; (iii) sorbed P; (iv) organic P; and (v) solution P (Shen et al., 2011). P in primary minerals such as apatite, strengite and veriscite is considered unavailable to plants as the physical breakdown and chemical weathering of these sources lags behind crop demand (Shen et al., 2011). Estimates for P release from weathering of primary minerals are in the range of 0.05 – 5 kg P ha^{-1} $year^{-1}$ (Parfitt et al., 2008).

Once in solution, P from the weathering of primary minerals can be precipitated into secondary P minerals such as calcium (Ca), iron (Fe) and aluminium (Al) phosphates. The form of secondary mineral formed by precipitation is pH dependent, as pH dictates the concentration of Ca, Al and Fe cations in soil (Hinsinger, 2001). Ca phosphates are more common in neutral to alkaline soils, while Fe and Al phosphates are more common in acidic soils (Hinsinger, 2001). The phytoavailability of secondary minerals present also changes with pH. As pH increases, calcium phosphates with lower phytoavailability such as hydroxyapatite dominate. On the contrary, iron and aluminium phosphates become more phytoavailable with increasing pH (Hinsinger, 2001). Secondary P minerals become phytoavailable through dissolution, the rate of which is affected by the distribution of the mineral particle being dissolved and soil pH (Kirk & Nye, 1986; Nye & Kirk, 1987; Shen et al., 2011).

P can also be adsorbed onto the surface of clay minerals and aluminium and iron oxides which have a large specific surface area for sorption (Gérard, 2016; Shen et al., 2011). Over time, P can become occluded in nanopores of Al and Fe oxides, where it is plant unavailable (Shen et al., 2011).

Unlike occluded P, organic P is an important source of potentially-phytoavailable P particularly in soils with low phosphorus solubility but with a large organic P pool (Tiessen et al., 1984). However, organic phosphorus exists in many forms which differ in their phytoavailability (Quiquampoix & Mousain, 2004). For example, inositol phosphates are resistant to mineralisation, form insoluble salts in acid soil and precipitate with calcium in alkaline soils (Quiquampoix & Mousain, 2004). Consequently, inositol phosphates are less phytoavailable than phospholipids which make up a smaller proportion of the organic P pool but have a faster turnover in soil (Quiquampoix & Mousain, 2004). Therefore, while organic P can account for up to 80% of the P in soil the phytoavailability of this pool depends on the forms that are present as well as soil chemical and physical properties (Shen et al., 2011).

The previous discussion of pools frequently mentions phytoavailable phosphorus, or the phosphorus in soil that is directly available for plant uptake. The most common methods used to quantify phytoavailable P are Mehlich's P, Bray's P and Olsen's P which are used for analysis of acid, alkaline-to-neutral and neutral soils, respectively. Olsen's P is the main extraction technique used to determine phytoavailable P in England, Wales and Northern Ireland and is used to categorise soils for fertiliser recommendations (Agriculture and Horticulture Development Board, 2017). Water-soluble phosphorus is another example of a commonly quantified P pool, because the concentration of P_{water} in soil is correlated with P leaching losses (Pote et al., 1996). Phytoavailable P and P_{water} is often referred to as 'labile P', which is P that rapidly equilibrates with the soil solution (Pierzynski et al., 2005). In Figure 1.1, Olsen's P and P_{water} would be located within the dashed circle indicating that they are labile forms of P. Soil P that is slow to equilibrate with soil solution is termed 'nonlabile'.

There are well-established extraction techniques used to estimate the proportion of P in soil that is labile and nonlabile. The most commonly used technique was developed by Hedley et al., (1982) and involves the sequential use of increasingly strong reagents to extract decreasingly labile forms of P. In this instance, the P fraction may be discussed in terms of the extractant used or the anticipated lability of the fraction. The true molecular composition of the P forms present in pools can only be hypothesised (Pierzynski et al., 2005) unless there is further analysis using techniques such as X-ray absorption near-edge structure (XANES) spectroscopy which provides information about the chemical nature of P in each fraction (Takamoto & Hashimoto, 2014).

1.2.2.3 State-of-the-art methods in soil phosphorus analysis

The number of state-of-the-art methods in soil phosphorus dynamics has increased considerable in recent years. Innovative methods have been reviewed extensively by (Kruse et al., 2015) and thus will be discussed briefly here. Techniques can broadly be divided into approaches that: (1) identify and quantify given P species; and (2) assess soil P reactions. Methods including ^{31}P NMR spectroscopy and direct infusion nanospray quadrupole time-of-flight mass spectroscopy allow quantification of specific P species and microbial communities in soil, respectively (Kruse et al., 2015). Both techniques provide considerable benefits over more traditional approaches, including simplified sample preparation and more accurate outputs (Kruse et al., 2015). A different suite of techniques is adopted for the study of soil P transformations. This includes, but is not limited to, P sorption isotherms (Jiao et al., 2007; Kang et al., 2011; Wei et al., 2019), estimation of phosphatase activity using artificial P substrates (Spohn, Carminati, et al., 2013; Tabatabai, 1994) and the use of the radioactive ^{33}P isotope to trace the flow of P through microorganisms, soil and plants (Noack et al., 2014; Spohn, Ermak, et al., 2013). The technique adopted will depend on the research question, and certain studies may adopt multiple techniques to achieve their objectives (Negassa et al., 2010). A multi-method approach, combining both state-of-the-art and traditional wet chemistry approaches, is encouraged by Kruse et al. (2015) to gain a holistic understanding of soil P pools and dynamics.

1.2.2.4 Losses

Phosphorus can be lost from soil through leaching, runoff, erosion or as a result of plant uptake (Figure 1.1). Leaching, surface runoff and atmospheric deposition of P contribute to elevated levels of nutrients in waterbodies, known as eutrophication (Correll, 1998; Miyazako et al., 2015; Tipping et al., 2014; Troost et al., 2013). Eutrophic waterbodies are starved of dissolved oxygen (DO) due to increased oxygen (O_2) demand from a growing autotrophic community. Aquatic life that would thrive in oligotrophic streams, lakes and oceans is no longer supported in these anoxic or hypoxic environments. Eutrophication incurs economic costs (Dodds et al., 2009; Pretty et al., 2003) and threatens human health (Chorus et al., 2000; Preece et al., 2017). Agriculture is the major source of P causing eutrophication (Heckrath et al., 1995; Smil, 2000; Svanbäck et al., 2013), responsible for 25-75% of P loads in European waterbodies (European Environment Agency, 2005). It is crucial to minimise the flow of P from agriculture to waterbodies, especially as global warming is expected to aggravate eutrophication (Lürling et al., 2017).

1.2.2.5 Plant uptake

Plant uptake of P as $H_2PO_4^-$ and HPO_4^{2-} is an important flow in the P cycle. P uptake occurs by active transport across the root plasma membrane via symporters including the PHosphate Transporter 1 (PHT1) family of membrane proteins (Ceasar et al., 2016; Nussaume et al., 2011).

Plants demonstrate numerous architectural and cellular adaptations to P deficiency. Architecturally, plants experiencing P deficiency have a higher root-to-shoot ratio, increased root branching, longer roots and more root hairs than plants in P-sufficient soils (Shen et al., 2011). Plants in a number of families including Proteaceae, Fabaceae and Myricaceae can also produce cluster roots under P deficiency (Shane & Lambers, 2005). Cluster roots are identified by sections of dense lateral root with high numbers of root hairs that release "exudative bursts" of carboxylate into the rhizosphere to mobilise P (Shane & Lambers, 2005). Plants can also increase P acquisition by forming symbioses with arbuscular mycorrhizal fungi (AMF). 80% of terrestrial plant species form associations with AMF, which provides plants with P in return for photosynthetically-derived carbon (Berruti et al., 2016). AMF increase the area of soil that can be exploited by the plant through the production of extraradical hyphae and can also mobilise P from forms that would otherwise be unavailable to the plant.

Plants can also alter internal cycling of P when they are experiencing P deficiency. Adaptations include the use of non-P lipids in the cell membrane to limit P consumption, acquiring P from organic forms including phosphomonoesters, acquiring P from old cellular tissue and alteration in cellular respiration pathways to avoid processes requiring inorganic P (Shen et al., 2011).

1.3 Nitrogen and its place in agriculture

Consideration is given to nitrogen because it is the most common limiting nutrient in agricultural soils (Rütting et al., 2018), the combined use of organic and inorganic fertilisers has been shown to affect N dynamics in soil (Frimpong & Baggs, 2010) and N fertilisation has been shown to affect P uptake by crops (Coblentz et al., 2004). Therefore, while phosphorus is the focus of this thesis an understanding of N dynamics and quantification of N availability is required to gain a comprehensive view of the effect of combined fertiliser applications on productivity and P phytoavailability.

1.3.1 Emissions from nitrogen production and agricultural use

The seven-fold increase in inorganic nitrogen (N) inputs to agricultural land between 1960 and 1995 is considered central to the doubling of global cereal production that occurred during the Green Revolution (Tilman et al., 2002, 2001; Vitousek et al., 1997). Agriculture remains dependent on inorganic N for yield maintenance with inputs expected to increase almost three-fold by 2050 compared to 2000 levels (Tilman et al., 2001).

There are significant sustainability and environmental concerns associated with the production and use of inorganic N fertilisers. Ammonia (NH_3) is the chemical feedstock required to produce ammonium nitrate (NH_4NO_3), urea (CH_4N_2O) and urea ammonium nitrate (UAN) which are the nitrogenous fertilisers most commonly used on cereal crops in Great Britain (Defra, 2018). More than 90% of the world's NH_3 is produced via the Haber-Bosch (HB) process (Gálvez et al., 2007). During the reaction hydrogen is combined with nitrogen in the presence of an iron (Fe) catalyst under high pressure and temperature (United States Environmental Protection Agency, 2017). Hydrogen is typically derived from steam reformation of light hydrocarbons such as natural gas and nitrogen is pooled from the air (Chen et al., 2018). The HB process requires non-renewable resources for energy production and results in the formation of carbon dioxide (CO_2) (Bicer et al., 2016; Rafiqul et al., 2005); it is estimated that NH_3 production consumes 1.2% of global primary energy demand (Chen et al., 2018) and emits 1.9 - 16.7 tonnes of CO_2 per tonne of NH_3 produced depending on the production facility and the fuel used (Rafiqul et al., 2005). Research into more efficient and sustainable alternatives to the HB process is ongoing (Chen et al., 2018; Cherkasov et al., 2015; Manabe et al., 2017). Meanwhile, agriculture remains dependent on an energy intensive, fossil-fuel based process to provide adequate N nutrition to crops.

Following their production, the misapplication of N fertilisers is associated with the degradation of aquatic and terrestrial ecosystems. The negative effect of N fertilisers on the environment can be reduced, for example, by making applications at the correct time and rate (de Boer et al., 2016; Ju et al., 2009; Trindade et al., 2009). However, agriculture remains the largest contributor of anthropogenic nitrous oxide (N_2O) emissions (European Environment Agency, 2018), a greenhouse gas (GHG) with a global warming potential (GWP) almost 300 times that of CO_2 over 100 years (Myhre & Shindell, 2013). N fertilisers contribute to aquatic eutrophication directly via leaching and surface runoff, and indirectly through deposition of nitrogen oxides (NO_x). NO_x deposition also fertilises terrestrial ecosystems, reducing biodiversity (Bobbink et al., 1988; Bobbink & Willems, 1987) and increasing N leaching which itself leads to eutrophication (Vitousek et al., 1997). Therefore while N fertilisers remain a critical cog in the modern agricultural system, their use must be carefully managed in order to minimise environmental degradation.

1.3.2 Nitrogen cycling in agricultural soils

The N cycle involves the transformation of N through soil, living organisms and the atmosphere (Figure 1.2). The processes of nitrification and denitrification are particularly important as they result in the formation of nitrate (NO_3^-) and N_2O, both significant pollutants (Figure 1.2).

Nitrification is a microbially-mediated reaction which takes place in two steps under aerobic conditions (Figure 1.2). First, oxidising bacteria catabolise ammonium (NH_4^+) into nitrite (NO_2^-) (Norton, 2008; Snyder et al., 2009). At this stage, NO_2^- is oxidised to NO_3^- by one of four genera of bacteria: *Nitrobacter* sp., *Nitrospira* sp., *Nitrococcus* sp., and *Nitrospina* sp. (Voroney & Derry, 2008). Nitrification represents the transformation of an immobile cation, NH_4^+, into a mobile anion, NO_3^- (Snyder et al., 2009). In the NO_3^- form, N is available for plant uptake but is also readily leached through the soil profile (Subbarao et al., 2006). NO_3^- is therefore important to study from a crop productivity and environmental perspective.

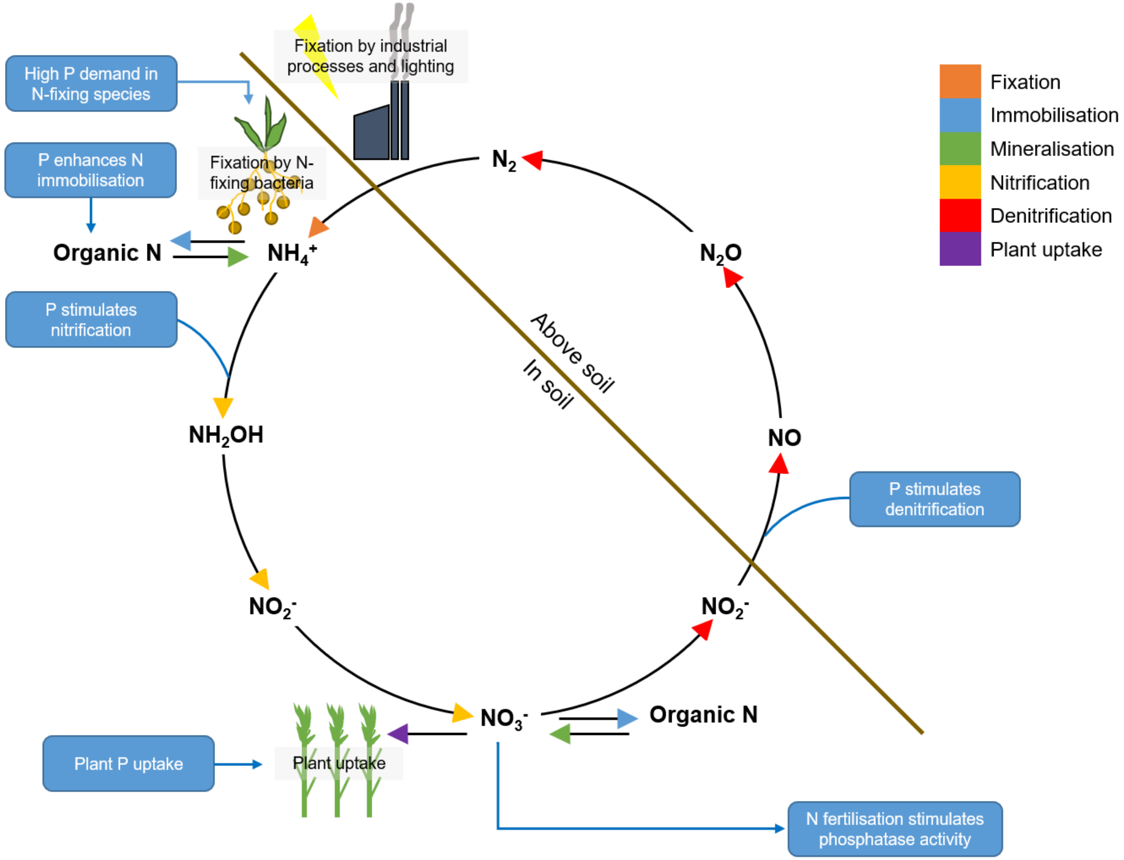

Figure 1.2: The Nitrogen Cycle. Nitrogen fixation converts dinitrogen gas in the atmosphere (N_2) in to ammonium (NH_4^+). NH_4^+ is transformed into nitrate (NO_3^-) via nitrite (NO_2^-) by the process of nitrification. NO_3^- is returned to the atmosphere via denitrification. Incomplete denitrification results in the production of nitrous oxide (N_2O), a potent greenhouse gas. NH_4^+ and NO_3^- can both become immobilised in organic forms, although this process is reversible via mineralisation. Blue boxes show links between the nitrogen and the phosphorus cycle

Further N can be lost when NO_3^- is reduced, by bacteria, to dinitrogen gas (N_2) via denitrification in anaerobic soils (Figure 1.2) (Coyne, 2008). During denitrification oxidised inorganic N compounds are returned to the atmosphere. Incomplete denitrification leads to the production of N_2O. Small losses of N_2O are environmentally important given the high GWP of this gas (Coyne, 2008; Kramer et al., 2006; Myhre & Shindell, 2013)

Mineralisation and immobilisation represent the conversion of N between organic and mineral forms (Figure 1.2). During mineralisation, enzymes produced by microorganisms convert organic-N to inorganic-N. As such, mineralisation represents an important process that contributes to N losses later in the cycle (Follett, 2008; Myrold & Bottomley, 2008; Snyder et al., 2009). Microorganisms assimilate inorganic N and in doing so convert inorganic-N to organic-N. This is the process of immobilisation, also referred to as microbial assimilation, which results in the production of plant-unavailable N.

The nitrogen and phosphorus cycle are intrinsically linked, through the stochiometric dependence of plants and microbes on N and P and the role of P in N cycling processes, and vice versa (Bouwman et al., 2009; Chen et al., 2016; He & Dijkstra, 2015; Marklein & Houlton, 2012). Figure 1.2 demonstrates some of these dependencies. P inputs affect N availability through several mechanisms. First, N-fixing plants and bacteria have greater demand for P than non-N-fixing counterparts. P inputs can stimulate N fixation by these species, increasing N availability through the production of ammonium and nitrate (Bouwman et al., 2009; Houlton et al., 2008). In P-deficient soils, P inputs can reduce N availability through enhanced microbial activity and N immobilisation (He & Dijkstra, 2015). Furthermore, P inputs reduce the effect of P limitation on nitrifiers and denitrifiers, stimulating N losses as nitrate and nitrous oxide (Chen et al., 2016; He & Dijkstra, 2015). N inputs also affect P phytoavailability through their role in the production of phosphatase enzymes. Phosphatases catalyse the hydrolysis of phosphomonoester bonds in organic P forms, increasing levels of phytoavailable P in soil. Phosphatase enzymes are rich in N, so their activity is responsive to N fertilisation (Marklein & Houlton, 2012; Widdig et al., 2019). These interdependencies further demonstrate the importance of studying and considering N and P reactions simultaneously.

1.4 Fertiliser effects on phosphorus and nitrogen cycling in agricultural soils

Fertiliser type influences the phytoavailability of N and P as well as the quantity of each nutrient leaving agricultural soils and entering the natural environment.

1.4.1 Use of phosphorus fertilisers in Great Britain

Farmers in the United Kingdom follow guidance in the Fertiliser Manual (RB209) published by the Agriculture and Horticulture Development Board (AHDB) when making decisions about fertiliser applications (AHDB, 2020). Following guidance in RB209 helps farmers to comply with regulatory restrictions related to the quantity and timing of fertiliser applications, save money and protect the environment (AHDB, 2020). The guide introduces seven key principles of fertiliser good practice: (i) obtaining relevant information related to soil type, historic agronomic practices, soil nutrient and physiochemical properties and local climate; (ii) assessing crop yield potential, economics and markets; (iii) assessment of available nutrients from organic manure; (iv) decisions on the rate, method and timing of fertiliser applications; (v) careful selection of fertiliser material; (vi) accurate application of fertilisers and manures; and (vii) record keeping (AHDB, 2020).

The results of soil testing inform farmers' decisions regarding suitable fertiliser application rates. RB209 recommends analysis of phosphorus every three to five years while sampling for soil mineral nitrogen (NO_3-N and NH_4-N) is advised annually (AHDB, 2020). The results of laboratory analysis are used to classify soils into indices. The soil index determines the rate at which farmers apply nutrients, including zero input when the soil is above a certain indices. The classification of soil P and K analysis into indices and their respective fertiliser application rates for a winter wheat crop are presented in Table 1.1. The rate applied will depend on the crop, the target yield and straw management.

Table 1.1: Phosphorus and potassium soil indices with their respective fertiliser application rates for winter wheat with straw incorporated and a target yield of 8 t ha^{-1} (AHDB, 2020). Recommended phosphorus applications rates have decreased since the previous edition of RB209 (Defra, 2010).

	Phosphorus		Potassium	
Index	Soil (mg P/L)	Application rate (P_2O_5, kg ha^{-1})	Soil (mg K/L)	Application rate (K_2O, kg ha^{-1})
0	0-9	100	0-60	105
1	10-15	80	61-120	75
2	16-25	50	121-180 (2-)	45
			180-240 (2+)	20
3	26-45	0	241-400	0
4	46-70	0	401-600	0
5	71-100	0	601-900	0
6	101-140	0	901-1500	0
7	141-200	0	1501-2400	0
8	201-280	0	2401-3600	0
9	Over 280	0	Over 3600	0

Measures to ensure safe and efficient use of organic manures are also included in RB209. The manual includes instructions to help maximise the nutrient value of organic fertilisers. Steps include calculating the quantity of phytoavailable nutrients supplied by the organic material and determining the application rate in line with regulations, guidance and crop requirements. Typical nutrient values including total amounts and the estimated phytoavailable amount are given for a range of organic materials. Values for pig slurry and biosolids, used in this thesis, are presented in Table 1.2. Although the availability of nutrients is discussed in the guide it is the total value, rather than the phytoavailable amount of phosphate and potash, that is used in fertiliser rate calculations. This is demonstrated in several example calculations provided in Section 2: Organic materials (AHDB, 2020).

Table 1.2: Typical dry matter, total nitrogen, total phosphate, phosphate availability, total potash, potash availability, total sulphur and total magnesium content or pig slurry and digested cake on a fresh-weight basis (AHDB, 2020).

Nutrient content	Pig slurry	Digested cake
Dry matter (%)	4	25
Total nitrogen (kg N/m^3)	3.6	11
Total phosphate (kg P_2O_5 m^{-3})	1.5	11
Phosphate availability (%)	50	50
Total potash (kg K_2O m^{-3})	2.2	0.6
Potash availability (%)	90	90
Total sulphur	0.7	8.2
Total magnesium	0.7	1.6

1.4.2 Phosphorus fertilisers: organic vs. inorganic

Inorganic and organic fertiliser additions differentially affect P cycling in soils. Modelling and experimental work have shown that while organic fertilisers generally increase the amount of P available for plant uptake their use may also contribute to elevated P leaching from agricultural soils compared to inorganic P (Allen et al., 2006; Esteller et al., 2009; Jiao et al., 2007; McDowell & Sharpley, 2004; Vanden Nest et al., 2016, 2014). The interactions that occur between organic materials, native soil phosphorus and plants are thought to be driven by (Guppy et al., 2005a): (i) competitive sorption for anionic binding sites between organic matter (OM) decomposition products and P; (ii) displacement of native soil P through metal complexation and dissolution; (iii) increased negative charge on the colloidal surface through binding of low molecular weight organic acids (LOAs); (iv) improved soil physical properties; (v) mineralisation of P in OM; (vi) alterations in pH; (vii) the formation of metal bridges between metals bound to LOAs on the colloidal surface and P; and (viii) microbial immobilisation of P (Figure 1.1).

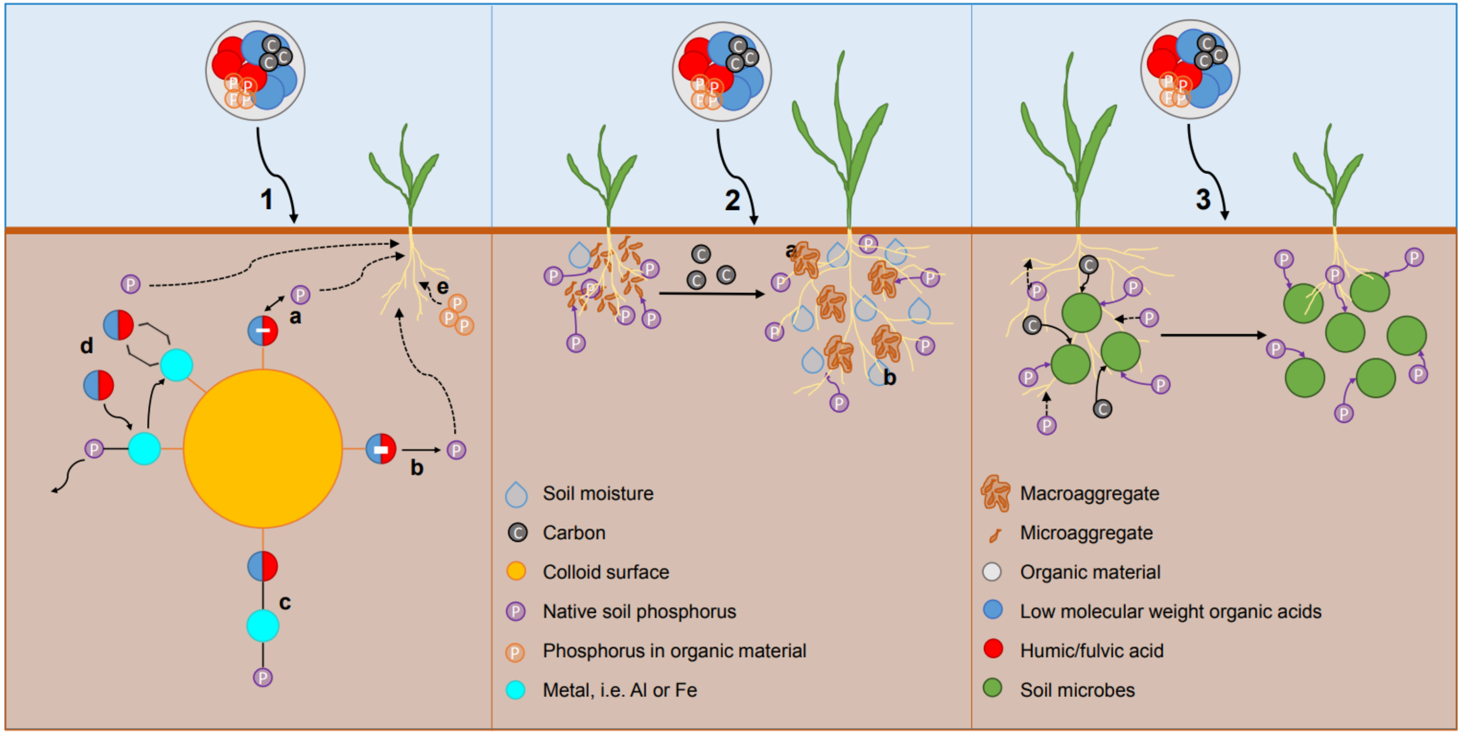

Figure 1.3: Hypotheses for the effect of organic matter (OM) additions on P phytoavailability in soils, adapted from Guppy et al., (2005a). Screen one: (a) low molecular weight organic acids (LOAs), fulvic acids (FA) and humic acids (HA) inhibit P sorption; (b) LOAs, HAs and FAs increase the negative charge of the colloid surface, repelling the negatively charged phosphate ion; (c) metal bridges form between P and metals bound to LAOs, HAs and FAs on the colloid surface; (d) metals chelate to LAOs, HAs and FAs, displacing P; and (e) P in OM is mineralised and becomes phytoavailable. Screen two: (a) aggregation results in a smaller surface area for P to bind; and (b) soil moisture regime is improved, both enhancing plant P uptake. Screen three: mineralisable carbon (C) acts as an energy source for soil microorganisms, enhancing their activity, P requirements and P uptake, reducing the amount of P available for plant uptake.

There have been two main approaches to studying P transformations in soils after fertiliser applications: incubation design laboratory experiments and field studies. In laboratory experiments, soils or soil components are amended with organic fertilisers (Audette et al., 2016; Jiao et al., 2007; Kang et al., 2011; Vanden Nest et al., 2016, 2014) or organic fertiliser derivatives, such as humic and fulvic acids (Hunt et al., 2007; Sibanda & Young, 1986; Ström et al., 2002). Both laboratory approaches remove many of the factors that affect soil P cycling and are therefore theoretical. The most obvious omission is the plant, which could influence P cycling through root secretions (Ström et al., 2002), their association with symbiotic fungi (Tawaraya et al., 2006) and general P uptake. However, incubation experiments are highly controlled with the opportunity for reduced variation between replicates. As such, they provide important information about the potential effect of fertiliser treatments on soil P chemistry.

While the majority of incubation experiments show increased P phytoavailability or leaching following the addition of organic matter or organic matter derivatives to soil (Audette et al., 2016; Hunt et al., 2007; Jiao et al., 2007; Kang et al., 2011; Sibanda & Young, 1986; Ström et al., 2002; Vanden Nest et al., 2016, 2014), they also reveal that differences in the organic matter source or derivative, stage of OM decomposition and soil properties can effect P phytoavailability. Hunt et al., (2007) incorporated dissolved organic matter (DOM) from fresh and decomposed plant and animal residues as well as Aldrich humic acid, oxalic and salicylic acid with three soil constituents: gibbsite, geothite and kaolin. In general, DOM from decomposed OM inhibited P sorption to a greater extent than DOM from fresh material. Not only was the increase in P phytoavailability dependent on the stage of decomposition, the response differed between soil constituents. In a leaching experiment, Vanden Nest et al., (2014) reported that out of five organic amendments, only farmyard manure significantly increased the concentration of total P and total dissolved P in leachate above the control. Therefore, the effect of organic inputs on P phytoavailability and leaching in the field may differ based on soil properties and the type of organic amendment. There may also be no response to organic amendments in terms of P sorption; studies have found no statistically significant effect (Borggaard et al., 2005) or only transient effects of organic matter addition on P dynamics (Guppy et al., 2005b).

Field conditions introduce a layer of complexity which cannot be achieved in the laboratory. As with laboratory incubations, organic amendments typically increase P phytoavailability compared with mineral P (Ali et al., 2019; Pizzeghello et al., 2011; Song et al., 2017). However the magnitude of the response can be influenced by the type of organic input (Vanden Nest et al., 2016, 2014). In a long-term field trial, Vanden Nest et al. (2016, 2014) reported increased P leaching and P phytoavailability, measured as calcium chloride extractable P ($CaCl_2$-P) and hot water extractable P (HWP), in soils treated with dairy farmyard manure compared to farm or municipal waste compost. The authors suggest the Ca:P ratio of the organic amendment is important in determining soil P phytoavailability and leaching (Vanden Nest et al., 2016). The response to organic fertilisers in terms of P phytoavailability is also affected by soil properties (Liu et al., 2012; Pizzeghello et al., 2011; Vanden Nest et al., 2016). Liu et al. (2012) applied pig slurry and inorganic P to a clay loam and a loamy sand soil with different initial P contents (65 and 142 mg kg^{-1}, respectively, as ammonium lactate-extractable P) but similar degrees of P saturation. The concentration and load of dissolved reactive P (DRP) in leachate from soil receiving pig slurry increased significantly compared to the zero-input control for the clay loam but not the loamy sand soil (Liu et al., 2012). The leaching response was attributed to abundant macropores in the clay loam. The same study demonstrates the benefits of incorporating pig slurry into the soil compared to surface application; in the clay-loam soil the concentration of DRP in leachate was reduced by over 50% by incorporation, possibly due to the destruction of macropores for water transport during the mixing process. Soil texture also affects the degree of P saturation after the application of farmyard manure (Pizzeghello et al., 2011) with greater accumulation in a peat and a clay soil compared to a sandy soil. Therefore while more complex, field studies support greenhouse and incubation work by demonstrating that factors other than high-level fertiliser type should be considered when predicting soil P phytoavailability after nutrient additions.

While field studies largely support conclusions of laboratory work, interpretation can be confused by experimental design. For example, differences in phytoavailable P or exports via leaching may simply be due to unequal P application rates between treatments (Allen et al., 2006; Esteller et al., 2009; Krey et al., 2013). These differences are exaggerated when fertiliser applications are made based on the N content of the amendment (Allen et al., 2006), especially when inputs differ in their N:P ratio. The failure of studies to recognise the importance of the P content of organic fertilisers was criticised in a review by Guppy et al., (2005a) who argued that increased P phytoavailability in organically-treated soils is not due to decreased P sorption but mineralisation of P in the organic fertiliser. Matching treatments for P inputs is becoming increasingly important as P replaces N as the limiting nutrient for fertiliser application rates across Europe (Sigurnjak et al., 2017) and the U.S. (Allen et al., 2006; Hao et al., 2015). When fertilisers are applied on an equal P basis, the concentration of soil phytoavailable and water soluble P can be higher in soils fertilised with mineral compared with organic P (Vaneeckhaute et al., 2016) indicating the importance of matching treatments for P when studying the effect of organic vs. inorganic fertilisation on soil P chemistry.

1.4.3 Nitrogen fertilisers: organic vs. inorganic

The effect of different fertiliser types on N transformations has been investigated with the majority of studies demonstrating lower N losses from organic compared to conventional systems (Aguilera et al., 2015; Dambreville et al., 2006; Doltra et al., 2015; Drinkwater et al., 1998; Flessa et al., 2002; Kramer et al., 2006; Küstermann et al., 2010, 2008; Marie et al., 2015; Mathieu et al., 2006; Petersen et al., 2006; Stalenga & Kawalec, 2008) although the opposite has also been reported (Bos et al., 2014; Miaomiao He et al., 2017; McGee, 2015)**.** While N_2O emissions are often lower from soils receiving organic inputs when expressed on a per area basis, the differences can be less pronounced or reversed when reported on a per production unit basis (Marie et al., 2015) although this depends on the crop (Aguilera et al., 2015). Furthermore, while studies conducted at a small scale find significant differences in N_2O emissions between organic and conventionally managed soils, a multi-site study found the effect of fertilisation strategy is small compared to soil abiotic factors such as pH, texture and carbon content (Doltra et al., 2015). This suggests meta-analysis may be a useful approach for determining how organic and conventional management compare in terms of N_2O emissions at a larger scale. Although the sample size of current meta-analyses is small, with typically fewer than 20 papers being included, results are conclusive between studies; while N_2O emissions are lower on a per area basis, lower yields mean that N_2O emissions are either similar (Mondelaers et al., 2009) or higher (Skinner et al., 2014; Tuomisto et al., 2012) in soils amended with organic compared to conventional fertilisers on a per production basis. Similar patterns are reported for nitrate leaching; losses are lower from organic than conventionally managed soils on a per area basis (Drinkwater et al., 1998; Kramer et al., 2006; Küstermann et al., 2010; Marie et al., 2015; Mondelaers et al., 2009; Tuomisto et al., 2012), but can be similar or higher when reported on a per product unit basis (Mondelaers et al., 2009; Tuomisto et al., 2012). The yield gap between organic and conventional systems must be closed to achieve comparable total N_2O emissions and NO_3 leaching on a per production basis (Tuomisto et al., 2012).

1.5 Combined fertiliser applications and soil nutrient cycling

1.5.1 Background

Farmers combine organic and inorganic fertilisers for reasons which differ based on the local farming system and national policy. In sub-Saharan Africa (SSA) farmers are encouraged to co-apply organic and inorganic fertilisers (Gentile et al., 2009) because: (i) neither mineral nor organic fertilisers are available in sufficient quantities for farmers to achieve optimum yields; (ii) there is often a positive interactive effect on yield when inputs are combined (Chivenge et al., 2011; Gentile et al., 2008, 2009); and (iii) both fertiliser types are an important component of long-term soil fertility management (Vanlauwe et al., 2010). In other regions, such as the United Kingdom (UK) (Defra, 2010) and China (Shuqin & Fang, 2018), the integration of organic and inorganic fertilisers is promoted for environmental reasons. In these systems combined fertiliser applications could reduce farmers' requirements for mineral fertilisers, minimising costs and relieving pressure on the non-renewable inputs required for the production of inorganic N and P (Nemecek et al., 2011). P and N cycling are affected by different mechanisms in combined fertiliser systems so the two nutrients will be discussed separately.

1.5.2 Phosphorus

Substituting crop P requirements with organic amendments is a viable approach to lessen farmers' requirements for fertilisers derived from rock P and encourage closed loop nutrient cycling while maintaining productivity. P leaching losses and phytoavailability are generally higher from organic compared to inorganic systems, due to processes outlined in section 1.4.1. While it is possible that combined applications will respond somewhere between organic and inorganic fertilisers, we may also expect to see interactive effects on the size of the labile P pool due to processes including competitive reactions between organic matter decomposition products and added P for anionic binding sites, increased negative charge on the soil surface and reduced surface area for P binding (Figure 1.1).

Incubation and greenhouse studies highlight the mechanisms driving soil P dynamics after integrated applications of organic and inorganic fertilisers. Reddy et al., (2005) reported an increase in labile P fractions when wheat or soybean residues were applied to an Alfisol, alone or in combination with inorganic P. Use of inorganic P in combination with wheat residue overcame an apparent immobilisation of P following the application of wheat residue alone. In contrast, the addition of inorganic P alone increased the amount of P in stable, less available fractions. The authors concluded that the integrated use of plant residues and inorganic P decreased the P sorption capacity of soil, increasing the amount of P that is phytoavailable. The results of this study suggest that the combined use of organic and inorganic fertilisers could improve P phytoavailability in the field while reducing requirements for inorganic P. However, it is important to synchronise the increase in available P with plant demand to minimise P leaching losses. Synchronising soil phytoavailability and plant demand may prove challenging as Reddy et al., (2005) showed that inputs differed in terms of the timing of peak P phytoavailability. Other incubation and greenhouse studies have shown similar increases in P phytoavailability following combined fertiliser applications but with unequal applications of P between treatments (Bolan et al., 1994; Delgado et al., 2002; Mokolobate & Haynes, 2003; Othieno, 1973).

Studies conducted in the field over years or decades provide insights into the long-term impact of fertilisation strategies on phosphorus levels in soil. Results collected after repeated applications of inorganic and/or organic amendments conclusively show that integrated fertiliser applications increase soil available P compared to inorganic fertilisers applied alone (Ahmed et al., 2019; Chen et al., 2017; Hu et al., 2018; Liu et al., 2010; Mao et al., 2015; Sun et al., 2015; Xin et al., 2017; Zhao et al., 2010). P application rates are unequal in many cases (Chen et al., 2017; Liu et al., 2010; Mao et al., 2015; Sun et al., 2015; Zhao et al., 2010) with one study applying over 125 kg more P ha^{-1} in the combined treatment than the inorganic NPK treatment (R. Sun et al., 2015). However, the phenomenon of increased P availability following repeated integrated fertiliser management is still observed when organic inputs act as substitutes rather than complements for inorganic P (Ahmed et al., 2019; Hu et al., 2018; Xin et al., 2017).

Field studies show that substitution of inorganic P with organic alternatives decreases the proportion of P in the residual-P, or unavailable, pool while increasing the proportion of P in the labile, or available, pool (Ahmed et al., 2019). While the results of long-term studies can largely be explained by alterations in P sorption/desorption because of the organic input, they also suggest alternative hypotheses that have not previously been considered. Recently Hu et al., (2018) observed an increase in Olsen’s P concentration after co-application of NPK with manure or straw and attributed this to higher levels of exchangeable calcium (Ca-ex) after the application of organic fertilisers. They suggested that organic amendments increased soil microbial activity and therefore CO_2 production in soil (Hu et al., 2018). CO_2 aids in the dissolution of limestone and therefore the production of exchangeable calcium. Interactions between Ca and P produce Ca-P forms which are available for plant uptake. The results of field studies therefore indicate that mechanisms other than P sorption/desorption are important in increasing P availability following the combined application of organic and inorganic fertilisers.

Work that focuses on P sorption/desorption reactions and soil chemistry may also overlook the role of soil microorganisms and their associated enzymes in determining P phytoavailability. Several field studies have investigated the role of phosphatases in P mineralisation in soils receiving combined fertiliser applications. Inositol hexaphosphate (IP_6), a phosphomonoester, is the most abundant form of organic P in soils (Ahlgren et al., 2013). Phosphatases are enzymes which catalyse the hydrolysis of inositol-phosphate bonds in phosphomonoesters, releasing orthophosphate for uptake by plants and microorganisms. Phosphatases are released by plants (acid phosphatase, ACP) (Dinkelaker & Marschner, 1992; Krämer & Green, 2000) and soil microorganisms, especially bacteria (alkaline phosphatase, ALP) (Nannipieri et al., 2011; Romanyà et al., 2017). Activity of ALP and ACP can be measured quantitatively using soil assays. Many studies have investigated the genetic control of ALP production by monitoring the expression of the ALP coding genes *phoA, phoX* and *phoD* (Sakurai et al., 2008; Tan et al., 2013). The co-application of inorganic and organic fertilisers has been shown to increase ALP activity and available P levels in soil (Chen et al., 2017; Hu et al., 2018; Liu et al., 2010), suggesting combined fertiliser applications can affect the concentration of available P in soil through microbial processes as well as sorption/desorption reactions. This is true whether P application rates are matched (Hu et al., 2018) or unequal (Chen et al., 2017; Liu et al., 2010) between treatments. Therefore, the increases in available P observed in long-term integrated fertiliser (For example Ahmed et al., 2019; Mao et al., 2015; Xin et al., 2017) experiments may be partially explained by alterations in soil microbial activity and their enzymatic capacity. Improved microbial functioning is an important factor in increasing crop yields in organic farming systems (Luo et al., 2018). The processes described here may therefore contribute to yield maintenance or improvement that has been reported under integrated nutrient management (Bedada et al., 2014; Ge et al., 2010; Pincus et al., 2016; Xie et al., 2016; Xin et al., 2017; Yadav et al., 2000; Zhao et al., 2016).

Many field experiments report the results of a single soil sampling occasion that occurs decades after the initiation of integrated fertiliser management. The sampling event often occurs after harvest, months after fertilisers have been applied and once crop P uptake is complete (for example Ahmed et al., 2019; Mao et al., 2015; Xin et al., 2017). Given that greenhouse experiments have shown effects of fertilisation within weeks of the first combined application of organic and inorganic fertilisers (Mokolobate & Haynes, 2003; Reddy et al., 2005) long-term studies may miss periods when P is most vulnerable to leaching or has the highest phytoavailability. The design of long-term field studies not only makes it difficult to determine when differences in P phytoavailability emerge within a season, but also between seasons. One field study conducted over a relatively short timescale shows effects of fertilisation strategy occurring within two years of the first application (Zhao et al., 2016), but the differences between P fertilised treatments are less pronounced than in long-term studies. These results suggest statistically and agronomically significant increases in P phytoavailability may take years to develop. It would be beneficial to monitor short-term P dynamics in field soils treated with agronomically-relevant P application rates to determine the effect of combined fertiliser applications on nutrient losses, phytoavailability and yield as farmers transition to integrated fertiliser management. This information could have further use if farmers are forced to reduce inorganic P consumption in response to a price spike similar to events occurring in 2008 (Khabarov & Obersteiner, 2017). In these situations, the use of an integrated fertiliser strategy may be transient but have important implications for food provisioning in that year (Khabarov & Obersteiner, 2017).

1.5.3 Nitrogen

There are two major hypotheses which explain how N cycling could be affected by combined fertiliser applications, termed the direct and indirect hypothesis (Vanlauwe et al., 2001). According to the direct hypothesis, the addition of material rich in carbon (C) will stimulate the soil decomposing microbial community, increasing their demand for N. As a result added fertiliser-N will be immobilised (Sall et al., 2003), reducing losses. Immobilised N can become plant-available later in the season via mineralisation. Immobilisation/mineralisation patterns under the direct hypothesis could reduce N losses through improved synchrony between plant demand for N and its availability in soil. The indirect hypothesis focuses on changes in soil structure as a result of increased organic inputs; improved soil properties may increase plant productivity and therefore demand for nutrients from added fertiliser-N. N transformations in systems receiving combined applications of organic and inorganic fertilisers have been shown to be more complex than these hypotheses suggest, and depend on chemical composition of the organic input (Frimpong & Baggs, 2010; García-Ruiz

& Baggs, 2007; Gentile et al., 2008, 2009), soil physical properties (Gentile et al., 2008) and local climatic conditions (Gentile et al., 2009).

Studies which investigate nutrient dynamics in soils receiving combined fertiliser applications raise concerns over the suitability of integrated soil fertility management in countries where legislation focuses on environmental protection. Frimpong & Baggs, (2010) reported higher N_2O emissions when three tropical plant residues were combined with ammonium nitrate (NH_4NO_3), compared to when NH_4NO_3 was applied alone. In the majority of treatments, integrating the plant residues with NH_4NO_3 also increased N_2O emissions relative to the organic controls, although the magnitude of the response was mediated by the chemical composition of the plant residue (Frimpong & Baggs, 2010). Gentile et al., (2008) found a positive interactive effect on N_2O emissions when urea was combined with maize, *Tithonia* or *Calliandra* residues in fine soils although the opposite was true in coarse soils. Carbon acts as the electron donor during denitrification and the availability of labile C has been shown to be an important factor in the rate of N_2O-genesis (Giles et al., 2017; Weier et al., 2010). It follows that increased N_2O emissions from integrated treatments have also been reported by Baggs et al., (2003), Garcia-Ruiz & Baggs, (2007), García-Ruiz et al., (2012) and Sarkodie-Addo et al., (2003).

However combining organic and inorganic fertilisers does not always stimulate N losses. Gentile et al., (2009) reported strong immobilisation of fertiliser-N and reduced NO_3 leaching losses when maize residues were combined with calcium ammonium nitrate, compared with the sole synthetic-N treatment, although losses were lowest from soils receiving only the organic amendment. Similarly, an increase of fertiliser-N retained in soil aggregates resulted in net N immobilisation when maize was combined with urea (Gentile et al., 2013). In an orchard system, Kramer et al., (2006) found that the denitrifying community was more active and more efficient in soils where composted chicken manure and calcium nitrate ($Ca(NO_3)_2$) were combined, compared to when $Ca(NO_3)_2$ was applied alone, although the organic treatment performed the best in terms of NO_3 leaching losses and N_2O emissions. These studies suggest that combining fertiliser types may present a way to manage N losses in agricultural systems while reducing inputs of non-renewable resources.

Farmers in the UK are encouraged to integrate the use of organic and inorganic fertilisers “to achieve an efficient and profitable production system with minimum adverse environmental impact” (Defra, 2010). Overall, the evidence to support this statement is limited and, in some cases, suggests that combining fertiliser types can enhance N losses to the environment. The farmer will require knowledge of soil type, chemical composition of the organic input, the correct ratio of organic-to-inorganic N and local climatic conditions in order to achieve the desired reduction in N_2O emissions or NO_3^- leaching. Given the potential for combined applications of organic and inorganic fertilisers to do environmental harm, it may be advisable for policy makers to avoid promoting this management technique to reduce N losses unless it is a short-term recommendation that aids in the transition towards a fully organic system.

1.6 Combined fertiliser applications and the wider soil system

1.6.1 Yields

The principal aim of fertilisation is to add nutrients to soil that improve or maintain crop productivity. Fertilisers can achieve this directly, by supplying nutrients in a form that is plant-available or will become plant-available over the life cycle of the crop, and indirectly by improving soil physiochemical properties which support plant growth. It is important for farmers and our growing population that any fertilisation strategy achieves this aim.

Support for the ability of integrated nutrient management to maintain or increase yields compared to the sole application of inorganic fertilisers is mixed. Results from single studies show lower (Dawe et al., 2003; Gong et al., 2009; Xie et al., 2016; Xin et al., 2017; Yadav et al., 2000), stabilised (Bedada et al., 2014; Xie et al., 2016; Xin et al., 2017; Yadav et al., 2000; Zhao et al., 2016) and higher yields (Bedada et al., 2014; Ge et al., 2010; Pincus et al., 2016; Zhao et al., 2016) under combined fertilisers applications, compared to inorganic management. Within studies, the magnitude and direction of the response can vary depending on the crop (Bedada et al., 2014; Xin et al., 2017; Yadav et al., 2000; Zhao et al., 2016), the year or season (Bedada et al., 2014; Xie et al., 2016; Yadav et al., 2000), the location (Yadav et al., 2000), the farmer (Bedada et al., 2014; Pincus et al., 2016), the size of the substitution (Liu et al., 2009; Xie et al., 2016) and the organic input (Liu et al., 2009; Zhao et al., 2016). Meta-analyses generally support the use of integrated nutrient management for yield (Chivenge et al., 2011; Vanlauwe et al., 2011; W. Wei et al., 2016) although an analysis of studies conducted across Asia concluded than organic amendments are not a suitable substitute for mineral N (Dawe et al., 2003). The results of meta-analyses supporting the combined use of organic and inorganic fertilisers may be compromised by higher rates of N application in integrated treatments (Chivenge et al., 2011; W. Wei et al., 2016) which is common in studies comparing inorganic and inorganic plus organic treatments (for example, Chivenge et al., 2009; Kanchikerimath & Singh, 2001; Martínez et al., 2017; Zhao et al., 2009). More research is required to elucidate the major factors determining the success of combined nutrient management in terms of yield, particularly in Europe as most research has been conducted in Asia and sub-Saharan Africa.

1.6.2 Soil organic matter, soil organic carbon and soil physical properties

Soil quality is “the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health”, where animal health includes human health (Doran & Parkin, 1994). A range of indices is used to quantify soil quality, including but not limited to soil depth, aggregation, structure, nutrient availability and microbial biomass C and N (Arshad & Martin, 2002; Doran & Parkin, 1994; Kibblewhite et al., 2008). By definition, soil quality is integral to the ability of a soil to perform its function, whether that be productivity, environmental quality or plant and animal health; therefore good soil quality is frequently cited as central to achieving sustainable land use (Arshad & Martin, 2002; E. K. Bünemann et al., 2018; Doran & Parkin, 1994; Vasu et al., 2020).

Soil organic matter (SOM), defined as “the organic fraction of the soil exclusive of undecayed plant and animal residues” (SSSA, 2008), is another well-recognised component of soil quality (Arshad & Martin, 2002; Bünemann et al., 2018; Reeves, 1997). The SOM content of soil is inextricably linked with nutrient cycling, soil structure, soil erosion, microbial activity, water relations and carbon sequestration. Combined fertiliser applications have been shown to increase the SOM and/or the soil organic carbon (SOC) content of soils compared to fertilisation with inorganic inputs only (Bedada et al., 2014; Gentile et al., 2013; Gong et al., 2009; Guo et al., 2016; Kanchikerimath & Singh, 2001; Li et al., 2017; Mao et al., 2015; Wei et al., 2016; Zhao et al., 2009), although the increase can be less than if the organic amendment is applied alone (Gong et al., 2009; Guo et al., 2016). The integrated use of organic and inorganic amendments may raise SOC levels by increasing the amount of stable C in soil compared to the use of inorganic fertilisers (Gong et al., 2009) and through the promotion of aggregation (Gentile et al., 2013) which physically protects SOM (Lützow et al., 2006; Six et al., 2002). Overall, evidence suggests integrated fertiliser management is a viable method to harness the benefits of organic amendments in terms of SOC accumulation although levels may not be as high as the use of organic fertiliser alone.

Organic carbon has been shown to stabilise aggregates as well as being physically protected inside them (Chaney & Swift, 1984; Erktan et al., 2016; Guo et al., 2019). The aforementioned effect of integrated fertiliser applications on SOC levels may promote favourable soil structure as the combined use of organic and inorganic fertilisers has been shown to increase aggregate mean weight diameter (MWD) (Bandyopadhyay et al., 2010; Gentile et al., 2013). However, the magnitude of the response has been shown to vary with soil texture and time and in some cases is negligible (Gentile et al., 2013) or negative; Mthimkhulu et al., (2016) reported significantly lower aggregate MWD in soils that were mulched and received inorganic fertilisers, compared to those only being mulched. More work is required to determine how the combined use of organic and inorganic fertilisers affects soil physical properties over time.

1.6.3 Soil microorganisms

Along with SOM, measures of the soil microbial community are often referred to as indicators of soil quality (Anderson, 2003; Bünemann et al., 2018). Shifts in microbial diversity, richness and activity could be expected in response to integrated fertiliser applications because soil microbial community structure, dynamics and function are linked with the availability of carbon sources. At a broad scale, combined applications of organic and inorganic fertilisers have been shown to increase microbial biomass carbon (Ge et al., 2010; Kanchikerimath & Singh, 2001; Lan et al., 2012; Zhao et al., 2016) and culturable counts of soil fungi and bacteria (Gong et al., 2009; Zhao et al., 2016) compared to the application of inorganic fertilisers. Integrated fertiliser applications have also been shown to increase microbial activity measured as respiration (Ge et al., 2010; Zhang et al., 2012). The diversity and richness of these larger, more active microbial communities is also affected by integrated fertiliser applications (Lazcano et al., 2013; Li et al., 2017; Sun et al., 2015; Zhang et al., 2012; Zhao et al., 2016), with both measures increasing in response to organic inputs (Sun et al., 2015; Zhang et al., 2012; Zhao et al., 2016) and correlated with soil nutrient status and pH (Li et al., 2017; Sun et al., 2015; Zhao et al., 2016). Importantly, the taxa whose relative abundance increases are involved in nutrient cycling, SOM decomposition, soil enzyme production and plant health (Li et al., 2017; Zhao et al., 2016). At the level of the gene, substitution of inorganic fertilisers with organic alternatives has been shown to increase the expression of N and P cycling genes compared to soils receiving no or only inorganic fertilisers (Chen et al., 2017; Hu et al., 2018; Zhao et al., 2016). Functionally, these alterations in the soil microbial community increase the production of enzymes involved with nutrient cycling compared with soils only receiving inorganic fertilisers (Ge et al., 2010; Kanchikerimath & Singh, 2001; Lazcano et al., 2013; Zhao et al., 2016). These studies demonstrate that substitution of inorganic fertilisers with organic alternatives affects microbial community size, composition and function in a way that improves soil quality.

1.6.4 Soil fauna

Earthworms have been shown to improve soil infiltration rates (Fischer et al., 2014), aid in carbon sequestration (Sánchez-de León et al., 2014; Zhang et al., 2013) and increase soil aggregation (Al-Maliki & Scullion, 2013; Coq et al., 2007; Sánchez-de León et al., 2014). Earthworms have also been shown to affect soil nutrient cycling through the production of casts rich in N, P, potassium (K) and Ca (Bhadauria & Ramakrishnan, 1989). Encouraging earthworm populations is therefore an important component of sustainable soil management. Earthworm numbers and biomass are commonly reported to increase in soils amended with organic fertilisers (Birkhofer et al., 2008; D'Hose et al., 2018; Leroy et al., 2008; van Eekeren et al., 2009). In contrast, mineral fertilisation can eradicate earthworm populations (Guo et al., 2016; Marinissen, 1992) or reduce numbers and/or biomass compared to unfertilised controls (Guo et al., 2016; van Eekeren et al., 2009) or soils receiving organic fertilisers (Birkhofer et al., 2008; Guo et al., 2016). The effect of integrated fertiliser management on earthworm populations has been little studied, but current publications suggest earthworm numbers increase with the proportion of nutrients supplied by organic fertilisers (Guo et al., 2016; Marinissen, 1992). Substitution of 50% of the nutrient budget with cattle manure compost (CMC) was shown to statistically significantly increase total earthworm numbers above the level of soils receiving no fertilisation without significantly reducing numbers compared to soils receiving only CMC (Guo et al., 2016). Integrated fertiliser management could therefore help to maintain earthworm numbers and protect the ecosystem services they provide.

1.7 Concluding remarks

The aim of fertilisation is to increase yield through the addition of nutrients in a form that is immediately available for plant uptake or will become available during the crop growing cycle. However, the effects of fertilisation extend beyond the crop because: (i) microorganisms, macrofauna and soil forming processes respond to fertiliser type and application rate (Gentile et al., 2013; Guo et al., 2016; Li et al., 2017); (ii) fertilisation affects the quantity of nutrients leaving agricultural soils in leachate, runoff and gas (Aguilera et al., 2015; Kramer et al., 2006; Vanden Nest et al., 2014); and (iii) the production of NH_3 and mining of rock P contribute to environmental degradation (Bicer et al., 2016; Kuo & Muñoz-Carpena, 2009; Rafiqul et al., 2005) and rely on finite resources (Chen et al., 2018; Smil, 2000). The combined use of organic and inorganic fertilisers has been shown to increase crop yields (Bedada et al., 2014; Pincus et al., 2016), earthworm numbers (Guo et al., 2016), nutrient availability (Ahmed et al., 2019; Hu et al., 2018; Reddy et al., 2005) and promote favourable microbial community function (Ge et al., 2010; Zhao et al., 2016) compared to the application of inorganic fertilisers alone. However the effect of integrated fertiliser management on short-term P dynamics is poorly understood due to (Figure 1.4): (i) infrequent or ill-timed soil sampling, often missing the major period of phosphorus uptake by the crop; (ii) re-current applications of P at the same rate over multiple seasons, despite possible changes in soil P index; and (iii) unequal P application rates in incubation, greenhouse and field studies.

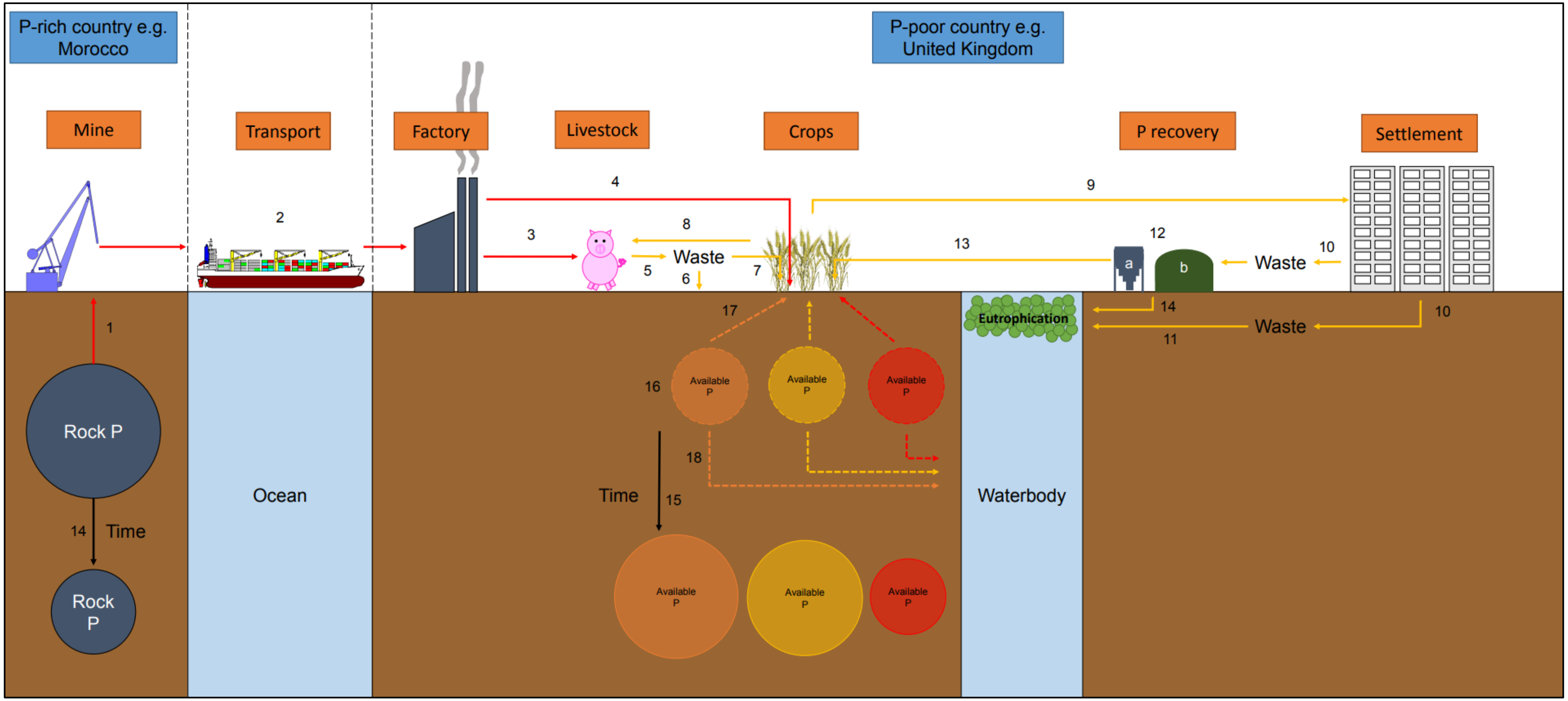
P-rich country e.g. Morocco
P-poor country e.g. United Kingdom
Mine
Transport
Factory
Livestock
Crops
P recovery
Settlement
1
2
3
4
5
6
7
8
9
10
10
11
12
13
14
14
15
16
17
18
Waste
Waste
Waste
a
b
Rock P
Time
Rock P
Ocean
Time
Available P
Available P
Available P
Available P
Available P
Available P
Eutrophication
Waterbody

Figure 1.4: Identified knowledge gaps. Phosphorus (P) mined from rock reserves (1) in P-rich countries such as Morocco is transported (2) to P-poor countries such as the United Kingdom where it is processed into feed for livestock (3) or fertiliser for crops (4). Livestock excrete P in waste (5). Waste from outdoor animals supplies P to soil directly (6). Waste from indoor animals can be spread as P-fertiliser to crops (7). Livestock consume P when they are fed plant diets (8) and when diets are supplemented with inorganic P (3). Human consumption of plant and animal products also represents intake of P (9). Human excretion of P (10) results in P-rich waste water that enters waterbodies where it causes eutrophication (11). P recovery technologies at waste water treatment plants (WWTPs) (12) including (a) struvite crystallisation and (b) anaerobic digestion (AD) produce biofertilisers suitable for agricultural use (13). Complete P removal may not be achieved or attempted, so WWTPs can also act as a source of P to waterbodies (14). Inorganic P fertilisers can be substituted with organic alternatives including AD products (13) and animal waste (7) to reduce requirements for inorganic P inputs from rock-P, supplies of which are declining. Integrated fertiliser applications have been shown to affect the amount of available P in soil compared to organic and inorganic management, with the size of the available P pool decreasing in the order integrated (orange circle), organic (yellow circle), inorganic (red circle) (15). The short-term effects of this practice on the concentration of available P in soil (16), crop P uptake (17) and leaching losses (18) are unknown, represented by dashed lines. Red arrows represent movement of inorganic P, or P losses and crop uptake from soils treated with inorganic P. Yellow arrows represent movement of organic P, or P losses and crop uptake from soils treated with inorganic P. Orange arrows represent P losses and crop uptake from soils treated with organic and inorganic P. Black arrows represent time passing. The diagram assumes P application rates are equal between treatments.

1.8 Aims and approach

The aim of this thesis is to determine how substitution of inorganic P with organic fertilisers affects short-term P phytoavailability and wheat productivity. “Short term” refers to the study of recently-applied fertiliser, rather than the assessment of soil nutrient status after repeated fertiliser applications. This approach is designed to increase knowledge of how combined fertiliser applications improve the sustainability of phosphorus use over two time-scales. First, the thesis asks if reducing inorganic P inputs through substitution with organic alternatives affects P phytoavailability to a growing crop. Could farmers’ dependence on P derived from depleted rock reserves be reduced without affecting yield and while maintaining or increasing levels of plant-available P in soil? Secondly, P phytoavailability is quantified after the major period of crop P uptake, providing an indication of P requirements for the proceeding crop. Does substitution of inorganic P with organic alternatives increase the soil P index compared to the application of inorganic P alone? If so, could the total amount of P from manufactured or organic sources be reduced in the next season? A short-term approach therefore demonstrates how combined fertiliser applications affect P supply to crops within a season and P demand from external sources between seasons.

The results presented in this thesis are derived from two studies spread across three chapters with objectives designed to meet the project aim and fill the knowledge gaps identified in Figure 1.4. A brief overview of the design, aims, objectives, hypotheses and predictions of each chapter is provided in Table 1.3.

Data was collected from studies using intact soil cores extracted from an arable field and transferred to a temperature and light-controlled greenhouse. This approach allows nutrient availability to be monitored during the growing period, and for any differences to be related to changes in plant productivity, nutrient uptake and leaching losses. The results of these studies provide information about productivity, nutrient cycling and P losses as farmers transition to an integrated fertiliser approach through choice, changes in policy or in response to global shortages of inorganic fertilisers.

Table 1.3: Experimental design, aims, objectives, hypotheses and predictions for each experimental chapter. Abbreviations: PS, pig slurry; DC, digested cake.

Chapter	Context	Aim and objectives	Hypotheses and predictions
Three	A study using intact soil cores fertilised with one of four treatments: (i) N only control, inorganic N; (ii) inorganic P and inorganic N; (iii) 50% P and N from pig slurry (PS), 50% P and N from inorganic sources; (iv) 100% P and N from pig slurry. Cores were maintained in a greenhouse and sown with winter wheat. Soils were analysed for nutrient availability four and eight weeks after fertilisation.	To determine how combining organic and inorganic fertilisers affects P phytoavailability and wheat productivity in the short term. The main questions were: 1. How does substituting inorganic fertilisers with PS affect P phytoavailability during the major period of P uptake in wheat? 2. Do shifts in P phytoavailability affect wheat productivity? 3. Does the ratio of organic to inorganic P affect potential P leaching losses?	Substituting inorganic P with PS will affect P phytoavailability compared to the application of inorganic fertilisers applied alone. P phytoavailability, potential leaching losses and uptake will be higher in the treatment receiving PS and inorganic P than the treatment receiving only inorganic P.
Four	A study using intact soil cores sown with winter wheat and fertilised with one of nine treatments. Main treatments included a treatment receiving all N and P from inorganic sources, a PS substitution treatment and a digested cake (DC) substitution treatment. DC was sourced from a municipal waste water treatment plant. A series of controls was included to allow the role of individual nutrients in productivity and nutrient cycling to be determined. Cores were sampled before fertiliser applications, during stem elongation and during anthesis. Plants were harvested at growth stage 93 (ripening, caryopsis loosening in the daytime) (Tottman & Broad, 1987). Cores	To determine how substituting inorganic P with organic alternatives affects P phytoavailability in the short term. The main questions were: 1. How does substituting inorganic fertilisers with organic alternatives affect P phytoavailability during and after the major period of P uptake in wheat? 2. How does substitution of inorganic fertilisers with organic inputs affect P leaching losses compared to the application of inorganic P alone? 3. How important is the organic amendment being incorporated in determining the magnitude and	Substituting inorganic P with PS and DC will affect P dynamics compared to the application of inorganic fertilisers alone. P phytoavailability and losses will be higher in both substitution treatments compared to the inorganic treatment, but the effect will be greater for PS than DC.

	were flooded at harvest for the collection of leachate.	direction of the response in terms of P dynamics?	
Five	Data for chapter five were obtained from the same experiment analysed in chapter four. Wheat was dried and biomass determined. Grains were then ground and analysed for total N and P content.	To determine how shifts in nutrient phytoavailability caused by substitution of inorganic P with DC and PS affect wheat productivity and nutrient uptake, compared to the application of inorganic P alone. The main questions were: 1. How does grain yield respond to substitution of inorganic P with DC and PS? 2. Is the nutrient content of grain affected by substitution of inorganic P with DC and PS? 3. Can changes in soil nutrient availability during stem elongation or anthesis explain yield trends?	Phosphorus phytoavailability will be increased when inorganic P is substituted with DC or PS, increasing P uptake and grain yield compared to the application of inorganic P alone.

Chapter 2 General methods

2.1 Summary

2.1.1 Project background and aims

Inorganic fertilisers increase crop yields but their production and use is associated with environmental degradation and sustainability concerns (Kuo & Muñoz-Carpena, 2009; Rafiqul et al., 2005; Smil, 2000). Substitution of inorganic fertilisers with organic alternatives, referred to here as integrated fertiliser management (IFM), could reduce farmers' requirement for rock-derived phosphorus (P) while recapturing P that would otherwise be lost from the system (Nemecek et al., 2011). Current studies show that after years of repeated applications, the concentration of crop available P is higher in soils receiving integrated fertiliser management compared to inorganic fertilisers alone (Ahmed et al., 2019; Chen et al., 2017; Hu et al., 2018; Liu et al., 2010; Mao et al., 2015; Sun et al., 2015; Xin et al., 2017; Zhao et al., 2010). Our understanding of the short-term effects of IFM on P phytoavailability and crop productivity is limited because studies typically report the results of one sampling point decades after the initiation of different fertiliser treatments or apply unequal rates of P between treatments. The aim of this thesis was to determine how substitution of inorganic fertilisers with organic alternatives affects short-term soil phosphorus dynamics and wheat productivity in an arable soil.

2.1.2 Experimental approach

The data reported in this thesis was derived from controlled greenhouse experiments using intact soil cores collected from arable fields at Leeds

University Farm (Figure 2.1). Cores were collected to 25 cm depth in 10.4 cm internal diameter PVC drainpipes cut to 28.0 cm in length. Once collected, cores were amended to 23 cm by removing up to 2 cm soil from the bottom of the core. Cores were transferred to a greenhouse (20^{o}C) where they were sown with pre-germinated winter wheat seedlings and maintained at a water-filled pore space (WFPS) that prevented leaching. Fertilisers were applied to the soil surface during tillering. Main treatments were matched for total phosphorus and readily available nitrogen (N), the sum of nitrate-N and ammonium-N. In chapter three, cores were destructively harvested four and eight weeks post-fertilisation and plant and soil material analysed for nutrient content and phytoavailability, respectively. Chapter three captured stem elongation. Stem elongation is the major period of nutrient uptake in wheat (Rose et al., 2007) and is characterised by successive extension of internodes giving plants a more upright appearance (Tottman & Broad, 1987). Cores used in the experiment analysed in chapters four and five were sampled before the application of fertilisers, during stem elongation and during anthesis. Anthesis is easily observable due to the production of small flowers on the ears, starting from the middle of the ear and extending to the ear tip and base (Tottman & Broad, 1987). Soil collected during the growing period was analysed for nutrient phytoavailability. Plants were harvested during ripening when grains were starting to loosen (Tottman & Broad, 1987). Grains were dried, weighed and analysed for nutrient content. Leachate was collected after harvest and the concentration and load of soluble reactive phosphorus (SRP) and nitrate-N (NO_3-N) and ammonium-N (NH_4-N) determined.

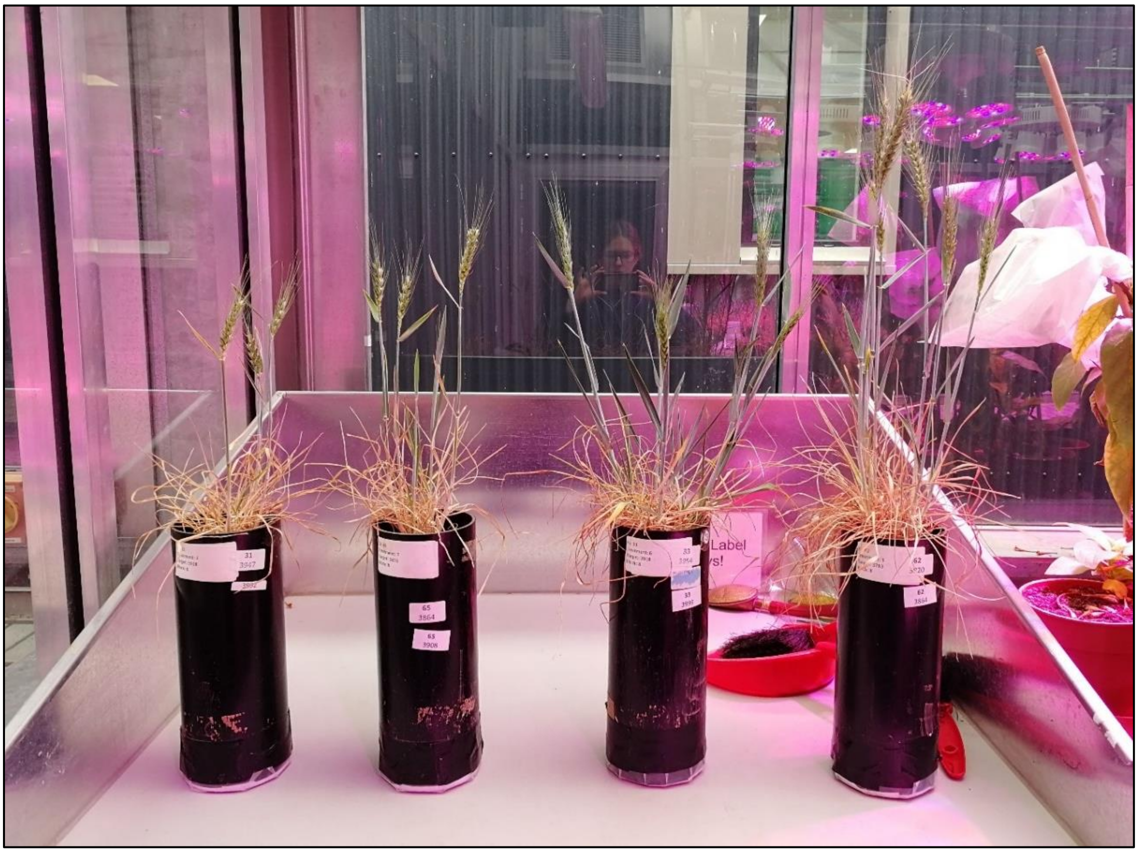

Figure 2.1: Intact soil cores in greenhouse. Cores were collected from an arable field and transferred to a light (16-hour photoperiod) and temperature (20ºC) controlled greenhouse where they were sown with winter wheat. Cores in this image are those used in chapters four and five, but chapter three adopted a similar design. Treatments from left to right: zero input control; inorganic P only; N only control; inorganic N plus inorganic P.

The use of intact soil cores was initially pursued because the project had aimed to monitor nitrous oxide (N_2O) emissions and the drainpipes used to collect cores can be fitted with accessories that allow for the collection of gasses. A chamber was developed for this purpose. However, results of a pilot study attempting to collect N_2O from cores were imprecise and so it was decided this avenue would not be pursued. Although labour intensive in their collection and maintenance, later work showed that using intact soil cores is a reliable method to monitor nutrient dynamics and crop productivity in fertilisation studies. It allows for a high number of replicates, careful monitoring and a high level of control which would be hard to achieve in the field. The following sections provide detailed protocols used across the thesis to avoid repetition in later chapters.

2.2 Leeds University Farm

Cores were collected from Leeds University Farm, Spen Common Lane, Tadcaster, LS24 9NS, United Kingdom. The farm is a commercial mixed arable and pasture farm (Holden et al., 2019). Mean annual precipitation and temperature are 674 mm (431 mm to 925 mm, min. to max. since 1961) and 9.2ºC (7.7ºC to 10.6ºC, min. to max. since 1961), respectively, as recorded by an on-site Met office weather station. The soil is a well-drained, loamy, calcareous brown earth from the Aberford series of Calcaric Endoleptic Cambisols (Cranfield University, 2018) underlain by dolomitic limestone of the Cadeby formation (British Geological Survey, 2018). Arable fields received 150 kg N ha^{-1} and 84 kg potassium oxide (K_2O) ha^{-1} in spring 2016, 58 kg phosphorus pentoxide (P_2O_5) ha^{-1} in autumn 2016, 140 kg N ha^{-1} and 104 kg K_2O ha^{-1} in spring 2017, and 8 t ha^{-1} of pig manure in autumn 2017. The field sampled in October 2018 (for chapter four and chapter five) received 230 kg N ha^{-1} and 36 kg K_2O ha^{-1} in spring 2018 and 53 kg P_2O_5 ha^{-1} in autumn 2017.

2.3 Core collection and preparation

2.3.1 Collection

Before core collection, an area of field was selected that was flat, free from debris and with good road access for practical reasons. A grid was laid out, composed of 1 m x 1 m grid squares. One core was collected from the centre of each square unless the surface was covered in debris or uneven until enough cores had been collected.

Cores were collected by inserting pre-weighed 10.4 x 28.0 cm (*internal diameter* x *height*) pieces of polyvinyl chloride (PVC) drainpipe with bevelled bottoms, referred to as sleeves, 25 cm into the ground. Sleeves were placed between rows of plants and topped with a small piece of timber, which acted to absorb the hit of a mallet. Cores were extracted using a spade, labelled with their location, cleaned externally and immediately weighed to determine fresh weight.

2.3.2 Preparation

Once collected, cores were returned to the laboratory where they were prepared for transfer to the greenhouse. Each core was first watered gravimetrically to field moisture content. After watering, soil was removed from the bottom of each core, so the final height of the soil was 23 cm. The bottom 2 cm of each sleeve was repacked with 250 g of sand to aid with drainage and sealed with polyester sheeting. The polyester at the bottom of cores used in chapters four and five was also covered with polythene sheeting to prevent contamination from the greenhouse bench. Every core was weighed at each step of the process (before watering, after watering, after removing soil, after adding sand and after adding polyester or polyester and polythene). Cores were maintained at 4^{o}C during the repacking process.

2.3.3 Moisture content determination of field moist soil

Additional cores were collected for moisture content determination. These cores were amended to 23 cm, as above, before being removed from their sleeve and dried at 105^{o}C until constant mass was achieved. The volume of water in these cores was used to estimate bulk density and WFPS of the experimental cores following Equation 2.1 to Equation 2.6.

Actual mean water content values (g H_2O g^{-1} oven-dry soil) of cores in chapter three are presented in Table 2.1. The mean water content of cores used in chapter four and five is presented in Table 2.2.

Table 2.1: Moisture content of cores used in chapter three. Value are mean ± one standard error of the mean and minimum and maximum water content (g H_2O g^{-1} oven-dry soil). Treatments are described in detail in individual chapters. N = 7.

Treatment	Harvest	Mean (g H_2O g^{-1} oven-dry soil)	One standard error of the mean	Minimum (g H_2O g^{-1} oven-dry soil)	Maximum (g H_2O g^{-1} oven-dry soil)
N only control	One	0.152	0.005	0.134	0.173
	Two	0.157	0.011	0.134	0.219
Inorganic	One	0.149	0.004	0.138	0.172
	Two	0.159	0.010	0.136	0.208
Combined	One	0.164	0.006	0.141	0.186
	Two	0.160	0.008	0.133	0.189
Organic	One	0.145	0.009	0.110	0.176
	Two	0.154	0.010	0.124	0.184

Table 2.2: Moisture content of cores used in chapter four and chapter five. Values are mean ± one standard error of the mean and minimum and maximum ($g\ H_2O\ g^{-1}$ oven-dry soil) water content of cores. Abbreviations: PS, pig slurry; DC, digested cake; Pi, inorganic phosphorus; Ni, inorganic nitrogen. n = 9.

Treatment	Harvest	Mean ($g\ H_2O\ g^{-1}$ oven-dry soil)	One standard error of the mean	Minimum ($g\ H_2O\ g^{-1}$ oven-dry soil)	Maximum ($g\ H_2O\ g^{-1}$ oven-dry soil)
Zero	Baseline	0.179	0.005	0.159	0.199
	Stem elongation	0.181	0.005	0.161	0.199
	Anthesis	0.181	0.005	0.161	0.198
Half rate Pi	Baseline	0.189	0.007	0.172	0.232
	Stem elongation	0.176	0.008	0.125	0.204
	Anthesis	0.183	0.003	0.171	0.201
Full rate Pi	Baseline	0.183	0.003	0.165	0.196
	Stem elongation	0.182	0.006	0.161	0.217
	Anthesis	0.180	0.004	0.578	0.203
Full rate Ni	Baseline	0.181	0.005	0.163	0.209
	Stem elongation	0.167	0.004	0.150	0.185
	Anthesis	0.168	0.004	0.153	0.179
	Baseline	0.182	0.008	0.155	0.241

Inorganic	Stem elongation	0.164	0.004	0.151	0.184
	Anthesis	0.168	0.005	0.150	0.183
	Baseline	0.169	0.009	0.102	0.201
PS substitution	Stem elongation	0.153	0.005	0.119	0.169
	Anthesis	0.165	0.006	0.144	0.195
	Baseline	0.178	0.006	0.158	0.203
PS only	Stem elongation	0.175	0.007	0.155	0.221
	Anthesis	0.165	0.009	0.136	0.221
	Baseline	0.183	0.004	0.168	0.199
DC substitution	Stem elongation	0.173	0.017	0.088	0.288
	Anthesis	0.166	0.003	0.155	0.183
	Baseline	0.181	0.006	0.160	0.213
DC only	Stem elongation	0.168	0.005	0.147	0.197
	Anthesis	0.163	0.006	0.140	0.184

2.3.3.1 Calculation of water-filled pore space

Water-filled pore space (WFPS) was calculated following the steps outlined in Equation 2.1 to Equation 2.6. Each sleeve was given an ID and weighed empty before collecting cores.

The volume of soil in each core was calculated using Equation 2.1.

Equation 2.1: Volume of soil per core.

$$Volume\ of\ soil\ (cm^3)\ =\ 23\ \times\ \pi\ \times\ 5.2^2$$

$$Volume\ of\ soil\ (cm^3)\ =\ 1954$$

Oven dry weight of soil could be calculated by subtracting the mass of the sleeve and the volume of water from the mass of cores amended to 23 cm following Equation 2.2. The volume of water in a core was estimated using the mean volume of water held in cores collected on the same day as the core being determined for WFPS.

Equation 2.2: Determination of mass of oven dry soil per core.

$$Mass\ oven\ dry\ soil\ =\ fresh\ mass - \ sleeve\ mass - \ volumetric\ water\ content$$

Bulk density could be calculated having determined oven dry mass of soil, following Equation 2.3.

Equation 2.3: Determination of bulk density based using results of Equation 2.2.

$$Bulk\ density\ (g\ cm^{-3}) = \frac{Mass\ oven\ dry\ soil}{Volume\ of\ soil}$$

Total porosity could be estimated using bulk density and an assumed particle density of 2.60 g cm^{-3} (Rowell, 1994b), following Equation 2.4.

Equation 2.4: Calculation of total porosity using results of Equation 2.3.

$$Total\ porosity\ (\%) = 100 - \left(\left(\frac{bulk\ density}{2.60}\right) \times 100\right)$$

The volume of water (cm^3) at x% WFPS can be calculated following Equation 2.5.

Equation 2.5: Determination of volume of water required to achieve a given water-filled pore space, using the results of Equation 2.4.

$$Volume\ of\ water\ (cm^3)\ at\ x\%\ WFPS$$
$$= volume\ of\ soil \times \left(\frac{total\ porosity}{100}\right) \times \frac{x}{100}$$

The target mass could therefore be calculated following Equation 2.6, where mass of water equals the value obtained in Equation 2.5 in grams.

Equation 2.6: Final calculation for the total mass of an individual core at a given water-filled pore space.

$$Target\ mass\ (g)$$
$$= mass\ of\ sleeve + (mass\ sand + mass\ polyester$$
$$+ mass\ plastic) + mass\ oven\ dry\ soil + mass\ water$$

2.3.3.1.1 WFPS worked example

A 23 cm deep soil core is collected in a 10.4 cm diameter sleeve weighing 474 g. The mass of the core, including sleeve, on a fresh basis is 4047g. Cores collected on the same day contained 588 ml water on average. The volume of the core is 1879 cm^3 and particle density is assumed at 2.60 g cm^{-3}. The total mass of sand, polyester and plastic added was equal to 261 g.

$$Mass\ oven\ dry\ soil\ (g) = 4047 - 474 - 588 = 2985$$

$$Bulk\ density\ (g\ cm^{-3}) = \frac{2985}{1954} = 1.53$$

$$Total\ porosity\ (\%) = 100 - \left(\left(\frac{1.53}{2.6}\right) \times 100\right) = 41.2$$

$$Volume\ water\ at\ 60\%\ WFPS\ (cm^3) = 1954 \times \frac{38.9}{100} \times \frac{60}{100} = 456$$

$$Target\ mass\ (g) = 474 + 261 + 2985 + 456 = 4176$$

2.4 Germination of seedlings

Winter wheat seeds (*Triticum aestivum L.*, “Skyfall”) were sterilised in 5% sodium hypochlorite (NaClO), rinsed 10 times with deionised water and placed on damp filter paper in 90 mm petri dishes to germinate. Dishes were sealed with parafilm and wrapped in aluminium foil to prevent light penetration. Seeds were maintained at room temperature for one week before being moved to a 4^oC fridge for one week to vernalise. Seeds were ready to sow at this point.

2.5 Power analysis

A pilot study was run from February 2017 to July 2017 to determine the effect of substituting inorganic P with organic fertilisers on P leaching and winter wheat productivity. The study had nine treatments and nine replicates per treatment, including inorganic, organic and combined fertiliser applications. The pilot failed due to an outbreak of powdery mildew in the greenhouse which reduced the number of replicates in certain treatments to two. However, enough data could be collected to perform power analysis for subsequent studies.

The power analysis was run using the pwr package in R Studio and was used to determine the number of replicates per treatment required to achieve power equal to 0.80, which is the generally accepted level for power (Jones et al., 2003).

2.6 Allocation of cores to treatments for balanced design

A randomised approach was adopted to ensure treatments had a similar bulk density (mean ± standard error). Cores were ordered from least to most dense, calculated according to Equation 2.3. Using chapters four and five with nine treatments as an example, numbers between one and nine were randomly generated and allocated to the first nine cores in the list, until each core had a unique number. Each number referred to a treatment. This process was repeated in blocks of nine until all cores in the list had been allocated to a treatment.

Cores were then randomly allocated to blocks using a random number generator, until each block contained one replicate of each treatment. Blocks were rotated and the position of cores in the block rearranged weekly in order to minimise the effect of variation in the greenhouse of plant growth.

2.7 Soil sampling in chapters four and five

The cores used in chapter four and five were sampled on three occasions during the growing period: one week before the application of fertilisers, during stem elongation (four weeks after fertilisation) and during anthesis (10 weeks after fertilisation).

Cores were sampled by inserting a minigouge (13 mm internal diameter, Van Walt, Haslemer, U.K.) 20 cm into the soil. Samples were immediately placed on ice after collection. The hole left by the auger was filled with sand and topped with the cap of a 15 ml falcon tube to prevent preferential flow of water through sand. After collection, samples were homogenised and portioned for freezing at -20^{o}C or drying at 40^{o}C.

2.8 Soil analysis

2.8.1 pH

pH was analysed following the method in Rowell, (1994a). 10 ± 0.1 g air-dried, sieved (< 2 mm) soil was weighed into a 50 ml flat-bottomed centrifuge tube with screw cap. 25 ml of distilled water was added to each sample before shaking by hand for 15 minutes. pH was determined using a pH electrode inserted into the solution. The reading was taken after one minute. The pH meter was regularly calibrated by two-point calibration (pH 4.01 and 7.01).

The protocol was modified for chapter four due to the large number of samples that needed to be analysed. Soils were shaken at 150 rpm for 15 minutes and filtered, as per the standard United States Environmental Protection Agency method (2004). pH was determined using a regularly calibrated (as above) pH electrode. The reading was taken after one minute. 10% duplicate samples (Table 2.3) and a certified reference material sample (AgroMAT Ag-1, SCP Science) were included in each batch. Precision values, measured as relative percentage difference (RPD) (Equation 2.7), are reported in Table 2.3.

Equation 2.7: Calculation of relative percentage difference between duplicate samples.

$$Relative\ percentage\ difference\ (\%, RPD) = \frac{(sample\ result - duplicate\ result) \times 100}{(sample\ result + duplicate\ result) \div 2}$$

Table 2.3: Relative percentage difference (RPD) of duplicate samples used in the analysis of soil, leachate and plant material (Equation 2.7).Values are mean RPD ± one standard error of the mean (SEM). Abbreviations: P, phosphorus; P_{water}, water-soluble P; $P_{organic}$, organic P, $P_{Olsen's}$, Olsen's P; N, nitrogen; RAN, readily available N; NH_4-N, ammonium-N; NO_3-N, nitrate-N; SRP, soluble reactive P. RAN is the sum of nitrate-, ammonium- and nitrite-N.

Chapter	Mean relative percentage difference ± one SEM									
	pH	P_{water}	$P_{organic}$	$P_{Olsen's}$	RAN	Plant N	Plant P	Leachate NH_4-N	Leachate NO_3-N	Leachate SRP
Three	5.38 ± 1.07	4.60 ± 3.62	/	1.62 ± 0.483	6.24 ± 2.09	4.19 ± 0.569	1.83 ± 1.12	/	/	/
Four, baseline	3.93 ± 1.00	5.74 ± 3.13	8.82 ± 2.28	4.34 ± 1.15	7.05 ± 1.62	/	/	/	/	/
Four, stem elongation	0.917 ± 0.153	7.65 ± 2.25	17.4 ± 6.63	3.55 ± 1.12	7.83 ± 2.15	/	/	/	/	/
Four, anthesis	1.47 ± 0.405	9.76 ± 3.77	6.56 ± 3.14	10.9 ± 1.74	8.72 ± 1.61	/	/	/	/	/
Four, harvest	/	/	/	/	/	/	/	5.85 ± 3.67	12.4 ± 4.04	1.28 ± 0.35
Five, harvest	/	/	/	/	/	13.9 ± 6.06	16.2 ± 4.74	/	/	/

2.8.2 Water-soluble phosphorus

2.8.2.1 Principle

This method estimates the amount of P in equilibrium between the solid soil surface and the soil solution (Kuo, 1996). Following extraction, P concentration is determined by the modified ascorbic acid method, developed by Murphy & Riley, (1962). Phosphomolybdic acid formed from orthophosphate and molybdenum is reduced by ascorbic acid to produce a phosphomolybdenum complex with an intense blue colour (ISO, 2018; Murphy & Riley, 1962). Antimony potassium tartrate catalyses colour development (Wu & Ruzicka, 2001). The intensity of the blue colour reflects the amount of orthophosphate in the phosphomolybdenum complex, allowing the concentration of orthophosphate to be quantified spectrophotometrically. The concentration of P in solution is calculated from a standard curve produced with solutions of known P concentration.

2.8.2.2 Reagents

Water-soluble phosphorus (P_{water}) was determined following the method of Olsen & Sommers, (1982). A colour developing solution was prepared by diluting the following reagents in 250, 100 and 1000 ml deionised water (diH_2O): 12.0 g ammonium paramolybdate [$(NH_4)_6Mo_7O_{24}•4H_2O$], 0.2908 g potassium antimony tartrate ($KsbO•C_4H_4O_6$) and 141 ml concentrated sulphuric acid (H_2SO_4, 5*N*), respectively. Dissolved ammonium paramolybdate and potassium antimony tartrate were added to the 5*N* H_2SO_4 solution and mixed thoroughly before being diluted to 2 litres. The final reagent was labelled Reagent A.

Colour developing solution was made by dissolving 1.056 g ascorbic acid in 200 ml Reagent A. Colour developing solution was prepared every 24 hours or with each new batch of reagents, whichever occurred first. Enough solution was made to determine the P_{water} content off all samples extracted that day, including blanks and standards.

2.8.2.3 Preparation of standards

A 5 mg L^{-1} phosphorus standard was prepared by diluting 250 µl of a phosphorus stock standard (1000 mg P L^{-1}) in deionised water to 50 ml. Solutions containing 0, 0.1, 0.2, 0.3, 0.4 and 0.8 mg L^{-1} were prepared by diluting 0, 1, 2, 3, 4 and 8 ml of the 5 mg P L^{-1} solution to 25 ml. A new standard curve was produced each day or with each new batch of reagents, whichever came first.

2.8.2.4 Extraction of water-soluble phosphorus from soil

2.5 ± 0.05 g of air-dried, sieved (< 2 mm) soil was weighed into a 50 ml centrifuge tube and shaken with 25 ml deionised water for 5 minutes at 150 rpm (Fuhrman et al., 2005; Kuo, 1996). Samples were centrifuged for 20 minutes at 3000 *g*, until the solution was free of soil mineral particles. Samples were filtered through Whatman no. 42 filter paper, with the first few millilitres of filtrate being discarded. 5 ml of filtrate was pipetted into a 25 ml volumetric flask followed by 4 ml of colour developing solution. Samples were diluted to volume in deionised water. 300 µl of sample was transferred to a 96 well plate. The maximum intensity develops after 10 minutes and is stable for 24 hours (Olsen & Sommers, 1982). Absorbance was measured after 35 minutes at 850 nm using a plate reader, to allow enough time for colour development in every sample. Each sample was measured in triplicate. 10% duplicate samples and one blank were included in each batch. Precision values, measured as relative percentage difference (RPD) (Equation 2.7), are reported in Table 2.3. A suitable certified reference material could not be found for this procedure.

2.8.3 Organic phosphorus

2.8.3.1 Principle

Organic P ($P_{organic}$) was determined by measuring the difference in the concentration of P between ignited and air-dried samples, following the method of Kuo, (1996). In this protocol, aliquots of a sample are ignited and shaken in sulphuric acid. A separate aliquot is not ignited before shaking in sulphuric acid. The concentration of P in filtered extracts is determined by the ascorbic acid method. The intensity of the phosphomolybdenum blue complex produced by the ascorbic acid method can be measured spectrophotometrically and the concentration of P in solution derived from results of a standard curve. Ignition at 550^{o}C converts organic P into inorganic P by high temperature oxidation (Kuo, 1996). The concentration of $P_{organic}$ in soil can therefore be calculated as the difference in P concentration between the ignited and unignited sample. Possible limitations of this method include increased extractability of inorganic P at temperatures above 160^{o}C, P volatilisation during ignition, incomplete oxidation of organic P and the hydrolysis of metal bridges binding P to soil organic matter by acid which could all affect the final $P_{organic}$ value (Kuo, 1996; Oniani et al., 1973; Williams et al., 1969).

2.8.3.2 Reagents

The extraction reagent (0.5M H_2SO_4) was prepared by diluting 27.8 mL of concentrated H_2SO_4 in 1 L diH_2O. 5*M* sodium hydroxide (NaOH) was prepared by diluting 200 g NaOH in 1 L diH_2O. *p*-nitrophenol was used as a pH indicator and was prepared by dissolving 0.25 g *p*-nitrophenol in 100 ml diH_2O.

Colour developing solution was prepared following the protocol of Kuo, (1996) which uses the method published by Murphy & Riley in 1962. To prepare the colour developing solution, the following reagents were diluted to 500 ml, 500ml, 100ml and 100ml diH_2O: 70 ml concentrated H_2SO_4, 20 g ammonium molybdate, 0.2728 g antimony potassium tartrate and 1.76 g ascorbic acid, respectively. 50 ml H_2SO_4, 15 ml ammonium molybdate solution, 30 ml ascorbic acid solution and 5 ml antimony potassium tartrate were mixed thoroughly to prepare the colour developing solution. Enough fresh colour developing solution was prepared to determine the concentration of organic P in all samples to be extracted that day, including blanks and standards.

2.8.3.3 Extraction of organic phosphorus from soil

For each core, 1.00 g of air-dried and sieved (< 2 mm) soil was ignited by heating at 550°C for 1 hour in glass tubes. Samples were transferred to 50 ml flat-bottomed falcon tubes with 25 ml 0.5M H_2SO_4. The procedure was repeated with 1.00 g of air-dried and sieved (< 2 mm) soil which was not ignited for each core. Air-dried and ignited samples were shaken at 150 rpm for 16 hours. After shaking, samples were filtered through Whatman no. 40 filter papers to obtain a clear solution. The first few millilitres of filtrate were discarded. A 1.00 ml aliquot of filtrate from each sample was transferred into a 30 ml sterilin tube. 5 drops 0.25% p-nitrophenol indicator solution were added to each tube. 5M NaOH was then added to each tube drop by drop until the solution changed from colourless to yellow. 4 ml of colour developing solution was added to each tube and the final volume adjusted to 25 ml using deionised water. 300 µl of sample was transferred to a 96 well plate. Maximum absorbance develops after 10 minutes and is stable for 24 hours (Kuo, 1996; Murphy & Riley, 1962). Absorbance was measured after 35 minutes at 850 nm using a plate reader, to allow enough time for colour development in every sample. Each sample was measured in triplicate. 10% duplicate samples and one blank were included in each batch. Precision values, measured as RPD (Equation 2.7), are reported in Table 2.3. A suitable certified reference material was not available for this procedure.

2.8.3.4 Preparation of standards

To prepare the standard curve, 5 ml 0.5M H_2SO_4 was added to six 30 ml sterilin tubes. 0, 1, 2, 3, 4 or 8 ml of a 5 mg P L^{-1} standard stock solution (prepared following the protocol in 2.8.2.3) was added to each tube. The final P concentration of standards was 0, 0.1, 0.2, 0.3, 0.4 and 0.8 mg P L^{-1}. pH was adjusted using p-nitrophenol and 5M NaOH, as above, before the addition of colour developing solution and dilution to 25 ml with diH_2O. Absorbance was read with experimental samples. A new standard curve was produced each day or with each new batch of reagents, whichever came first.

The concentration of $P_{organic}$ in a single core was calculated following Equation 2.8.

Equation 2.8: Calculation of organic phosphorus in soil following the method of Kuo, (1996).

$$P_{Organic} = total\ P_{ignited} - total\ P_{unignited}$$

2.8.4 Olsen’s phosphorus

2.8.4.1 Principle

Olsen’s phosphorus ($P_{Olsen's}$) is a measure of crop available P (Olsen et al., 1954). Bicarbonate (HCO_3^-) and hydroxide (OH^-) released from the alkaline (pH 8.5) sodium hydrogen carbonate ($NaHCO_3$) extractant during the reaction promote P desorption and thus increase the concentration of orthophosphate in solution (Kuo, 1996). Ca^{2+} activity is also reduced by $NaHCO_3$, minimising calcium phosphate precipitation reactions (Olsen et al., 1954). The concentration of P in filtered extracts is determined by the ascorbic acid method.

2.8.4.2 Reagents

0.5 g sodium hydroxide (NaOH) is dissolved in 1 L of 0.5M $NaHCO_3$, made by diluting 42 g $NaHCO_3$ in 1 L deionised water (diH_2O). Polyacrylamide solution (0.25 g polyacrylamide dissolved in 500 ml diH_2O) was used as a decolourising and flocculating agent to prevent colouration caused by organic matter dissolution and dispersion of silt and clay (Banderis et al., 1976).

2.8.4.3 Extraction of Olsen's phosphorus

2.5 ± 0.05 g of air-dry < 2 mm sieved soil was weighed into a 125 ml shaking flask. 50 ml of $NaHCO_3$ was added to each sample. 250 µl of polyacrylamide was added to each flask before shaking at 150 rpm for 60 minutes. Shaking time was increased compared to the standard method to achieve values within the accepted range of the certified reference material. Samples were filtered through Whatman no. 40 filter paper into 50 ml falcon tubes. The first few millilitres of filtrate were discarded. Filtered samples were diluted 10x with deionised water before transferring to autoanalyzer tubes. Orthophosphate-P was determined by Skalar San++ continuous flow analyser, using the method in Table 2.4. Three blanks, 10% duplicate samples and one certified reference material sample (ArgroMAT Ag-1, SCP Science) were included in each batch. Precision values, measured as RPD (Equation 2.7), are reported in Table 2.3.

Table 2.4: Methods for determination of nutrient concentration is soil and plant extracts by Skalar San++ continuous flow autoanalyzer. Abbreviations: NO_2-N, nitrite-N; NO_3-N, nitrate-N; NH_4-N, ammonium-N; PO_4-P, orthophosphate-P.

Compound	Method
NO_2-N	The diazonium compounds formed by diazotizing of sulphanilamide by nitrite in water under acid conditions is coupled with alpha-naphthyl ethylenediamine dihydrochloride to produce a reddish-purple colour which is measured at 540 nm (United States Environmental Protection Agency, 1974).
NO_3-N + NO_2-N	Nitrate is reduced to nitrite by hydrazinium sulphate and the nitrite (originally present plus reduced nitrate) is determined by diazotizing with sulphanilamide and coupling with alpha-naphthyl-ethylenediamine dihydrochloride to form a highly-coloured azo dye which is measured at 540 nm (Kamphake et al., 1967).
NH_4-N	Ammonia is chlorinated to monochloramine which reacts with salicylate to from 5-aminosalicylate. After oxidation and oxidative coupling, a green-coloured complex is formed. The absorption of the formed complex is measured at 660 nm (Krom, 1909).
PO_4-P	Ammonium molybdate and potassium antimony tartrate react in an acidic medium with diluted solutions of phosphate to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-coloured complex by ascorbic acid. The complex is measured at 880nm (Boltz & Mellon, 1948).

2.8.5 Available nitrogen

2.8.5.1 Principle

Available nitrogen (NO_3-N, NH_4-N and NO_2-N) concentration in soil was determined following a protocol provided by the School of Geography, University of Leeds which resembles that of Maynard et al., (2008). 1M potassium chloride (KCl) displaces ammonium and nitrate from ion exchange sites into solution where their concentration can be quantified. Potassium displaces exchangeable ammonium (Tucker, 1974) while chloride displaces nitrate (Pare et al., 1995). The concentration of ammonium-N and nitrate-N was determined by continuous flow autoanalyzer (Table 2.4).

2.8.5.2 Reagents

1 M KCl was prepared by diluting 74.54 g KCl in 1 L diH_2O.

2.8.5.3 Extraction of available nitrogen

Samples were removed from the freezer on the day they were required and allowed to defrost on ice before weighing approximately 10 g field moist soil into a 125 ml shaking bottle. The next day, 50 ml 1M KCl was added to each unsieved sample, 10% duplicate samples, blanks and the certified reference material sample (AgroMAT Ag-1, SCP Science). Samples were shaken at 150 rpm for 60 minutes before filtering through Whatman 42 papers into 50 ml falcon tubes. The first few millilitres of filtrate were discarded. Samples were analysed for NO_3-N, NH_4-N and NO_2-N using the methods in Table 2.4. Results were expressed on an oven-dried basis after moisture content determination (section 2.8.6). Precision values, measured as RPD (Equation 2.7), are reported in Table 2.3.

2.8.6 Moisture content and loss on ignition

Approximately 5.0 g fresh soil was weighed into a pre-weighed crucible. To determine air-dried moisture content, soil was dried at 40°C for 48 hours before re-weighing. To determine oven-dried (OD) moisture content, air-dried samples were dried at 105°C for 48 hours before reweighing. To determine loss on ignition, oven-dried samples were heated at 550°C for 16 hours in a muffle furnace and the difference between the unignited and ignited sample used to quantify percent soil organic matter.

Moisture content expressed relative to oven-dried weight (g H_2O g^{-1} soil) was calculated following Equation 2.9.

Equation 2.9: Calculation of moisture content expressed relative to fresh weight. Abbreviations; OD, oven-dried soil.

$$Moisture\ content\ (g\ H_2O\ g^{-1}\ \mathrm{OD}\ soil) = \frac{(crucible + fresh\ soil\ (g)) - (crucible + OD\ soil\ (g))}{OD\ soil\ (g)}$$

Organic matter (%) was calculated following Equation 2.10.

Equation 2.10: Calculation of soil organic matter. Abbreviations: OD, oven-dried soil.

$$Organic\ matter\ (\%) = \left(\frac{(crucible + OD\ soil\ (g)) - (crucible + ignited\ soil\ (g)}{\big(crucible + OD\ soil\ (g)\big) - crucible\ (g)}\right) \times 100$$

2.9 Plant analysis

2.9.1.1 Principle

Total N and P uptake in plant tissue were determined using the one-step method (Allen et al., 1974). Organic matter is broken down by the oxidising agents sulphuric acid and hydrogen peroxide. Sulphuric acid also prevents the flask from drying out which is a safety risk. Selenium acts as a catalyst for the conversion of organic N to ammonium sulphate and lithium increases the temperature of the reaction. The concentration of N and P in solution can be determine spectrophotometrically using the methods in Table 2.4.

2.9.1.2 Reagents

Mixed digestion reagent was prepared by mixing 175 ml 30% hydrogen peroxide (H_2O_2), 0.21 g selenium and 7 g lithium sulphate in a one-litre bottle before adding 210 ml concentrated sulphuric acid while heating and cooling.

2.9.1.3 Total digestion of plant nitrogen and phosphorus

Plant tissue was prepared for analysis by drying at 40^{o}C for 48 hours before grinding to a fine powder in an electric coffee grinder. Approximately 0.30 g sample was weighed directly into a 100 ml glass tube suitable for hotblock digestion. Three reagent blanks, one certified reference material sample (SRM 1573a, tomato leaves) and 10% duplicate samples (Table 2.3) were included per batch. 4.4 ml of mixed digestion reagent was added to each tube. Tubes were loaded into the Velp hotblock and heated slowly to 300^{o}C. The temperature was held at 300^{o}C until the solution had cleared. Once cooled, samples were transferred with washings into 50 ml falcon tubes and diluted to 25 ml with deionised water. Samples could then be transferred to autoanalyser tubes for determination of total N and P concentration by Skalar San++ continuous flow autoanalyser (Table 2.4). This method gives concentrations. The total uptake of N or P in a specific tissue was calculated by multiplying mass of that tissue by the concentration of the desired nutrient in that tissue.

2.10 Leachate analysis

Leachate samples were filtered through pre-rinsed 0.45 µm nylon syringe filters into 30 ml sterilin tubes before analysis for NO_3-N, NH_4-N, NO_2-N and SRP by Skalar San++ Continuous Flow Analyser. NO_3-N was calculated by subtracting absorbance of NO_2-N from (NO_3-N + NO_2-N). Methods for determination of each nutrient can be found in Table 2.4. The concentration of each nutrient was multiplied by the volume of leachate collected to determine nutrient load. 10% duplicate samples were included (Table 2.3). Precision values, measured as RPD (Equation 2.7), are reported in Table 2.3.

2.11 Organics analysis

Organic fertilisers were analysed by Natural Resources Management Laboratories. Only method outlines could be provided and are copied below.

2.11.1 Determination of oven-dry matter

As-received samples are homogenised and a representative sub-sample taken in a suitable tray. The weight is accurately recorded before and after drying in an oven at 105 ^{o}C ± 5^{o}C to determine the oven-dry matter as a % weight loss. The drying time is at least 12 hours and samples are checked to ensure they are completely dry.

2.11.2 Determination of aqua-regia soluble (‘total’) elements

Digested cake is dried (105^{o}C) and ground to pass a 1 mm screen. Pig slurry is analysed on an ‘as-received’ basis. A representative portion of the prepared sample is digested in an open vessel with concentrated hydrochloric and nitric acid (aqua-regia) using a temperature-controlled digestion block. The formation of strong oxidising agents will destroy organic matter and break down the mineral matric of the sample. The elements dissolved in the acid are analysed by ICP-OES/ICP-MS.

2.11.3 Determination of total carbon and nitrogen

The sample is oven-dried and ground. Samples are totally combusted in an oxygen enriched atmosphere in a reaction tube. Nitrogen and carbon products are carried by a constant flow of carrier gas (helium) through an oxidation catalyst, and then through reduced copper wires, where excess oxygen is removed and nitrogen oxides are reduced to elemental nitrogen.

The nitrogen and carbon products are separated through a chromatographic column. As the products are eluted from this column they pass through a T.C.D. detector, which generates an electrical signal proportional to the amount of nitrogen and carbon present. Various products can be eliminated if required using various traps, such as a magnesium perchlorate trap to eliminate hydrogen. Peak elimination reduces the risk of overlapping peaks and shortens run times. This procedure is known as the Dumas Technique.

2.11.4 Determination of organic matter content

The sample is dried and ground to pass a 2 mm screen. The test portion is dried at 103^oC, then ashed at 430^oC. The ash is determined as the residue on ignition. The organic matter is taken to be the loss of mass on ignition. Both are expressed as a percentage by mass of the dried sample.

2.11.5 Determination of pH

For slurries, samples are analysed on an as-received basis. For solid sludges, a suitable aqueous solution will be prepared. pH measurements are made at room temperature potentiometrically. The ratio of the slurry/manure to water is 1:6.

2.11.6 Determination of ammonium nitrogen, nitrate nitrogen and nitrite nitrogen

Ammonium-N and nitrate-N were extracted using 1 M KCl at an extraction ratio of 1:10 (10 g sample: 100 ml 1 M KCl). The determination of nitrate-N and nitrite-N is based on the formation of a diazo compound between nitrite and sulphanilamide. This compound is then coupled with N-1-napthylethylenediamine dihydrochloride to give a red azo dye. The colour is measured at 540 nm using an Alpkem RFA segmented flow analyser (Astoria Pacific).

In channel one, nitrate is reduced quantitatively to nitrite by cadmium metal in the form of an open tubular cadmium reactor (OTCR). The nitrite and reduced nitrate are therefore both measured as total oxidised nitrogen (TON).

In channel two, nitrite is measured. Nitrate-N is therefore determined by deducting the nitrite figure from the TON.

In channel three, ammonium reacts with alkaline hypochlorite and phenol to form indophenol blue. Sodium nitroprusside acts as a catalyst in formation of indophenol blue which is measured at 640 nm. Precipitation of calcium and magnesium hydroxides is eliminated by the addition of a combined potassium sodium tartrate/sodium citrate complexing reagent.

2.11.7 Determination of uric acid nitrogen

Samples are dried and ground to pass a 1 mm screen. The sample is extracted using a warm alkaline buffer solution. The uric acid content of the extract is determined using reverse phase HPLC with UV detection. The results are reported as % w/w uric acid nitrogen to 2 decimal places. The results can be converted from uric acid to uric acid-N by multiplying the value of uric acid by 0.333.

2.11.8 Determination of total nitrogen

Total nitrogen and ammonium-nitrogen are determined on fresh samples using the Kjeldahl method. The method is based on oxidising organic substances by heating with sulphuric acid to reduce the nitrogen present to ammonium sulphate. When this is distilled with sodium hydroxide gaseous ammonium is produced. This is then dissolved in a boric acid solution where the ammonia reacts with the acid. The excess acid is then titrated to determine the concentration of nitrogen.

Samples for total nitrogen determination are heated with sulphuric acid on a digestion block. The solution is then analysed on an automated Kjeltec analyser as per the above protocol. Ammonium-N is first extracted from the sample in potassium chloride and the filtered solution then analysed for N in the same way.

The catalyst used to accelerate the decomposition process is copper based and not the traditional mercuric compound as this causes environmental and health concerns.

2.12 Data entry and management

Data collected during soil, plant or leachate analysis was recorded in a lab book. Sample ID, sample treatment and sampling occasion (for example baseline, stem elongation, anthesis in chapter four) were recorded with each parameter measured. Any unusual observations were recorded. Data was transferred to Microsoft Excel (2016) when all samples had been analysed for a parameter. Data collected in the greenhouse was recorded on individual recording sheets. A new recording sheet was used weekly and data transferred to Microsoft Excel (2016) at the end of each week. Every time data was entered, it was checked for the correct number of samples in each treatment and the correct allocation of samples to treatments in order to pick up both labelling and data entry errors. Data was saved to OneDrive under a password protected account.

A filing system was developed to ensure data could be easily and correctly accessed. A data folder was produced for each experiment. Data produced from the experiment analysed in chapter three was subdivided into folders based on the material being analysed. Files were named based on the parameter being analysed. Data produced from the experiment analysed in chapter four was subdivided into folders based on sampling occasion. Files were named according to the sampling point and the parameter being measured, for example Olsen's P data from the baseline sampling point was labelled "BLGH_Olsen's phosphorus". For analysis, files from individual sampling points were collated in a new master document, so as not to lose original information. This was another opportunity to check for data entry and recording errors, which took place before collating files to ensure that the correct number of samples and the correct samples were allocated to each treatment. Master documents were saved in an Analysis folder, so it was clear that these documents were the ones to be used for analysis.

2.13 Quality assurance

Quality was integrated into the project through planning, proper implementation of procedures and methods and documentation and recording. Individual steps taken towards quality assurance are outlined in Table 2.5.

Table 2.5: Steps taken towards quality assurance at each stage of the project.

Stage	Implementation
Project planning	Planning ensured: - Equipment, people and facilities were ready when required. - The workload was manageable, particularly in terms of sample collection and preparation for storage and analysis. - Quality, informative data could be collected in the event of unplanned challenges such as plant disease.
Procedures and methods	- Proper training was received before the use of new equipment. - Certified reference materials were used where available to ensure accuracy. - 10% duplicate samples were included in all runs to ensure precision. - When analytical work was outsourced, it was to a laboratory with UKAS accreditation to the international standard of competence ISO/IEC 17025. - Glassware was washed in the laboratory glass wash, soaked in 10% hydrochloric acid (HCl) for at least 24 hours and rinsed 3 times in deionised water before use. Acid-washed glassware was stored in sealed plastic bags or covered with cling film to prevent contamination from deposition and dust. - Samples were prepared and stored appropriately for analysis. For most soil analytes, this involved storage at 4^{o}C before drying at 40^{o}C, transfer to air-tight bags and storage in the dark at room temperature. Samples for soil available nitrogen concentration were homogenised before storage at -20^{o}C in air-tight plastic bags with transfer to the freezer occurring within three days of collection. - Leachate samples were stored in polystyrene tube at 4^{o}C overnight, filtered in the order they were collected into fresh polystyrene tubes before re-storing at 4^{o}C and analysing the next day (within 2 days of collection). Help was recruited for filtering as this is a labour-intensive but time-sensitive process. Samples were filtered in the order they were collected to avoid confounding effects of storage time on soluble reactive phosphorus concentration. - When analytical work was conducted in batches, an even number of samples from each treatment was included in the batch. - In the greenhouse, cores were randomly allocated to blocks so that each block contained one sample from each treatment. Blocks were rotated in the greenhouse and cores re-randomised in their blocks weekly to avoid the effect of variation in greenhouse conditions on productivity and soil processes.

Documentation and repeatability	- Data has been stored in a well-organised, password protected filing system. - Protocols are saved with data files for future reference. - Detailed records were kept in the field, greenhouse and lab in lab books or in recording sheets which were not removed from University premises. - Most laboratory protocols follow those in published literature and are cited for future reference and repeatability. - Other steps taken towards data management and entry are covered in section 2.12.

Chapter 3 Short-term effects of combined fertiliser applications on wheat productivity and phosphorus and nitrogen availability in soil

3.1 Introduction

Phosphorus (P) is a critical element that supports all life (Deevey, 1970). The importance of P is well recognised in arable and livestock farming (Cordell et al., 2009, 2015; Metson et al., 2012) which consumes 90% of the world's P supply (Schröder et al., 2010). Over time, the dominant source of P used for fertiliser has shifted from human and animal waste to phosphate rock (Cordell et al., 2009). Global demand for P for fertiliser is increasing and is forecast to reach 46 million tonnes by 2020 (FAO, 2017b). However, supplies of P rock used in the production of fertilisers are declining with peak P production predicted to occur as soon as 2030 (Cordell et al., 2009).

The shift from organic to inorganic fertiliser sources may have affected P phytoavailability in agricultural soils because organic fertilisers increase P phytoavailability compared to the application of inorganic fertilisers (Gong et al., 2009; Jiao et al., 2007; Pizzeghello et al., 2011; Song et al., 2017; Yan et al., 2018). Several mechanisms leading to enhanced P phytoavailability in organically treated soils have been suggested, including competition between native soil P and decomposition products of the organic amendment, improved soil physiochemical properties and increased negative charge on soil surface particles (Guppy et al., 2005a).

The mechanisms which lead to increased P phytoavailability in soil receiving organic fertilisers also contribute to elevated levels of P in leachate and surface runoff compared to inorganic fertilisers (Esteller et al., 2009; Kang et al., 2011; Kleinman et al., 2010; McDowell & Sharpley, 2004; Svanbäck et al., 2013; Vanden Nest et al., 2016, 2014). These differences are apparent from days (Kang et al., 2011; Kleinman et al., 2010) to years (Esteller et al., 2009; McDowell & Sharpley, 2004; Svanbäck et al., 2013; Vanden Nest et al., 2016, 2014) after the onset of fertilisation. Agriculture is the dominant source of P causing aquatic eutrophication (Heckrath et al., 1995; Smil, 2000; Svanbäck et al., 2013; Weihrauch & Opp, 2017) with negative environmental, financial and human health impacts (Chorus et al., 2000; Dodds et al., 2009; Lürling et al., 2017; Preece et al., 2017; Pretty et al., 2003). Warming has been shown to increase the biomass and production of toxins by microorganisms that cause eutrophication (Lürling et al., 2017). Developing fertilisation strategies that minimise the flow of P from agriculture is therefore crucial in minimising the environmental impact of the food production system.

Governments and multinational organisations have implemented policies to extend the life of remaining P reserves and to minimise the environmental impact of P. The Urban Waste Water Treatment Directive sets maximum levels of P in effluent from water treatment works (Council of the European Union, 1991), the use of inorganic phosphates in detergents is banned in the European Union (European Commission, 2012) and P concentration is included in the health classification of a waterbody (European Commission, 2000). Farmers in the UK are encouraged to integrate the use of organic manures and mineral fertilisers “to achieve an efficient and profitable production system with minimal adverse environmental impact” (Defra, 2010). This approach could also help to lessen farmers’ requirements for inorganic fertilisers which have been shown to reduce soil fertility and health in the long term through depletion of soil organic matter, structure and biological activity (Ge et al., 2010; Munkholm et al., 2002; Ram et al., 2016; Smil, 2000; Zhong & Cai, 2007).

Evidence from greenhouse and incubation studies suggests that combining organic and inorganic fertilisers can increase phytoavailable P concentration (Garg & Bahl, 2008; Halajnia et al., 2009; Reddy et al., 2005; Toor & Bahl, 1997) plant P uptake (Halajnia et al., 2009) and P leaching (Leinweber et al., 1999) compared to when either fertiliser type is applied alone. However, it is difficult to put knowledge derived from these studies into practice because it is common for: (i) a single component of the system to be studied, such as the soil (Halajnia et al., 2009; Reddy et al., 2005; Toor & Bahl, 1997) (ii) only constituent parts of organic matter, for example low and heavy molecular weight organic acids (Bolan et al., 1994; Delgado et al., 2002) (Bolan et al 1994; Delgado et al 2002), to be applied to soil and incubated; and (iii) P application rates to be unequal between treatments (Garg & Bahl, 2008; Halajnia et al., 2009; Leinweber et al., 1999; Mokolobate & Haynes, 2003; Toor & Bahl, 1997). Therefore our understanding of short-term P dynamics under combined fertiliser regimes is limited by experimental designs which are inappropriate for the system being studied.

Field studies provide insight into the long-term effects of integrated fertiliser treatments on yield and P phytoavailability (Ahmed et al., 2019; Mao et al., 2015; R. Sun et al., 2015). In the field, integrating the use of organic and inorganic fertilisers has been shown to increase the concentration of crop available P compared to the application of inorganic P alone (Ahmed et al., 2019; Chen et al., 2017; Hu et al., 2018; Mao et al., 2015; Sun et al., 2015; Xin et al., 2017; Zhao et al., 2010). P applications rates are often higher in the treatment receiving organic and inorganic fertilisers together (Chen et al., 2017; Mao et al., 2015; Sun et al., 2015), but increases in P phytoavailability are also reported when treatments receive equal amounts of P (Ahmed et al., 2019; Hu et al., 2018; Xin et al., 2017). Results from field studies are typically derived from a single soil sampling event conducted years to decades after the initiation of fertilisation and after harvest (Ahmed et al., 2019; Mao et al., 2015; Xin et al., 2017). The timescale over which increases in crop available P occur and whether increased P phytoavailability coincide with crop demand is therefore unclear given the duration and infrequent sampling of field studies.

Available data shows that integrating the use of organic and inorganic fertilisers is a promising approach that can enhance (Bedada et al., 2014; Pincus et al., 2016; Zhao et al., 2016) or maintain crop yields (Bedada et al., 2014; Dawe et al., 2003; Xie et al., 2016; Xin et al., 2017; Zhao et al., 2016). However, the response is highly variable within and between studies and has been shown to depend on factors including the crop (Yadav et al., 2000), the year (Bedada et al., 2014) and the farmer (Pincus et al., 2016). Moreover, increases in yield may simply be explained by higher nutrient application rates in combined compared to sole treatments (Chivenge et al., 2009; Kanchikerimath & Singh, 2001; Martínez et al., 2017; Zhao et al., 2009) which has been identified as an issue in meta-analyses (Chivenge et al., 2011; W. Wei et al., 2016). In countries where fertiliser sources are combined in order to overcome issues associated with pollution and supply, ensuring nutrient application rates are balanced across treatments is critical to determine the true effect of integrated nutrient management on yield. A number of long-term field studies have been conducted with a substitution design and show that combined fertiliser applications can decrease (Dawe et al., 2003; Gong et al., 2009; Xie et al., 2016; Xin et al., 2017; Yadav et al., 2000), increase and maintain yields, compared to the application of inorganic fertilisers alone.

The aim of this study was to determine how combining organic and inorganic fertilisers affects P phytoavailability and wheat productivity in a soil with a high P status (soil P index four). Farmers would be recommended against applying phosphorus under this scenario as the concentration of soil available P is above the target index of two (AHDB, 2020). However, the high P status ensures that substitution is unlikely to affect wheat productivity, and therefore the phytoavailability of other nutrients, due to treatment-induced differences in P phytoavailability.

The main objectives were to determine how partial substitution of inorganic fertilisers with organic alternatives affects: (i) the concentration of plant-available P in soil, measured as Olsen's P; (ii) N availability in soil, measured as ammonium-N and nitrate-N concentration; (iii) wheat productivity; and (iv) P leaching risk, using soil water-soluble phosphorus concentration as an indicator (Pote et al., 1996). This study tested the hypothesis that the ratio of organic to inorganic P applied with fertilisers affects P phytoavailability in soils, and that combining fertiliser sources increases P phytoavailability compared to the application of inorganic P alone. The hypothesis was tested in a greenhouse experiment using intact soil cores. Pig slurry, a readily available source of nutrients in the United Kingdom (UK) (ADAS, 2008), was used to supply organic P to the most popular variety of winter wheat on the UK market (*Triticum aestivum* L., cv "Skyfall") (RAGT, 2017).

3.2 Materials and Methods

3.2.1 Summary

Soil cores collected from an arable field were sown with winter wheat and fertilised with one of four different fertiliser treatments: (i) N only control; (ii) inorganic N and P; (iii) combined application of pig slurry and inorganic N and P; and (iv) pig slurry only. Two winter wheat plants were grown in each pot. At the end of tillering each treatment received fertilisers at a rate equivalent to 140 kg P_2O_5 ha^{-1} and 170 kg readily available nitrogen (RAN, the sum of ammonium-N and nitrate-N) ha^{-1} (Table 3.1). Cores were destructively harvested 28 and 56 days after fertilisation. Soil was analysed for pH and Olsen's P, water-soluble P, and RAN concentration. Plant material was analysed for biomass and total N and P uptake.

Table 3.1: Fertiliser application rates. Phosphorus pentoxide (P_2O_5) and readily available nitrogen (RAN) were supplied by monocalcium phosphate [$Ca(H_2PO_4)_2$], ammonium nitrate (NH_4NO_3) and pig slurry. Treatments were matched for P_2O_5 and RAN at application rates of 140 and 170 kg ha^{-1}, respectively. The exception was the N only control treatment which did not receive P from any source.

	P_2O_5 (kg ha^{-1})		RAN (kg ha^{-1})	
Treatment	$Ca(H_2PO_4)_2$	Pig slurry	NH_4NO_3	Pig slurry
N only control	0	0	170	0
Inorganic	140	0	170	0
Combined	70	70	85	85
Organic	0	140	0	170

3.2.2 Core collection and preparation

On 18 January, 62 cores were collected from a commercially managed arable field at Leeds University Farm (Spen Common Lane, Tadcaster, United Kingdom, LS24 9NT) that has been fertilised with organic and inorganic fertilisers historically. Details on the core collection and preparation method are described in the general methods (2.3). Six of the cores collected on 18 January were randomly selected for moisture content determination (general methods 2.3.3).

Experimental cores were moved to a temperature and light controlled (20°C, 16-hour photoperiod) greenhouse and arranged in seven blocks, with each block containing one randomly allocated replicate from each treatment. Cores were dried to and then maintained at 50% water-filled pore space (WFPS) for the remainder of the experiment by watering with deionised water every day. Once dried to the set level, each core was sown with three pre-germinated winter wheat seedlings (*T. aestivum* L. cv “Skyfall”) (general methods 2.4). The third seedling to emerge was removed immediately, leaving two plants per pot.

3.2.3 Fertilisers and their application

Pig slurry was used to supply an organic source of P. A bulk sample of 10 L of pig slurry was collected from an indoor pig unit at Leeds University Farm and thoroughly mixed before being portioned into 1 L samples and frozen at -20°C. The pigs were weaner/growers under commercial indoor management, including veterinary treatment and diet. Duplicate samples of fresh material were sent to Natural Resource Management (Berkshire, England) for analysis. Total P, potassium (K), magnesium (Mg), sulphur (S), copper (Cu), zinc (Zn), sodium (Na) and calcium (Ca) were extracted with aqua-regia solution and dissolved elements analysed by ICP-OES/ICP-MS. Total carbon (C) and N were determined following the Dumas technique. pH was measured potentiometrically in a slurry-to-water ratio of 1:6. Organic matter was determined by loss on ignition (LOI). Further details on pig slurry analysis can be found in the general methods (0). Monocalcium phosphate [$Ca(H_2PO_4)_2$] was used to supply inorganic P and ammonium nitrate (NH_4NO_3) was used to supply inorganic N.

Cores were fertilised according to one of four treatments (Table 3.1): (i) N only control; (ii) all nutrients from inorganic P and inorganic N (inorganic) (iii) half N and P from inorganic sources, half N and P from pig slurry (combined); and (iv) all N and P from pig slurry (organic). Treatments provided 170 kg ha^{-1} and 140 kg ha^{-1} of RAN and total phosphorus pentoxide (P_2O_5), respectively, with the exception of the N only control which only supplied inorganic N.

Fertiliser treatments were applied 33 days after sowing (DAS) which coincided with the final stages of tillering. The surface of each core was disturbed to 2 cm with a palette knife before applying fertilisers to the surface. Organic and/or inorganic fertilisers allocated to each core were combined in a 100 ml pot with a lid. Deionised water was added to each pot so that the volume of water supplied with fertilisers was equal between treatments. Fertilisers were poured evenly across the soil surface, before rinsing each pot three times with deionised water to ensure all product was added. On the same day, six randomly selected cores were destructively harvested for baseline soil analysis (Table 3.2).

Table 3.2: Baseline soil analysis. Six soil cores destructively harvested on the day of fertilisation. Sum available N is the sum of nitrate-N (NO_3-N), ammonium-N (NH_4-N) and nitrite-N (NO_2-N). Olsen's phosphorus and potassium are followed by their soil index (Agriculture and Horticulture Development Board, 2017). n=6 for all parameters except potassium, where n=3.

Parameter	Mean ± one standard deviation (mg kg^{-1})
pH	7.19 ± 0.126
Sum available N	5.63 ± 1.61
NO_3-N	4.64 ± 1.55
NH_4-N	0.893 ± 0.279
Olsen's phosphorus	55.0 ± 7.41 (4)
Water-soluble phosphorus	2.58 ± 0.727
Potassium	163 ± 15.3 (2-)

3.2.4 Harvest

Seven cores per treatment were destructively harvested 28 and 56 days after the application of fertilisers (harvest one and harvest two, respectively). Aboveground tissue was cut at the surface and weighed before drying at 40^{o}C for biomass measurements. Soil cores were removed from sleeves and cut in four pieces; in half lengthways, and each resulting half divided widthways (Figure 3.1). One top quarter was immediately homogenised and frozen at -20^{o}C. Roots were removed from the other top quarter before drying at 40^{o}C and grinding to < 2mm. Soil from the bottom half of the core was not analysed but was either dried at 40^{o}C or frozen at -20^{o}C. Roots were rinsed in tap water and dried at 40^{o}C for 48 hours.

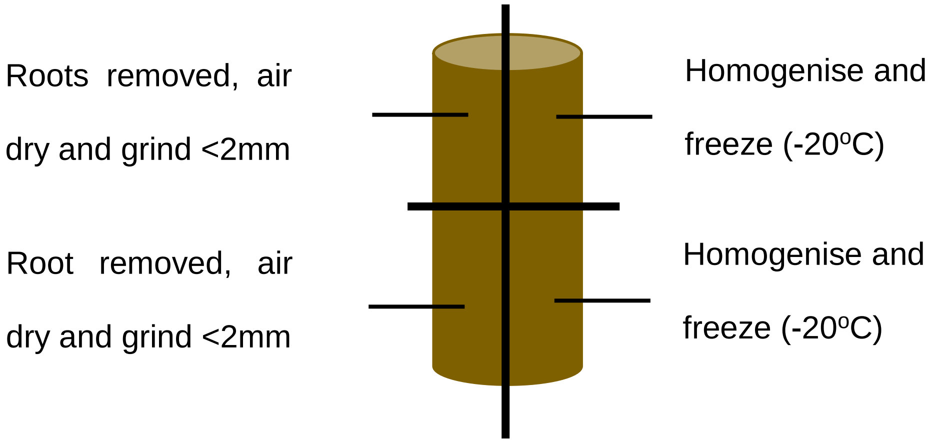

Figure 3.1: Preparation of soil cores for analysis. Cores were divided into four equal segments and each quarter prepared for storage based on analysis requirements. Only the top half of soil cores were eventually analysed.

Soil collected was analysed for water-soluble P, Olsen's P, potassium chloride (KCl)-extractable N and pH. Plant tissue collected 28 days after fertilisation (harvest one) was analysed for aboveground biomass. Plant tissue harvested 56 days after fertilisation (harvest two) was analysed for aboveground dry weight, total N and total P uptake. Only the top half of each core was analysed for soil chemical properties. Details of soil and plant analysis can be found in the general methods (2.8 and 2.9, respectively).

3.2.5 Statistical analysis

Statistical analyses were run in IBM SPSS Statistics 24. Five cores that leached during the experiment were removed from the analysis (three from the combined treatment, two from the organic treatment). The effect of harvest, treatment and their interaction on each dependent variable was assessed by one- or two-way analysis of variance (ANOVA). Assumptions were tested by residual analysis. Homogeneity of variances, normality and outliers were assessed by Levene's test, Shapiro-Wilk's test and inspection of boxplots, respectively. Data received a $\log_{10}$ or square root transformation when test assumptions were violated. Non-parametric tests or a Generalised Linear Modelling approach (GLM) were adopted when transformations were unsuccessful. Details for individual tests are available in the results section.

3.3 Results

3.3.1 Summary

A summary of the main findings is presented in Figure 3.2. Data are expressed as percentage change from the N only control, based on mean values.

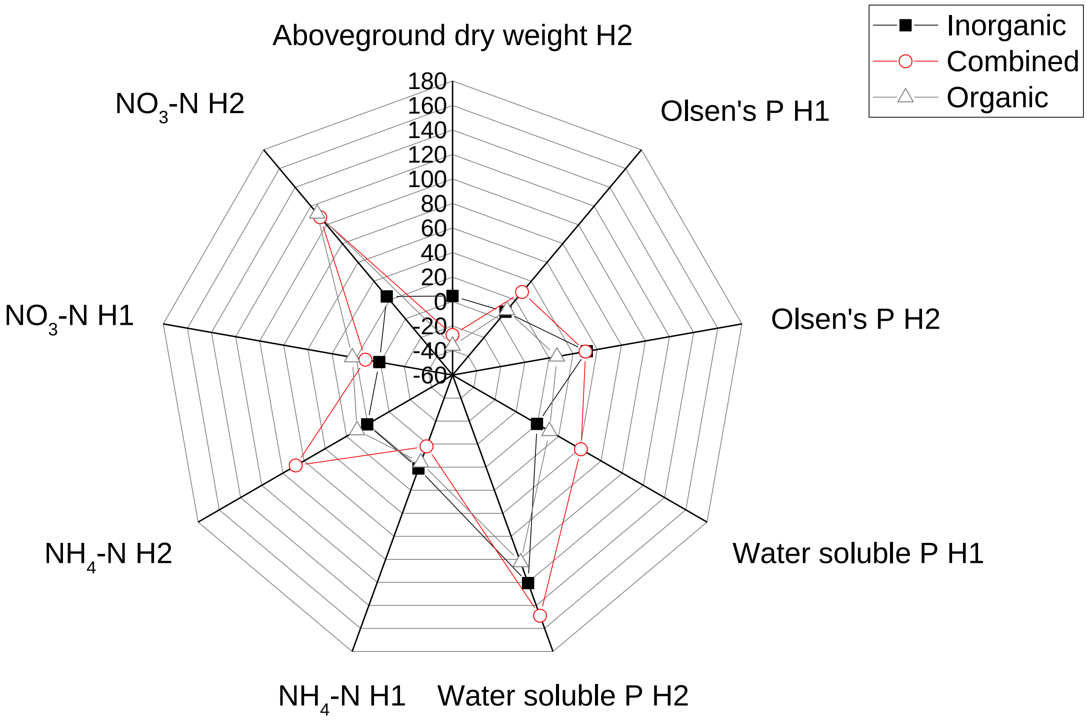

Figure 3.2: Summary of main findings. Results are expressed as percent change compared to the N only control. Olsen's P, water-soluble P, NO_3-N and NH_4-N are measures of available nutrients in soil and were reported as mg per kg oven-dried soil. Aboveground dry weight is reported in grams. A summary of treatments is presented in Table 3.1. Abbreviations: H1, harvest one, 28 days after fertilisation; H2, harvest two, 56 days after fertilisation. n = 7.

3.3.2 Organic analysis

The results of slurry analysis are presented in Table 3.3 as concentrations and the total amount of nutrients added to each core in the organic treatment.

Table 3.3: Chemical composition of pig slurry. Values are the mean of duplicate samples. The concentration of each component is presented based on fresh weight and the total quantity applied per core in the organic treatment. Phosphorus pentoxide (P_2O_5), potassium oxide (K_2O), magnesium oxide (MgO), sulphur dioxide (SO_2) and sodium oxide (Na_2O) were derived by multiplying phosphorus, potassium, magnesium, sulphur and sodium by 2.29, 1.205, 1.66, 2.5 and 1.35, respectively. n = 2.

Determinand	mg/g fresh weight	Total (mg) supplied in organic treatment
pH	6.33	-
Oven dry solids (%)	2.16	-
Total nitrogen	2.60	180
NH_4-N	2.11	148
NO_3-N	<0.01	-
Total phosphorus	0.75	51.9
Total P_2O_5	1.72	119
Total potassium	1.57	109
Total K_2O	1.89	131
Total magnesium	0.545	37.8
Total MgO	0.90	62.5
Total sulphur	0.196	13.5
Total SO_2	0.490	33.9
Total copper	0.0020	0.138
Total zinc	0.01	0.691
Total sodium	0.46	31.8
Total Na_2O	0.62	42.8
Total calcium	0.52	18.6
Total carbon	16.55	1140
Organic matter LOI	14.50	1000

3.3.3 Aboveground dry weight

A two-way ANVOA was conducted to examine the effect of fertiliser treatment and harvest on wheat aboveground dry weight. Residuals were normally distributed ($p > 0.05$) and there was homogeneity of variances ($p = 0.186$). There was one outlier 1.5 to 3 box-lengths from the edge of a box-plot which was included in the analysis as there was no evidence of a measurement or data entry error.

There was a statistically significant interaction between harvest and treatment on aboveground dry weight, $F_{(3,43)} = 4.80$, $p = 0.006$, partial $\eta^2 = 0.251$ (Figure 3.3). Simple main effect of treatment were tested with statistical significance receiving a Bonferroni adjustment. Values are means ± one standard error of the mean (SEM). There was a statistically significant effect of treatment on mean above ground dry weight at harvest one ($F_{(3,43)} = 3$, $p < 0.05$, partial $\eta^2 = 0.173$) and harvest two ($F_{(3,43)} = 3$, $p < 0.001$, partial $\eta^2 = 0.560$). At harvest one mean aboveground dry weight of the organic treatment (2.096 g ± 0.406) was statistically significantly lower than the N only control (3.733 g ± 0.376). By harvest two, the N only control and inorganic treatment (8.273 g ± 0.376 and 8.647 g ± 0.376, respectively) had statistically significantly higher above ground dry weight than the combined (6.013 g ± 0.376) and organic (5.292 g ± 0.406) treatment (Table 3.4).

An analysis of simple main effects for harvest was conducted with statistical significance receiving a Bonferroni adjustment. There was a statistically significant effect of harvest on mean above ground dry weight for all treatments (Table 3.5) but the effect size was greater for the N only control and inorganic treatment than the combined or organic treatment.

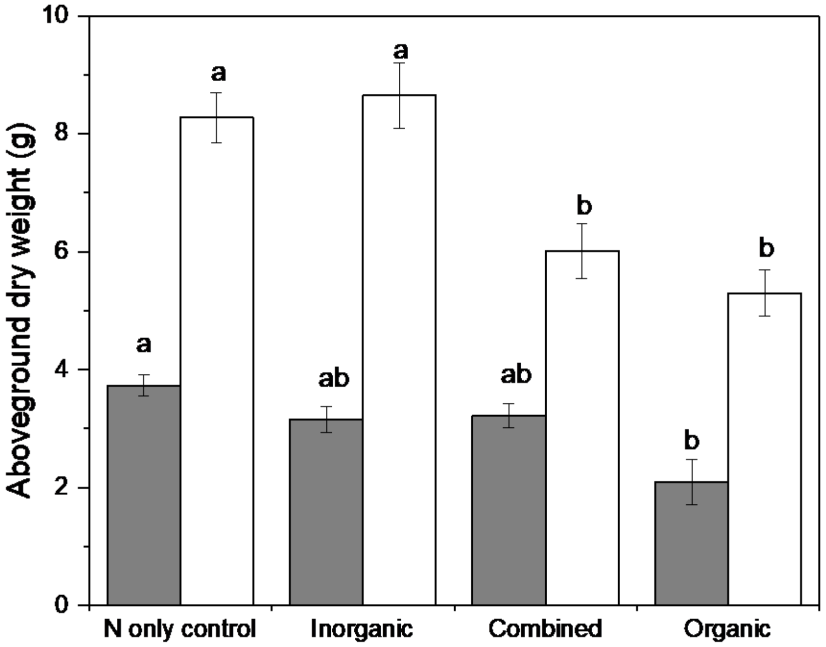

Figure 3.3: Mean aboveground dry weight of plants harvested 28 (grey bars) and 56 (white bars) days after fertilisation. Error bars are ± one standard error of the mean. Treatments are described in Table 3.1. Pairwise comparisons were run for treatment at each harvest. The mean value of bars sharing the same letter are not statistically significantly different ($p > 0.05$). Pairwise comparisons for harvest are presented in Table 3.5, respectively. $n = 7$.

Table 3.4: Pairwise comparisons between treatments for aboveground dry weight at each harvest. Comparisons were run to determine statistically significant differences in aboveground dry weight (g) between wheat plants receiving different fertiliser treatments 28 days (harvest one) and 56 days (harvest two) after fertilisation. p-values received a Bonferroni adjustment. Statistically significant differences between treatments are highlighted in bold. Treatments are described in Table 3.1 and mean values are presented in Figure 3.4. n = 7.

Harvest	Treatment		Mean difference	Standard error	p-value
One	Zero	Inorganic	0.58	0.53	1.000
		Combined	0.51	0.62	1.000
		Organic	1.6	0.55	**0.030**
	Inorganic	Combined	- 0.066	0.62	1.000
		Organic	1.1	0.55	0.370
	Combined	Organic	1.1	0.62	0.520
Two	Zero	Inorganic	- 0.34	0.53	1.000
		Combined	2.3	0.52	**0.0010**
		Organic	3.0	0.55	**<0.001**
	Inorganic	Combined	2.6	0.53	**<0.001**
		Organic	3.4	0.55	**<0.001**
	Combined	Organic	0.72	0.55	1.000

Table 3.5: The effect of harvest on dry weight for each fertiliser treatment. Results were obtained from a test of simple main effects with Bonferroni adjustment. Statistically significant effects are highlighted in bold. Treatments are described in Table 3.1. n = 7.

Treatment	Degrees freedom	F	p	Partial η^2
N only control	1,43	72.9	**< 0.001**	0.629
Inorganic	1,43	107	**< 0.001**	0.713
Combined	1,43	20.0	**< 0.001**	0.318
Organic	1,43	31.0	**< 0.001**	0.419

3.3.4 Root biomass

A two-way ANOVA was conducted to determine the effect of treatment, harvest and their interaction on root biomass. There was homogeneity of variances (Levene's test, p = 0.166), residuals were normally distributed in each cell of the design (Shapiro-Wilk's test, p > 0.05) and there were no outliers as assessed by visual inspection of a boxplot. The interaction effect between harvest and treatment was not statistically significant, $F_{(3,43)} = 0.157$, p = 0.925, $\eta^2 = 0.0110$ (Table 3.6). Values are marginal means ± one standard error of the mean. Analysis of main effects showed there was a statistically significant increase in root biomass between harvest one (1.58 ± 0.143) and harvest two (2.52 ± 0.132), $F_{(1,43)} = 23.9$, $p < 0.001$, $\eta^2 = 0.357$). There was no statistically significant effect of treatment on root biomass $F_{(3,43)} = 1.16$, p = 0.337, $\eta^2 = 0.0750$, although at harvest two root biomass decreased as the proportion of nutrients supplied pig slurry increased.

Table 3.6: Mean root biomass and root-to-shoot ratio of plants 28 and 56 days after the application of fertilisers. Treatments are described in Table 3.1. Values are mean ± one standard error of the mean (SEM). n = 7.

	Mean root dry weight ± SEM (g)		Root:shoot ratio	
Treatment	Harvest one	Harvest two	Harvest one	Harvest two
N only control	1.83 ± 0.270	2.78 ± 0.222	4.77 ± 0.834	6.07 ± 0.372
Inorganic	1.44 ± 0.233	2.59 ± 0.272	5.34 ± 1.07	7.19 ± 1.04
Combined	1.63 ± 0.187	2.51 ± 0.359	4.18 ± 0.700	5.41 ± 0.874
Organic	1.41 ± 0.262	2.22 ± 0.229	3.31 ± 0.565	4.93 ± 0.475

Root:shoot ratio was calculated by dividing the mass of shoots by the mass of roots, i.e. for the N only control at harvest one the root:shoot ratio is 1:4.77, versus 1:3.31 for the organic treatment. Therefore a high value in Table 3.6 represents a plant with a greater mass of shoot per unit of root. A two-way ANOVA was run to determine the effect of treatment and harvest on root:shoot biomass. There was no statistically significant interaction, $F_{(3,43)} = 0.063$, p = 0.979, partial η^2 = 0.004. The effect of treatment on root:shoot ratio was not statistically significant, $F_{(3,43)} = 2.53$, p = 0.070, partial η^2 = 0.150. Root:shoot ratio increased significantly between harvest one (4.40 ± 0.432) and harvest two (5.90 ± 0.398), $F_{(1,43)} = 6.57$, p = 0.014, partial η^2 = 0.132.

3.3.5 Plant nutrient status

Plant nutrient status was assessed at the end of the experiment, 56 days after the application of fertilisers. Assumptions of one-way ANOVA were tested for total N uptake (TN), total P uptake (TP), P concentration ([P]) and N concentration ([N])). The outcome of assumption testing is presented in Table 3.7 along with a description of the final test conducted. p-values for multiple comparisons received a Bonferroni adjustment.

Table 3.7: Test assumptions for analysis of plant nutrient content. Data for plant nutrient content were assessed for assumptions of one-way ANOVA. Homogeneity of variances was assessed using Levene's test and was accepted when $p > 0.05$. Normal distribution was assessed using Shapiro-Wilk's test and was accepted when $p > 0.05$. Outliers were assessed by inspection of a boxplot. The final test adopted was based on the outcome of these tests and is explained in the "Decision" column.

Parameter	Homogeneity of variances	Normally distributed	No outliers	Decision
[N] (mg/g)	✗	✓	✓	Continue with Welch's robust-test of equality of mean and Games-Howell post-hoc.
Total N uptake (mg)	✓	✓	✗	There was one outlier 1.5 box-lengths from the edge of a boxplot. Continue with Kruskal-Wallis and Dunn's post-hoc.
[P] (mg/g)	✓	✗	✗	There was one outlier 1.5 box-lengths from the edge of a boxplot. Data was not normally distributed. Assumptions could not be met by data transformation. Continue with Kruskal-Wallis and Dunn's post-hoc on non-transformed data.
Total P uptake (mg)	✗	✓	✗	There were two outliers 1.5 box-lengths from the edge of a boxplot and homogeneity of variances was violated. Assumptions could not be met by data transformation. Continue with Kruskal-Wallis and Dunn's post-hoc on non-transformed data.

Table 3.8: Nutrient status of wheat plants 56 days after the application of fertilisers. Differences in mean nitrogen concentration ([N]) between treatments were assessed following Welch's robust test of equality of means with a Games-Howell post-hoc test. Total N uptake (TN), total P uptake (TP) and phosphorus concentration ([P]) were analysed using the non-parametric Kruskal-Wallis test with Dunn's procedure for multiple comparisons (Dunn, 1964). P-values for multiple comparisons received a Bonferroni adjustment. Values are means ± one standard error of the mean for [N] and medians and 95% confidence intervals of the median for TN, TP and [P] where the level of replication is one soil core containing two plants. In a column, means/medians followed by the same letter are not statistically significantly different ($p > 0.05$). Treatments are described in Table 3.1. n = 7.

Treatment	Mean [N] (mg/g)	Median total N uptake (mg)	Median [P] (mg/g)	Median total P uptake (mg)
N only control	12.5 ± 0.422	104 (92.8, 112)^{ab}	2.43 (2.24, 2.91)^{b}	21.2 (19.7, 22.3)^{ab}
Inorganic	12.2 ± 0.236	113 (89.6, 121)^{a}	3.03 (2.68, 3.19)^{ab}	27.0 (22.0, 28.3)^{a}
Combined	11.9 ± 0.630	70.4 (57.8, 84.3)^{bc}	3.15 (2.83, 3.81)^{a}	19.2 (17.2, 21.8)^{b}
Organic	12.2 ± 1.14	65.7 (56.5, 69.0)^{c}	3.55 (2.97, 4.44)^{a}	19.1 (18.0, 20.2)^{b}

3.3.5.1 Phosphorus

A Kruskal-Wallis H test was run to determine if there were differences in the concentration of P in plant tissue between fertiliser treatment groups. Median [P] values decreased in the order organic, combined, inorganic, N only control (Table 3.8). Distributions of P concentration in plant tissue were not similar for all groups, as assessed by visual inspection of a boxplot. Pairwise comparisons were tested using Dunn's procedure (Dunn, 1964) with a Bonferroni correction for multiple comparisons (Table 3.8). There were statistically significant differences in the distribution of [P] between groups, $\chi^2_{(3)}$ = 12.6, p = 0.006. Post-hoc analysis showed that the [P] was statistically significantly higher in the organic treatment (mean rank = 20.7) and the combined treatment (mean rank = 17.46) compared to the zero-P treatment (mean rank = 6.14) (p = 0.006 and p = 0.47, respectively). There was no statistically significant difference between the inorganic treatment (mean rank = 12.7) and the zero-P treatment (p = 0.278), combined treatment (p = 1.000) or the organic treatment (p = 0.430).

Another Kruskal-Wallis H test was run to determine if there were an effect of fertiliser treatment on the TP content of plant tissue. Median TP content decreased in the order inorganic, N only control, combined, organic (Table 3.8). Distributions of TP in plant tissue were not similar for all groups, as assessed by visual inspection of a boxplot. There were statistically significant differences in the distribution of [P] between groups, $\chi^2_{(3)}$ = 12.2, p = 0.007. Pairwise comparisons were tested using Dunn's procedure (Dunn, 1964) with a Bonferroni correction for multiple comparisons (Table 3.8). The total P uptake by plants in the inorganic treatment (mean rank = 21.9) was statistically significantly higher than that of the combined treatment (mean rank = 10.00) (p = 0.031) and the organic treatment (mean rank = 8) (p = 0.010) but not statistically significantly higher than the N only control (mean rank = 15.3) ($p = 0.728$). The total P uptake by plants in the N only control was not statistically significantly higher than the combined fertiliser treatment ($p = 1.00$) or the organic treatment ($p = 0.594$).

3.3.5.2 Nitrogen

There was no statistically significant difference in the concentration of N in plant tissue between fertiliser treatment groups, Welch's $F_{(3,\ 11.2)} = 0.186$, $p = 0.904$ (Table 3.8). A Kruskal-Wallis H test was run to determine if there were differences in total N uptake (mg) of wheat plants between fertiliser treatments (Table 3.8). Distributions of TN content were similar for all groups, as assessed by visual inspection of a boxplot. Median TN content decreased in the order inorganic, N only control, combined, organic. Median TN values were statistically significantly different between fertiliser treatments, $\chi^2_{(3)} = 18.3$, $p < 0.001$. Median TN content of the inorganic and N only control was statistically significantly higher than the median TN content of the organic treatment ($p = 0.004$ and $p = 0.012$, respectively). The median TN content of the inorganic treatment was also statistically significantly higher than the median TN content of the combined treatment ($p = 0.020$). There was no statistically significant difference in median TN content between the combined and organic treatment ($p = 1.00$).

3.3.6 Olsen's phosphorus

A two-way ANVOA was conducted to examine the effect of fertiliser treatment and harvest on the concentration of phytoavailable P in soil, measured as Olsen's P (mg kg^{-1}) (Figure 3.4). There was homogeneity of variances (Levene's test, $p = 0.780$) and normality was achieved in each cell of the design (Shapiro-Wilk's, $p > 0.05$). Outliers were detected by inspection of a boxplot. There were 3 outliers 1.5 to 3 box-lengths from the edge of the box but there was no evidence of data or measurement error so these were included in the analysis. Means are presented as marginal means ± one standard error of the mean.

The interaction effect between treatment and harvest was not statistically significant, $F_{(3,43)}$ = 1.86, p = 0.151, η^2 = 0.115. Therefore analysis of main effects was tested for treatment and harvest. The concentration of Olsen's P was similar at harvest one (55.1 ± 1.92) and harvest two (50.5 ± 1.77), $F_{(1,43)}$, p = 0.083, η^2 = 0.068. There was a statistically significant effect of treatment on the concentration of Olsen's P in soil $F_{(3,43)}$ = 7.32, p < 0.001, η^2 = 0.338. p-values for multiple comparisons were Bonferroni adjusted (Table 3.9). The marginal means ± one SEM for the N only control, inorganic, combined and organic treatment were 43.9 ± 2.45, 55.6 ± 2.45, 60.6 ± 2.88 and 51.2 ± 2.65, respectively. There was no statistically significant difference in the concentration of Olsen's P between P-fertilised treatments. Only the inorganic and combined treatment increased the concentration of Olsen's P above the level of the N only control (p = 0.010 and p <0.001, respectively).

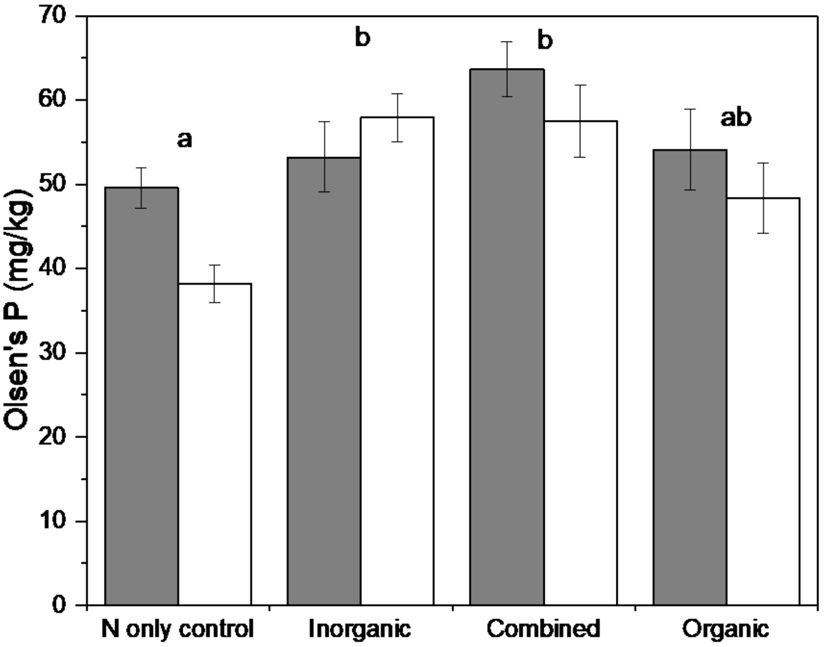

Figure 3.4: Mean Olsen's phosphorus concentration (mg kg^{-1}) of soil harvested 28 (grey bars) and 56 (white bars) days after fertilisation. Error bars are ± one standard error of the mean. Treatments are described in Table 3.1. The mean value of bars sharing the same letter are not statistically significantly different ($p > 0.05$). Absolute values for pairwise comparisons between treatments are presented in Table 3.9. n = 7.

Table 3.9: Pairwise comparisons between treatments for Olsen's phosphorus concentration (mg kg^{-1}) in soil. p-values received a Bonferroni adjustment. Statistically significant differences between treatments are highlighted in bold. Treatments are described in Table 3.1 and mean values are presented in Figure 3.4. n = 7.

Treatment		Mean difference	Standard error of the mean	p-value
N only control	Inorganic	- 11.7	3.47	**0.010**
	Combined	- 16.7	3.78	**<0.001**
	Organic	- 7.35	3.61	0.288
Inorganic	Combined	- 5.02	3.78	1.000
	Organic	4.32	3.61	1.000
Combined	Organic	9.34	3.91	0.129

3.3.7 Water-soluble phosphorus

A two-way ANOVA was run to assess the effect of treatment and harvest on water-soluble phosphorus concentration (P_{water}, mg kg^{-1}) in soil (Figure 3.5). The assumption of homogeneity of variances was met (Levene's test, p = 0.629) and normality was achieved in each cell of the design (Shapiro-Wilk's test, p > 0.05). Outliers were assessed by inspection of a boxplot. There were several outliers 1.5 to 3 box-lengths from the edge of the box which were included in the analysis as there was no evidence of data entry or measurement error. Means are presented as marginal means ± one standard error of the mean.

The interaction effect between treatment and harvest was not statistically significant, $F_{(3,43)} = 0.716$, p = 0.548, $\eta^2 = 0.048$. Therefore the main effect of treatment and harvest was tested. The concentration of water-soluble P in soil was statistically significantly lower at harvest two (2.99 ± 0.225) than harvest one (3.84 ± 0.245), $F_{(1,43)} = 6.48$, p = 0.015, $\eta^2 = 0.131$. There was a statistically significant effect of treatment on the concentration of water-soluble P in soil, $F_{(3,43)} = 6.67$, p = 0.001, $\eta^2 = 0.318$. P_{water} concentration was statistically significantly higher in the inorganic and combined treatment compared to the N only control (p = 0.047 and p = 0.001, respectively) (Table 3.10). There was no statistically significant difference in the WSP concentration between the N only control and the organic treatment (p = 0.051) or the organic treatment and either of the other P-fertilised treatments. The marginal means for the N only control, inorganic, combined and organic treatment were 2.27 ± 0.312, 3.50 ± 0.312, 4.35 ± 0.366 and 3.53 ± 0.337, respectively.

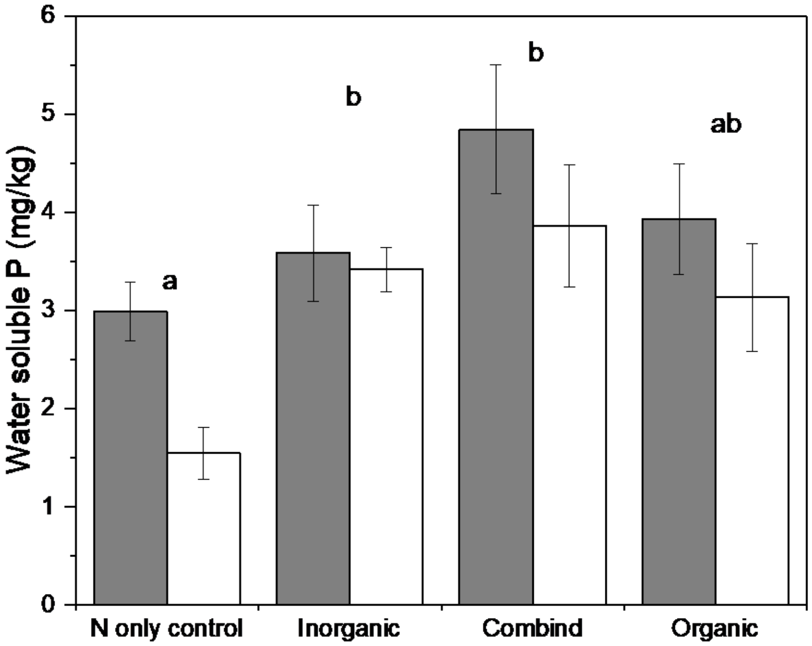

Figure 3.5: Mean water-soluble phosphorus concentration (mg kg^{-1}) of soil harvested 28 (grey bars) and 56 (white bars) days after fertilisation. Treatments are described in Table 3.1. The mean value of bars sharing the same letter are not statistically significantly different ($p > 0.05$). Absolute values for pairwise comparisons are presented in Table 3.10. n = 7.

Table 3.10: Pairwise comparisons between treatments for water-soluble phosphorus concentration (mg kg^{-1}) in soil. p-values received a Bonferroni adjustment. Statistically significant differences between treatments are highlighted in bold. Treatments are described in Table 3.1.

Treatment		Mean difference	Standard error of the mean	p-value
N only control	Inorganic	- 1.23	0.441	**0.047**
	Combined	- 2.08	0.481	**0.001**
	Organic	- 1.27	0.459	0.051
Inorganic	Combined	- 0.852	0.500	0.500
	Organic	- 0.034	0.459	1.000
Combined	Organic	0.818	0.498	0.644

3.3.8 Available N

The effect of harvest and treatment on the concentration of ammonium-N (NH_4-N), nitrate-N (NO_3-N) and total available N (NH_4-N + NO_3-N + nitrite-N (NO_2-N)) was determined by two-way ANOVA (Table 3.11). In order to meet the assumptions of the two-way ANOVA, a square root transformation was applied to NO_3-N and total available N data. Based on the inspection of a boxplot there was one outlier in the NH_4-N data (studentised residual, 6.14) and another in the NO_3-N data (studentised residual, 3.93) which were removed from the analysis. The interaction effect was not statistically significant for NH_4-N, NO_3-N or total available N (Table 3.11). Therefore analysis of main effects for treatment and harvest was conducted with pairwise comparisons receiving a Bonferroni correction. Means are presented as marginal means ± one standard error of the mean.

There was a statistically significant effect of treatment on the concentration of NO_3-N, NH_4-N and total available N in soil (Table 3.11). However, the results of pairwise comparisons show that only NH_4-N concentration is statistically significantly different between treatments (Table 3.12). The concentration of NH_4-

N in soil decreased in the order organic, inorganic, combined, N only control (Table 3.12). There was no statistically significant difference in the concentration of NH_4-N between treatments fertilised with P. However the concentration of NH_4-N was statistically significantly lower in the N only control than the inorganic and organic treatment ($p = 0.035$ and $p = 0.028$, respectively).

The concentration of NO_3-N and total available N was statistically significantly higher at harvest one (1.92 ± 0.0690 and 2.14 ± 0.070, respectively) than harvest two (1.68 ± 0.0650 and 1.92 ± 0.0660, respectively) ($p = 0.014$ and $p = 0.029$, respectively). There was no statistically significant difference in NH_4-N concentration between harvest one (0.802 ± 0.028) and harvest two (0.727 ± 0.026) ($p = 0.058$).

Table 3.11: The effect of treatment, harvest and their interaction on available nitrogen (N) concentration in soil. Data was analysed by two-way ANOVA. Abbreviations: NO_3-N, nitrate-N; NH_4-N, ammonium-N. Total available N is the sum of nitrate-N, ammonium-N and nitrite-N. Statistically significant effects ($p < 0.05$) are highlighted in bold. Treatments are described in Table 3.1. Pairwise comparisons are presented in Table 3.12. n = 7.

N compound	Effect	Degrees freedom	F	p-value	η^2
NO_3-N	Interaction	3,42	1.03	0.390	0.068
	Harvest	1,42	6.58	**0.014**	0.135
	Treatment	3,42	3.45	**0.025**	0.197
NH_4-N	Interaction	3,42	1.31	0.284	0.085
	Harvest	1,42	3.81	0.058	0.083
	Treatment	3,42	3.92	**0.015**	0.219
Total available N	Interaction	3,42	1.32	0.281	0.086
	Harvest	1,42	5.12	**0.029**	0.109
	Treatment	3,42	3.47	**0.024**	0.198

Table 3.12: Pairwise comparisons between treatments for nitrate-N (NO_3-N), ammonium-N (NH_4-N) and total available N (the sum of NO_3-N, NH_4-N and nitrite-N concentration) concentration (mg kg^{-1}) in soil. Means are non-transformed values for harvest one and two, which took place 28 and 56 days after fertilisation, respectively. Marginal means are presented for the purpose of multiple comparisons. The values for NO_3-N and total available N are marginal means ± one standard error of the mean for square root transformed data. The values presented for NH_4-N are marginal means ± one SEM for untransformed data. p-values for multiple comparisons received a Bonferroni adjustment. Treatments are described in Table 3.1. n = 7.

Treatment	Harvest	NO_3-N^{c}	NH_4-N	Total available N^{c}
N only control	One	3.47 ± 0.379	0.730 ± 0.040	4.25 ± 0.415
	Two	1.99 ± 0.075	0.607 ± 0.020	2.64 ± 0.080
	Marginal mean	1.63 ± 0.092	0.662 ± 0.035^{a}	1.83 ± 0.093
Inorganic	One	3.45 ± 0.486	0.885 ± 0.045	4.40 ± 0.501
	Two	2.46 ± 0.563	0.732 ± 0.046	3.25 ± 0.598
	Marginal mean	1.67 ± 0.088	0.808 ± 0.035$_{b}$	1.92 ± 0.089
Combined	One	3.90 ± 0.576	0.745 ± 0.070	4.69 ± 0.550
	Two	4.14 ± 0.622	1.14 ± 0.357	5.36 ± 0.916
	Marginal mean	1.98 ± 0.103	0.769 ± 0.043$_{ab}$	2.21 ± 0.104
Organic	One	4.28 ± 0.377	0.847 ± 0.086	5.19 ± 0.422
	Two	4.23 ± 0.563	0.790 ± 0.042	4.23 ± 0.598
	Marginal mean	1.93 ± 0.095	0.818 ± 0.038^{b}	2.15 ± 0.096

[c] Results of pairwise comparisons showed no statistically significant difference in the concentration of NO_3-N and total available N in soil between treatments, despite the overall effect of treatment being statistically significant (NO_3-N, p = 0.025; total available N, p = 0.024).

3.3.9 Soil pH

A two-way ANVOA was conducted to determine the effect of fertiliser treatment and harvest on soil pH (Figure 3.6). There was homogeneity of variances ($p > 0.05$) but the assumption of normality was violated in two groups ($p < 0.05$, right-skewed). Inspection of a boxplot showed one outlier each in two cells of the design. Outliers were included in the analysis as there was no evidence to suggest they were caused by data entry or measurement error; their inclusion had no effect on the main conclusions that could be drawn from the test. Test assumptions could not be met by a $\log_{10}$ transformation of the data. A GLM with gamma probability distribution and log link function was generated. Pairwise comparisons were Bonferroni corrected.

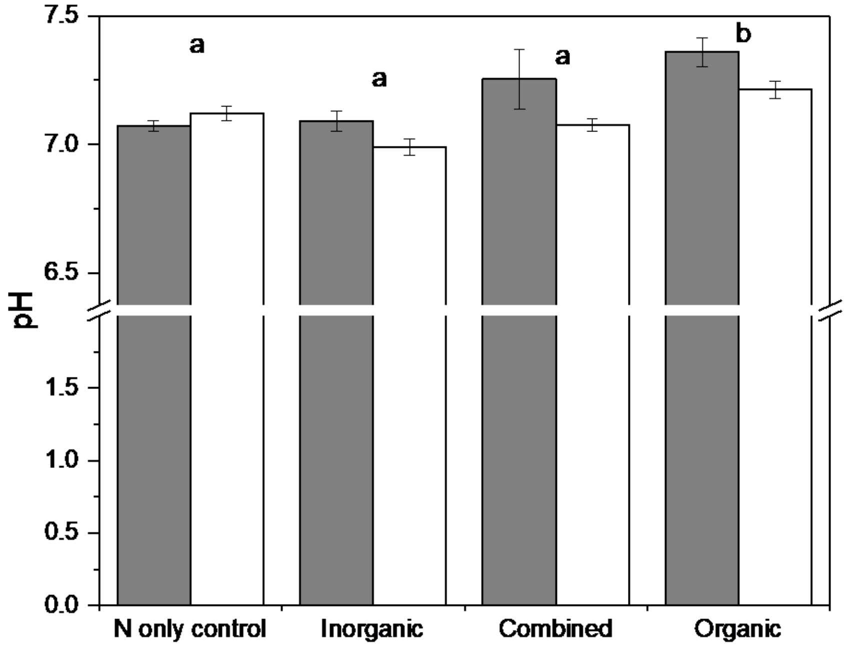

Figure 3.6: Mean pH of soil harvested 28 (grey bars) and 56 (white bars) days after fertilisation. Treatments are described in Table 3.1. Error bars are ± one standard error of the mean. The mean value of bars sharing the same letter are not statistically significantly different ($p > 0.05$). Absolute values for pairwise comparisons are presented in Table 3.13. $n = 7$.

There was a statistically significant association between treatment and pH of the upper soil profile, $\chi^2_{(3)} = 22.7$, $p < 0.001$. There was no statistically significant association between harvest and pH of the upper soil profile, $\chi^2_{(1)} = 2.48$**,** $p = 0.115$ and no statistically significant interaction between treatment and harvest, $\chi^2_{(3)} = 6.01$, $p = 0.111$. Soil in the organic treatment was statistically significantly more alkaline than the N only control, inorganic and combined treatments (Table 3.13). There was no statistically significant difference between the pH of other treatments.

Table 3.13: Pairwise comparisons between treatments for soil pH. p-values were Bonferroni-adjusted within each simple main effect. Results were obtained from a test of simple main effects with Bonferroni adjustment. Statistically significant differences between treatments are highlighted in bold. Treatments are described in Table 3.1. n = 7.

Treatment		Mean difference	Standard error of mean	p-value
N only control	Inorganic	0.0859	0.0531	0.592
	Combined	- 0.0147	0.0568	1.000
	Organic	- 0.172	0.0550	**0.010**
Inorganic	Combined	- 0.101	0.0568	0.458
	Organic	- 0.258	0.0549	**<0.001**
Combined	Organic	- 0.157	0.0594	**0.049**

3.4 Discussion

Contrary to this study's hypothesis the concentration of Olsen's P was similar between P-fertilised treatments. This finding challenges the results of other studies (Garg & Bahl, 2008; Halajnia et al., 2009; Mao et al., 2015; Reddy et al., 2005; Toor & Bahl, 1997) which report higher concentrations of soil labile P when fertiliser sources are combined, compared to the application of inorganic P only. However, in many of these studies the amount of P added in combined treatments far exceeds that applied in the inorganic comparison (Garg & Bahl, 2008; Halajnia et al., 2009; Mao et al., 2015; Toor & Bahl, 1997). When P application rates are matched, both higher (Ahmed et al., 2019; Reddy et al., 2005; Xin et al., 2017) and similar concentrations of available P have been reported in combined compared to inorganic treatments (Zhao et al., 2016). The results suggest that in this system P sorption/desorption reactions are not affected by the combination of organic and inorganic fertilisers, providing support for the argument made by Guppy et al., (2005a) that increases in labile P reported in previous studies are due to unaccounted P in organic amendments.

The concentration of Olsen's P and water-soluble P in soil have been shown to be correlated with P losses in leachate and runoff (Leinweber et al., 1999; Pote et al., 1996; Schoumans & Groenendijk, 2000). No evidence is presented for reduced leaching losses when the inorganic nutrient supply is substituted with pig slurry. Indeed the highest concentration of Olsen's P was recorded in the combined treatment at harvest one when the value exceeded 60 mg P kg^{-1}, a "turning point" above which total dissolved phosphorus levels in leachate increase considerably (Glæsner et al., 2013; Heckrath et al., 1995). P fertilisation also increased the concentration of water-soluble P in soil, with the highest concentration reported in the combined treatment. The data suggests that combining organic and inorganic fertilisers could increase the risk of phosphorus leaching, but this needs to be verified in a soil with a low P status and through quantification in leachate rather than soil.

In the present study, substituting 100% of the N and P budget with pig slurry resulted in statistically significantly lower wheat aboveground dry weight 28 days after fertilisation compared to the N only control. At the same time point, there was no statistically significant difference in aboveground dry weight between the combined, inorganic or N only control. By harvest two (56 days after fertilisation) mean aboveground dry weight of plants from treatments receiving pig slurry, alone or in combination with inorganic fertiliser, was significantly lower than the inorganic and N only control. This agrees with long-term studies which report lower maize yields compared to inorganic controls in treatments where PS is included as a substitute for chemical fertilisers (Gong et al., 2009) or provides all of the crop's N (Gong et al., 2009; Li, 2013; Martínez et al., 2017). The results also support long-term field studies using a range of organic amendments, that show lower yields in crops fertilised with organic and inorganic fertilisers, compared to those receiving only inorganic fertilisers (Dawe et al., 2003; Gong et al., 2009; Xie et al., 2016; Xin et al., 2017; Yadav et al., 2000).

The reduced yields reported here are unlikely to be due to differences in P phytoavailability as the baseline concentration of soil available P was high and was slightly increased in the combined compared to the inorganic treatment. Trends may instead be explained by differences in N availability and form. Previous studies investigating the effect of pig slurry on yield have reported N availability as a limiting factor (Li, 2013; Martínez et al., 2017). The results show no statistically significant difference in the concentration of soil-available N between treatments, suggesting that N availability is not affecting aboveground dry weight.

Although differences in N availability are not evident in the soil, plants in the combined and organic treatment displayed some symptoms which suggest N limitation or toxicity. High root-to-shoot ratio is a sign of low N in soil (Bahrman et al., 2005; Grechi et al., 2007) and increased with the proportion of N supplied by pig slurry. The form of N added may also have been affecting productivity especially in the organic treatment. The majority of nitrogen added with pig slurry is in the form of NH_4-N which is toxic to wheat (Cox & Reisenauer, 1973; Setién et al., 2013; Wang et al., 2016). Symptoms of NH_4-N toxicity include reduced shoot and root biomass (Guo et al., 2019; Setién et al., 2013; Wang et al., 2016) and high P concentrations in plant tissue (Britto & Kronzucker, 2002; Cox & Reisenauer, 1973; Kirkby & Mengel, 2008; Van Beusichem et al., 2008). By the end of the experiment, root biomass was on average 14.4% lower in the organic compared to the inorganic treatment and 20.3% lower in the organic compared to the N only control. High levels of NH_4-N at harvest two could explain the slightly suppressed root growth recorded in the combined treatment, although suppression was less severe than in the organic treatment. The concentration of P in wheat tissue was also higher in treatments receiving pig slurry, compared to the inorganic treatment. Although inconclusive, the results suggest a possible role of NH_4-N in determining dry weight when pig slurry is used as an organic amendment.

Symptoms of NH_4-N toxicity were less severe for the combined treatment, in terms of root and shoot biomass and PO_4-P accumulation in tissue. While soil NO_3-N and NH_4-N concentration decreased between harvests in the inorganic and organic treatment, the opposite pattern was observed in the combined treatment. This suggests N may have been immobilised in the combined treatment before being re-mineralised later in the growing cycle. It is possible that asynchrony between plant uptake and soil demand reduced aboveground dry weight in the combined treatment. Such immobilisation/remineralisation patterns have been reported in other studies investigating the effect of combined fertiliser applications on productivity and N cycling (Gentile et al., 2013, 2009). Although the experiment was terminated before grain development it is possible that higher levels of N, especially NO_3-N, during anthesis could improve grain protein content (Bogard et al., 2010; Taulemesse et al., 2016) and therefore quality (Branlard et al., 2001; Shewry, 2007). This mechanism could counteract the cost of reduced aboveground dry weight at the end of stem elongation in the combined treatment.

A limitation of this study is unequal potassium (K) application rates between treatments. The soil used here has a potassium index of 2-, which is the target index for arable rotations (AHDB, 2020). At this level, potassium should be added to maintain the target index only (AHDB, 2020). For winter wheat, the recommended application rate is 45 kg K_2O ha^{-1} and this addition can be made at any time of year (AHDB, 2020). It is unlikely that productivity was suppressed by potassium limitation in the organic and combined treatment which received potassium at a rate of 160 and 80 kg K_2O ha^{-1} respectively. Conversely, it is possible that productivity of plants in the inorganic treatment could have been enhanced by the incorporation of K. The effect of this would be an increased performance gap between treatments. Overall this is unlikely because yield responses are only expected below the soil target index (AHDB, 2020).

3.5 Conclusions

In this study, there was no statistically significant difference in Olsen’s or water-soluble P concentration between treatments receiving inorganic or combined fertiliser applications. The data provides no support for the integration of organic and inorganic fertilisers as a strategy to reduce P leaching losses from soil and suggests that this practice could compromise wheat yields in the short term. More work is required to determine the effect of organic substitution on grain yield and P dynamics on soils with low starting P status and with different organic amendments.

Chapter 4 Nutrient cycling and leaching losses following integrated organic-inorganic fertilisation on a soil with a low P status

4.1 Introduction

There is growing interest in the use of organic fertilisers to support yields. In China, partial substitution of the total nutrient budget with organic fertilisers has been promoted as a method to reduce extreme levels of aquatic and atmospheric pollution experienced in the country (Shuqin & Fang, 2018). On the other hand, farmers in sub-Saharan Africa (SSA) are encouraged to combine the use of organic and inorganic fertilisers to overcome the limited supply of both resources in the region and enhance yield (Gentile et al., 2009). Combining or substituting organic and inorganic fertilisers has clear benefits in terms of reducing farmers' reliance on fertilisers derived from non-renewable resources and overcoming supply issues.

The use of organic fertilisers provides additional benefits when the amendment is derived from human waste. A number of technologies have been developed that recover nutrients from human waste water in a form which can be used as fertiliser (Desmidt et al., 2015) including anaerobic digestion (AD). AD is the microbial breakdown of organic waste into methane and digestate, a biofertiliser permitted for use in UK agriculture. Introducing digestate derived from waste water management into farm nutrient budgets represents a step towards closed-loop nutrient cycling and diverts nutrients away from waterbodies where they contribute to eutrophication.

Digestate can be divided into two fractions: the liquid fraction and the solid fraction which is referred to as digested cake (DC). DC can be applied directly as fertiliser (Drosg et al., 2015) but presents issues for farmers because of the typically low nitrogen-to-phosphorus ratio of these products (Vanden Nest et al., 2015). Given the high concentrations of phosphorus (P) in DC, farmers may over apply P when calculating fertiliser application rates based on nitrogen (N) content. Excessive P application rates are an issue in experiments comparing the fertiliser value of digestate with other organic amendments, where higher concentrations of soil available P may be explained by unequal P application rates between treatments applied according to N concentration (Elliott et al., 2005; Esteller et al., 2009; Galvez et al., 2012; Haraldsen et al., 2011).

Studies comparing the ability of different fertiliser sources to increase soil available P concentration have been conducted with treatments providing equal P application rates. Results show that digestate can increase P phytoavailability compared to alternative biofertilisers (Vanden Nest et al., 2015). Other studies report statistically insignificant differences in P phytoavailability between soils amended with AD products compared with a range of organic inputs (Möller & Stinner, 2010; Vaneeckhaute et al., 2016). Studies applying bio- and inorganic fertilisers at equal P rates have reported lower (Loria & Sawyer, 2005; Vaneeckhaute et al., 2016), higher (Alburquerque et al., 2012) and similar (Bachmann et al., 2011; Losak et al., 2014; Sigurnjak et al., 2016; Vanden Nest et al., 2015; Vaneeckhaute et al., 2016) concentrations of available P in soil fertilised with digestate compared to soils fertilised with chemical P, although the degree of difference changes over time (Alburquerque et al., 2012; Vaneeckhaute et al., 2016) and with P application rate (Loria & Sawyer, 2005). Higher concentrations of P in leachate or soil solution have been reported when soils have been amended with digestate compared to triple superphosphate (TSP) (Vanden Nest et al., 2015; Vaneeckhaute et al., 2016). In contrast, Elliott et al. (2002) reported lower leaching losses, P phytoavailability and P uptake into Bahiagrass when soils were fertilised with a range of AD products compared with inorganic P, although the decrease in P phytoavailability and uptake did not result in lower yields (O'Connor et al., 2004). Low P phytoavailability and leaching losses from AD products compared to inorganic P fertilisers has been reported elsewhere and may be explained by the use of Fe and Al in the waste water treatment process (Alleoni et al., 2008; Brandt et al., 2004; Elliott et al., 2005). Yang et al. (2008) showed that the effect of iron (Fe) and aluminium (Al) on P binding can be strong enough to reduce P leaching losses from soils amended with AD plus inorganic P compared to inorganic P only, even though the total P application rate was higher in the combined treatment (Yang et al., 2008).

Incorporating raw animal waste into nutrient budgets is a viable fertilisation strategy for farms without waste treatment facilities or access to AD products. As well as providing nutrients to crop, spreading manure or slurry onto land can save farmers money in disposal costs and help to close farm nutrient cycles. In the United Kingdom (UK), pigs are estimated to produce 12, 3 and 7 Kt of N, P and potassium (K) annually in slurry, making pig slurry a valuable source of nutrients for UK agriculture (ADAS, 2008). Pig slurry has been shown to successfully increase total and phytoavailable P compared to soils not receiving fertiliser, with greater effects at higher application rates (Chikuvire et al., 2019; Hountin et al., 1997). As with digested cake, repeated applications of pig slurry based on N content can lead to significant increases in soil test P while applications made based on P can have no effect (Karimi et al., 2018). Application of pig slurry can increase the concentration of soluble reactive phosphorus (SRP) in leachate although the response has been shown to vary depending on soil fertiliser history (Liu et al., 2012). Studies comparing leachate and runoff P losses in mineral- and pig slurry-amended soils suggest similar losses between treatments in terms of concentration and load (Aronsson et al., 2014; Kleinman et al., 2010; Kumaragamage et al., 2012).

Non-P nutrients in organic amendments can have a significant effect on the phytoavailability of phosphorus in soil. It has been suggested that high levels of exchangeable calcium in fresh manures and sewage sludge result in the formation of calcium phosphate precipitates with low phytoavailability (Bachmann et al., 2016; de Conti et al., 2015; Siddique & Robinson, 2003). As previously mentioned, high levels of iron and aluminium in biosolids have been shown to “stabilise” phosphorus and reduce estimated P leaching risk due to an increase in P sorption capacity (Withers et al., 2016). Similarly, adding aluminium chloride to pig slurry can reduce the concentration of soluble reactive phosphorus in slurry by 73% and in leachate by 53%, compared to untreated slurry (Smith et al., 2004). The addition of liquid ferric chloride ($FeCl_3$) can also reduce P losses from soils amended with pig slurry (O’ Flynn et al., 2013). As well as intentional manipulation through the addition of P binding minerals, the composition of pig slurry can be affected by the animal’s diet as minerals can pass through the animal undigested. For example, pig diets may be supplemented with iron to enhance growth and performance (Li et al., 2018) which could act to stabilise P in waste materials (Moreno-Caselles et al., 2005). This demonstrates that factors other than organic matter derivatives, especially iron, aluminium and calcium, can affect phosphorus phytoavailability after the addition of both pig slurry and digested cake.

Although digestate and pig slurry have both been shown to increase soil available P concentration, it is unlikely that either input will provide balanced nutrition when applied alone. In conventional cropping systems, farmers may need to supply inorganic fertilisers with the organic amendment to provide the full complement of plant nutrients without exceeding permissible levels of N. When applied alone, organic fertilisers have been shown to increase P phytoavailability (Gong et al., 2009; Jiao et al., 2007; Pizzeghello et al., 2011; Song et al., 2017; Yan et al., 2018) and leaching losses (Esteller et al., 2009; Kang et al., 2011; Kleinman et al., 2010; Laboski & Lamb, 2004; McDowell & Sharpley, 2004; Svanbäck et al., 2013; Vanden Nest et al., 2016, 2014) compared to the application of inorganic P fertilisers. Data suggests that combining the use of inorganic inputs with organic alternatives can increase phytoavailable P, crop P uptake and P leaching compared to the sole application of either fertiliser type (Ahmed et al., 2019; Garg & Bahl, 2008; Halajnia et al., 2009; Leinweber et al., 1999; Mao et al., 2015; Reddy et al., 2005; Toor & Bahl, 1997; Xin et al., 2017). However, our understanding of the mechanisms and timings of increases in P phytoavailability and losses is limited by unbalanced fertiliser application rates between treatments (Garg & Bahl, 2008; Halajnia et al., 2009; Leinweber et al., 1999; Mao et al., 2015; Mokolobate & Haynes, 2003; Toor & Bahl, 1997; Zhang et al., 2011), incubation style experiments which omit important components of the soil-plant-fertiliser system (Bolan et al., 1994; Delgado et al., 2002; Halajnia et al., 2009; Reddy et al., 2005; Toor & Bahl, 1997) and long-term studies which report results at a single time point after years to decades of manipulation (Ahmed et al., 2019; Hu et al., 2018; Xin et al., 2017). Studies on the short-term effects of combined fertiliser applications are required: (i) to elucidate the time-scale over which increases in P phytoavailability occur; (ii) to determine whether any increases that do occur coincide with crop demand; and (iii) to better understand the environmental consequences of transitioning to systems which incorporate bio-based fertilisers.

The aim of this study was to determine how partial substitution of inorganic fertilisers with digested cake or pig slurry affects the concentration of Olsen's P ($P_{Olsen's}$), water-soluble P (P_{water}), organic P ($P_{organic}$), ammonium-N (NH_4-N) and nitrate-N (NO_3-N) in soil and SRP, NH_4-N and NO_3-N in leachate, compared to the sole application of inorganic fertilisers on a soil with a low starting P status over one crop cycle. This study tested the hypothesis that the concentration of available P in soil is affected by substitution of inorganic fertilisers with organic alternatives. It was predicted that the combination of organic and inorganic fertilisers would result in the greatest increase in $P_{Olsen's}$ and P_{water}, but that this effect would be greater for pig slurry than for digested cake. Accordingly, the concentration of SRP in leachate would decline in the order pig slurry substitution, digested cake substitution, inorganic only. The hypotheses were tested in a greenhouse study using intact soil cores sown with the most popular variety of winter wheat (*Triticum aestivum* L., cv 'Skyfall') amongst farmers in the UK (RAGT, 2017).

4.2 Materials and Methods

4.2.1 Core collection and preparation

Cores were collected from an arable field at Leeds University Farm, Spen Common Lane, Tadcaster, LS24 9NS, UK in October 2018. The soil is a well-drained, loamy, calcareous brown earth from the Aberford series of Calcric Endoleptic Cambisols (Cranfield University, 2018). A 10 m x 12 m grid was established for collection of cores, as described in the general methods chapter (2.3.1). Baseline sampling was conducted one week prior to the collection of experimental cores to determine soil nutrient status. Cores were extracted from ten randomly selected locations within the sampling area using a soil auger. At each location, one sample was collected from 5 - 10 cm and 15 - 20 cm depth. Samples from both depths were pooled to give one sample per location before being dried at 40°C and ground to 2 mm using a mechanical grinder (Humbolt H-4199). These samples were analysed for $P_{\text{Olsen's}}$ concentration (general methods, 2.8.4) to calculate fertiliser application rates based on British Fertiliser Regulations (Defra, 2010).

Cores for experimental manipulation were collected on 22 and 24 October 2018 from the same grid used for baseline sampling. Cores were collected to 25 cm depth in 10.4 x 28.0 cm (*internal diameter x height*) polyvinyl chloride (PVC) pipes. Three cores from each sampling day were sacrificed to estimate the volume of water in cores. Water-filled pore space (WFPS) of each core was calculated with this information (general methods, 2.3.3.1)

4.2.2 Setup of greenhouse experiment

Cores were stored at 4°C until 7 November when they were amended to 23.0 cm depth (general methods, 2.3.2). Cores were moved to a temperature and light controlled (20^{o}C, 16-hour photoperiod) greenhouse on 14 November and allowed to dry to 60% WFPS which took up to one week. Cores were maintained at 60% WFPS by watering daily with deionised water until after stem elongation when cores were watered every other day. Each core was sown with two pre-germinated seedlings of winter wheat *Triticum aestivum* L., ‘Skyfall’ (RAGT, 2017). The second seedling to emerge in each pot was removed, leaving one plant per pot for experimental manipulation.

The experiment adopted a completely randomised block design with nine treatments and nine replicates per treatment (Table 4.1). Main treatments were matched for readily available nitrogen (RAN, the sum of ammonium-N and nitrate-N) and total phosphorus pentoxide (P_2O_5) with application rates of 200 kg ha^{-1} and 60 kg ha^{-1}, respectively. Treatments were designed to test the individual and combined effect of adding P from different sources on P dynamics and leaching losses and consisted of: (i) a zero input control (zero); (ii) a half rate inorganic P_2O_5 treatment (half rate Pi); (ii) a full-rate inorganic P_2O_5 treatment (full rate Pi); (iv) a treatment receiving only inorganic N at the full rate (full rate Ni); (v) an inorganic control receiving inorganic P and inorganic N at the full rate (inorganic); (vi) a pig slurry substitution treatment, receiving pig slurry (PS) and inorganic P so that the total P application rate was 60 kg P_2O_5 ha^{-1} and the RAN application rate was 200 kg ha^{-1} (PS substitution); (vii) a treatment receiving only pig slurry at a rate of 30 kg P_2O_5 ha^{-1} and 200 kg RAN ha^{-1} (PS only); (vii) a digested cake (DC) substitution treatment, receiving digested cake and inorganic P so that total P application rate was 60 kg P_2O_5 ha^{-1} and inorganic N so that the total RAN application rate was 200 kg ha^{-1} (DC substitution); and (ix) a treatment receiving only digested cake at a rate of 30 kg P_2O_5 ha^{-1} and inorganic N at a rate of 198 kg RAN ha^{-1}, so that the final RAN application rate was 200 kg ha^{-1} (DC only)

(Table 4.1). Ammonium nitrate (NH_4NO_3) and monocalcium phosphate [$Ca(H_2PO_4)_2$] supplied inorganic N and P, respectively.

Table 4.1: Fertiliser application rates for each treatment. Pig slurry (PS) and digested cake (DC) supplied organic nitrogen (N) and phosphorus (P). Inorganic phosphorus (Pi) and inorganic nitrogen (Ni) were supplied by monocalcium phosphate ($Ca(H_2PO_4)_2$) and ammonium nitrate (NH_4NO_3), respectively. Totals are the sum of nutrients from all sources in a treatment. Values presented before a comma are mg pot^{-1}. Values presented after a comma are kg ha^{-1}. Readily available nitrogen (RAN) and phosphorus pentoxide (P_2O_5) supplied by organic and inorganic sources are termed organic RAN, inorganic RAN, organic P_2O_5 and inorganic P_2O_5, respectively.

		Organic RAN	Organic total N	Inorganic RAN	Total RAN	Total N	Organic P_2O_5	Inorganic P_2O_5	Total P_2O_5
Treatment	Organic source	mg pot^{-1}, kg ha^{-1}							
Zero input	-	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Half rate Pi	-	0,0	0,0	0,0	0,0	0,0	0,0	25.5, 30.0	25.5, 30.0
Full rate Pi	-	0,0	0,0	0,0	0,0	0,0	0,0	51.0, 60.0	51.0, 60.0
Full rate Ni	-	0,0	0,0	170, 200	170, 200	170, 200	0,0	0,0	0,0
Inorganic	-	0,0	0,0	170, 200	170, 200	170, 200	0,0	51.0, 60.0	51.0, 60.0
PS substitution	PS	170, 200	213, 250	0,0	170, 200	212, 250	25.5, 30.0	25.5, 30.0	51.0, 60.0
PS only	PS	170, 200	213, 250	0,0	170, 200	212, 250	25.5, 30.0	0,0	25.5, 30.0
DC substitution	DC	1.88, 2.22	18.1, 21.2	168, 198	170, 200	186, 212	25.5, 30.0	25.5, 30.0	51.0, 60.0
DC only	DC	1.88, 2.22	18.1, 21.2	168, 198	170, 200	186, 212	25.5, 30.0	0,0	25.5, 30

4.2.3 Organic fertiliser collection and characterisation

Pig slurry was collected from the slurry pit adjacent to a weaner-grower building housing 240 pigs at Leeds University Farm. The pigs were under commercial indoor management, including veterinary treatment and diet. Slurry was stirred with a large stick before collecting 10 L of slurry in a bucket. This sample was thoroughly stirred before being subdivided into 1 L portions. Subsamples were stored at four degrees Celsius for six months. The storage period was designed to imitate the closed period for fertiliser applications in the UK, when farmers must store their slurry over winter before the application window opens in late winter/Spring. After the storage period samples were frozen at -20^{o}C until use.

Digested cake was collected from Naburn Sewage Treatment Works, York, UK (Yorkshire Water). The digesters at Naburn receive municipal sewage sludge and treat it under mesophilic conditions (35^{o}C) in single-step reactors (Sapp *et al*, 2015; Barnes, 2018). 5 kg collected by an operative was thoroughly mixed and subdivided into 250 g portions. These samples were frozen at -20^{o}C until use.

Two subsamples of each product were removed from the freezer and analysed for chemical composition by NRM laboratories, Bracknell, Berkshire, RG42 6NS, UK (general methods, 2.11)**.** The mean value of these two samples was used to calculate fertiliser application rates for each organic input (Table 4.2).

4.2.4 Fertiliser applications

Fertiliser treatments were applied five weeks after sowing which coincided with the final stages of tillering. The surface of each core was disturbed to 2 cm with a palette knife before applying fertilisers to the surface. Organic and/or inorganic fertilisers allocated to each core were combined in a 100 ml pot with a lid. Deionised water was added to each pot so that the volume of water supplied with fertilisers was equal between treatments. Fertilisers were poured evenly across the soil surface, before rinsing each pot three times with deionised water to ensure all product was added.

4.2.5 Soil sampling

Cores were sampled at three points during the experiment: (i) one week before fertilisation, for baseline analysis; (ii) during stem elongation, four weeks after fertilisation; and (iii) during anthesis, 10 weeks after fertilisation. These time points are equal to four, nine and 15 weeks after sowing, respectively. Cores were sampled once to 20 cm depth at each sampling occasion using a mini-auger. The hole left after removing the sample was filled with sand and capped to reduce movement of water through sand during watering. Samples were placed on ice after collection before being homogenised and portioned for drying at 40^{o}C or freezing at -20^{o}C.

4.2.6 Soil analysis

Soils were analysed for $P_{Olsen's}$, $P_{organic}$, P_{water}, and available N concentration (general methods, 2.8). 10% duplicate samples were included for each parameter. Briefly, $P_{Olsen's}$ concentration was extracted by shaking 2.5 g air-dried soil in 50 ml 0.5 M sodium hydrogen carbonate ($NaHCO_3$) for one hour at 150 rpm. $P_{organic}$ was determined by shaking ignited (550^{o}C) and air-dried samples in

0.5 M H_2SO_4 for 16 hours at 150 rpm (Kuo, 1996). $P_{organic}$ was calculated as the difference between the concentration of P in ignited and air-dried samples. P_{water} was extracted by shaking 2.5 g air-dried soil in 25 ml deionised water for five minutes, followed by centrifuging for 20 minutes at 3000 g (Olsen & Sommers, 1982). Loss on ignition was calculated based on the difference in weight between air-dried and ignited (550^{o}C, 12 hours) samples. Available N (NO_3-N and NH_4-N) was extracted by shaking 10 g field moist soil with 50 ml 1M KCl for 1 hour at 150 rpm. Absorbance was measured spectrophotometrically using a Skalar San ++ Autoanalyser ($P_{Olsen's}$, available N) or plate reader (P_{water}, organic P). pH was measured in a 1:2.5 soil:water solution after shaking at 150 rpm for 15 minutes (Rowell, 1994a; United States Environmental Protection Agency, 2004).

4.2.7 Harvest and leachate collection

Plants were harvested 24 weeks after sowing, equivalent to 19 weeks after fertilisation. Aboveground biomass was removed at the surface. Plants were separated into shoots and ears. Chaff was removed from grain by hand, and grains were counted and weighed before and after drying at 40^{o}C for two days. Shoots were also weighed before and after drying at 40^{o}C for two days. After drying, grain was processed into powder using a coffee grinder before analysis of total N and P concentration using the one-step method (general methods, 2.9.1.3).

Nutrient losses in leachate were determined by flooding cores to 100% WFPS and collecting leachate for one hour. Leachate was filtered (syringe filter, 0.45 µm) before analysis of soluble reactive P and available N concentration by Skalar San^{++} continuous flow analyser (general methods, Table 2.4).

4.2.8 Statistical analysis

Data was analysed by one-way and mixed two-way ANOVA using IBM SPSS Statistics 21. All assumptions were checked prior to analysis. Extreme outliers, deemed as points having studentised residuals of more than 3.00 or less than minus 3.00**,** were removed from the analysis. Tests for multiple comparisons received Bonferroni corrections. Generalised linear modelling via the GLMER function in the lme4 package in R studio was used where assumptions of the mixed two-way ANOVA were violated and could not be met by data transformation (Bates et al., 2015; R Core Team, 2018).

4.3 Results

4.3.1 Summary

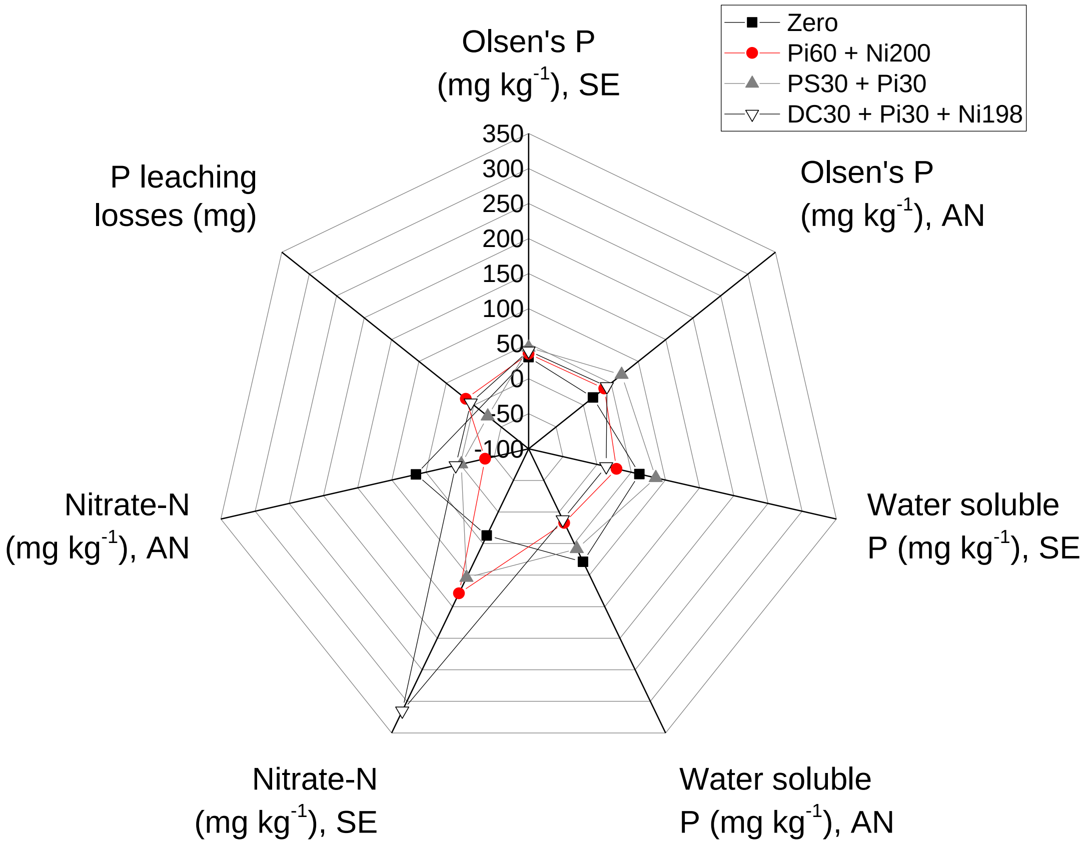

Figure 4.1: Summary of main findings. Results are reported as percentage change between the mean baseline level and the mean concentration during stem elongation (SE) or anthesis (AN) sampling point. The exception is phosphorus (P) leaching which is reported as percentage change compared to the zero-input control. Only treatments receiving their full allocation of nitrogen and P plus the zero-input control are included here for ease of interpretation. n = 9.

Main results are summarised in Figure 4.1. During stem elongation the effect of treatment on the concentration of P_{water} ($p = 0.025$) and NO_3-N ($p < 0.001$) in soil was statistically significant. During anthesis, there was a statistically significant effect of treatment on the concentration of P_{water} ($p = 0.022$), $P_{Olsen's}$ ($p < 0.001$), NO_3-N ($p = 0.002$) and NH_4-N ($p = 0.008$) in soil. There was a statistically significant effect of treatment on the concentration of soluble reactive P ($p < 0.001$) and NH_4-N ($p < 0.001$) in leachate, but not NO_3-N concentration ($p = 0.729$) in leachate. The effect of sampling point was determined for each treatment by repeated measures analysis. For main treatments (inorganic, PS substitution, DC substitution), there was a statistically significant effect of time on the concentration of $P_{Olsen's}$, NO_3-N, and NH_4-N in soil ($p < 0.001$ for all treatments and all nutrients). There was no statistically significant increase in P_{water} concentration compared to the baseline for the DC substitution treatment ($p = 0.535$), but the concentration of P_{water} increased over time in the inorganic ($p = 0.043$) and PS substitution ($p = 0.002$) treatments.

4.3.2 Organics analysis

Results of the analysis of organic fertilisers are presented in Table 4.2 as concentration and total amount of each nutrient applied.

Table 4.2: Chemical composition of organic fertilisers applied to winter wheat. Results are expressed as concentrations (mg g^{-1}) on a fresh weight basis and as the total amount of each nutrient added (mg). The concentration of nitrate was below the level of detection for pig slurry and digested cake and was therefore assumed to be zero for the purpose of fertiliser calculations. Abbreviations: N, nitrogen; NH_4-N, ammonium-N; NO_3-N, nitrate-N; P, phosphorus; K, potassium; Mg, magnesium; S, sulphur; Cu, copper; Zn, zinc; Na, sodium; Ca, calcium; B, boron; C, carbon; LOI, loss on ignition. n = 2.

	Pig slurry		Digested cake	
Determinand	Concentration ± s.d. (mg g^{-1})	Total applied (mg)	Concentration ± s.d. (mg g^{-1})	Total applied (mg)
pH	7.62 ± 0.0566		7.30 ± 0.0141	
Total N	3.00 ± 0.00	213	11.7 ± 0.251	18.1
NH_4-N	2.40 ± 0.0357	170	1.22 ± 0.00707	1.88
NO_3-N	<0.01	/	<0.1	/
Total P	0.157 ± 0.00283	11.1	7.21 ± 0.228	11.1
(P_2O_5)	(0.396 ± 0.00648)	(25.5)	(16.5 ± 0.523)	(25.5)
Total K	1.98 ± 0.0141	141	0.274 ± 0.0117	0.423
(K_2O)	(2.39 ± 0.0170)	(169)	(0.330 ± 0.0141)	(0.510)
Total Mg	0.196 ± 0.000707	13.9	0.825 ± 0.00852	1.28
(MgO)	(0.325 ± 0.00117)	(23.1)	(1.37 ± 0.0141)	(2.12)
Total S	0.196 ± 0.00106	13.9	2.73 ± 0.0707	4.22
(SO_3)	(0.489 ± 0.00265)	(34.6)	(6.83 ± 0.177)	(10.5)
Total Cu	0.00161 ± 0.00	0.114	0.0600 ± 0.00	0.0927
Total Zn	0.00548 ± 0.00	0.388	0.185 ± 0.00353	0.286
Total Na	0.482 ± 0.00460	34.1	0.152 ± 0.00262	0.235
(Na_2O)	(0.650 ± 0.00620)	(46.1)	(0.205 ± 0.00354)	(0.317)
Total Ca	0.461 ± 0.00141	32.7	8.74 ± 0.156	13.5
Total B	0.00113 ± 0.00	0.08	0.0233 ± 0.000672	0.0360
Total C	11.9 ± 0.0707	844	325.5 ± 0.354	503
Organic matter LOI	11.3 ± 0.0354	797	604 ± 0.707	933

4.3.3 Olsen's phosphorus

There were four outliers with studentised residual values greater than ± 3.00 which were removed from the analysis. Residuals were not normally distributed, as assessed by Normal Q-Q plot. This observation was supported by results of a Shapiro-Wilk's test which showed the assumption of normality was violated in three cells of the design. Data were $\log_{10}$ transformed. Following the transformation there was one outlier with a studentised residual value of 3.00. Removing this outlier had no effect on the conclusions that could be drawn from the results of a two-way mixed ANOVA so it was included in the analysis. The assumption of sphericity was violated for the two-way interaction (Mauchly's Test of Sphericity, $\chi^2_{(2)} = 14.4$, $p = 0.001$). The Greenhouse-Geisser correction (ε) was applied to overcome the violation of sphericity.

There was a statistically significant interaction between fertiliser treatment and sampling point, $F_{(13.3,\ 104)} = 2.86$, $p = 0.001$, $\varepsilon = 0.266$. Therefore, the effect of treatment at each time point was tested by one-way ANOVA (Table 4.3). The effect of time for each treatment was determined by one-way repeated measures ANOVA (Table 4.3). There was no statistically significant difference in the concentration of Olsen's P (mg kg^{-1}) between treatments before the application of fertilisers ($F_{(8,63)} = 0.653$, $p = 0.730$, $\eta^2 = 0.077$) or during stem elongation ($F_{(8,63)} = 1.40$, $p = 0.217$, $\eta^2 = 0.150$). By anthesis, there was a statistically significant effect of treatment on the concentration of Olsen's P in soil ($F_{(8,63)} = 8.50$, $p<0.001$, $\eta^2 = 0.519$). Adding Pi alone led to an increase in Olsen's P concentration at anthesis compared to the zero treatment, but the difference was only statistically significant for the full rate Pi treatment ($p = 0.001$) and not for the half rate Pi treatment ($p = 0.784$) (Table 4.3). Mean Olsen's P concentration was lowest in the full rate Ni treatment. Adding Pi led to a statistically significant increase in Olsen’s P concentration compared to the application of Ni only (inorganic treatment compared to full rate Ni treatment, $p = 0.001$). There was no statistically significant difference in the concentration of Olsen’s P between the inorganic treatment and substitution treatments (DC substitution, $p = 1.00$; PS substitution, $p = 1.00$).

There was a statistically significant effect of time on $P_{Olsen's}$ for all treatments except the full rate Ni and DC only treatments (Table 4.3). Olsen’s P increased successively from the baseline condition to stem elongation to anthesis for the half rate Pi and PS substitution treatments. For the full rate Pi, inorganic, PS only and DC substitution treatments the greatest increase in $P_{Olsen's}$ was between the baseline and stem elongation sampling point and there was no statistically significant difference in $P_{Olsen's}$ between stem elongation and anthesis.

Table 4.3: Olsen's phosphorus ($P_{\text{Olsen's}}$) concentration (mg kg^{-1}) and P index at anthesis in soils receiving different fertiliser treatments. Soils were sampled before fertiliser applications (baseline), during stem elongation and during anthesis. A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated ($p < 0.05$). Values are untransformed means ± one standard error of the mean (SEM). Statistics at the end of rows are for the effect of time for each treatment (one-way repeated measures ANOVA). In a row, values followed by the same lowercase letter are not statistically significantly different ($p > 0.05$). Statistics at the bottom of columns are for the effect of treatment at each time point (one-way ANVOA). In a column, values sharing the same uppercase letter are not statistically significantly different ($p > 0.05$). Statistically significant values and differences are highlighted in bold. n = 9. Treatments are described in Table 4.1.

	Mean $P_{\text{Olsen's}}$ ± 1 SEM (mg kg^{-1})								
Treatment	Baseline	Stem elongation	Anthesis	P index, anthesis	Sphericity assumed	Degrees freedom	*F*	p	η^2
Zero[e]	11.4 ± 1.16	14.9 ± 1.34	13.4 ± 0.931^{AB}	1	✓	2,10	4.88	**0.033**	0.494
Half rate Pi	10.4 ± 0.836^{a}	14.7 ± 0.722^{b}	16.5 ± 0.494^{cBC}	2	✓	2,14	26.1	**0.001**	0.789
Full rate Pi	11.2 ± 0.856^{a}	17.4 ± 1.66^{b}	20.3 ± 0.883^{bD}	2	✓	2,16	32.3	**<0.001**	0.802
Full rate Ni	11.1 ± 1.26	13.7 ± 0.984	12.1 ± 1.00^{A}	1	×	2,14	3.34	0.065	0.323
Inorganic	12.9 ± 1.16^{a}	17.5 ± 1.13^{b}	17.8 ± 1.51^{bBCD}	2	✓	2,14	15.9	**<0.001**	0.694
PS substitution	11.2 ± 0.764^{a}	16.2 ± 0.990^{b}	19.0 ± 0.947^{cCD}	2	✓	2,14	30.0	**<0.001**	0.810
PS only	10.3 ± 0.750^{a}	15.8 ± 1.63^{b}	14.8 ± 1.03^{bABC}	1	✓	2,16	32.5	**<0.001**	0.802
DC substitution	10.3 ± 0.817^{a}	14.4 ± 1.13^{b}	14.7 ± 0.824^{bABC}	1	✓	2,16	17.6	**<0.001**	0.687
DC only	11.8 ± 1.25	14.6 ± 0.567	13.5 ± 0.653^{AB}	1	×	2,12	4.96	0.059	0.453
Degrees freedom	8,63	8,63	8,63						
F	0.653	1.39	8.50						
p	0.730	0.217	**<0.001**						
η^2	0.077	0.150	0.519						

[e] Pairwise comparisons showed no statistically significant difference in $P_{\text{Olsen's}}$ over time, despite the overall effect of time being statistically significant.

4.3.4 Water-soluble phosphorus

A mixed two-way ANOVA was conducted to determine the effect of treatment, harvest and their interaction on P_{water} concentration (mg kg^{-1}) in soil. There were five cases with studentised residuals greater than ± 3.00, which were removed from the analysis. Residuals were approximately normally distributed, determined by visual inspection of a Q-Q plot. There was homogeneity of variances (Levene's test, $p > 0.05$), homogeneity of covariances (Box's test, $p > 0.05$) and the assumption of sphericity was met for the two-way interaction (Mauchly's test, $\chi^2_{(2)} = 3.83$, $p = 0.147$).

The interaction effect between treatment and sampling point was statistically significant, $F_{(16,126)} = 2.43$, $p = 0.003$, $\eta^2 = 0.236$. Therefore simple main effects were tested. The simple main effect of treatment at each time point was determined by one-way ANVOA. There was no statistically significant difference in P_{water} concentration between treatments before the application of fertilisers, $F_{(8,63)} = 1.79$, $p = 0.096$, $\eta^2 = 0.185$ (Table 4.4). By stem elongation, there was a statistically significant effect of treatment on P_{water} concentration, $F_{(8,63)} = 2.40$, $p = 0.025$, $\eta^2 = 0.234$, but pairwise comparisons showed no statistically significant difference between treatments (Table 4.4). The same pattern was observed at anthesis, $F_{(8,63)} = 2.47$, $p = 0.022$, $\eta^2 = 0.239$. The simple main effect of time for each treatment was determined by one-way repeated measures ANOVA. The effect of time was statistically significant for each treatment except for the half rate Pi, full rate Ni, DC substitution and DC only treatment (Table 4.4). In treatments where the effect of sampling point was statistically significant, P_{water} generally increased between the baseline and stem elongation sampling point with little change after stem elongation. The exception was the PS only treatment;

P_{water} concentration increased between the baseline and stem elongation sampling point before returning to the baseline level during anthesis.

Table 4.4: Water-soluble phosphorus (P_{water}) concentration (mg kg^{-1}) in soils receiving different fertiliser treatments. Soils were sampled before fertiliser applications (baseline), during stem elongation and during anthesis. Repeated measures analysis was conducted by two-way mixed ANOVA on raw data. A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated ($p < 0.05$). Values are means ± one standard error of the mean (SEM). Statistics at the end of rows are for the effect of time for each treatment. In a row, values followed by the same lowercase letter are not statistically significantly different ($p > 0.05$). Statistically significant values are highlighted in bold. Treatment details can be found in Table 4.1. n = 9.

	Mean P_{water} ± 1 SEM (mg kg^{-1})							
Treatment	Baseline	Stem elongation	Anthesis	Sphericity assumed?	Degrees freedom	*F*	p	η^2
Zero	0.306 ± 0.054^{a}	0.496 ± 0.064^{b}	0.548 ± 0.033^{ab}	✓	2,8	7.52	**0.015**	0.653
Half rate Pi	0.422 ± 0.044	0.568 ± 0.036	0.515 ± 0.037	✓	2,14	2.06	0.164	0.227
Full rate Pi	0.397 ± 0.027^{a}	0.688 ± 0.070^{b}	0.564 ± 0.035^{b}	×	2,16	11.3	**0.007**	0.586
Full rate Ni	0.466 ± 0.042	0.518 ± 0.030	0.498 ± 0.033	✓	2,16	0.546	0.590	0.064
Inorganic	0.498 ± 0.026^{a}	0.640 ± 0.045^{b}	0.584 ± 0.024^{b}	×	2,14	5.60	**0.043**	0.444
PS substitution	0.372 ± 0.059^{a}	0.692 ± 0.069^{b}	0.590 ± 0.024^{ab}	✓	2,16	9.83	**0.002**	0.551
PS only	0.434 ± 0.026^{a}	0.579 ± 0.039^{b}	0.454 ± 0.032^{a}	✓	2,14	19.7	**<0.001**	0.738
DC substitution	0.460 ± 0.050	0.522 ± 0.049	0.480 ± 0.026	✓	2,16	0.650	0.535	0.075
DC only	0.499 ± 0.036	0.499 ± 0.032	0.515 ± 0.028	✓	2,12	0.093	0.911	0.015
Degrees freedom	8,63	8,63	8,63					
F	1.79	2.40	2.47					
P	0.096	**0.025**[c]	**0.022**[c]					
η^2	0.236	0.234	0.239					

[c] Pairwise comparisons showed no statistically significant differences in P_{water} between treatments despite the overall effect of treatment being statistically significant.

4.3.5 Organic phosphorus

Data followed a gamma distribution, as determined by inspection of a histogram and the gamma_test function on R ($p = 0.050$). Data transformation was unsuccessful in achieving a normal distribution (Shapiro-Wilk's, $p < 0.05$). A mixed effects model with a gamma distribution and log link function was generated to determine the effect of treatment, harvest and their interaction on organic P concentration in soil (mg kg^{-1}), using the GLMER function in the lme4 package of RStudio. Core ID was included as a random effect to account for repeated measures while treatment, harvest and their interaction were included as factors. There was no statistically significant interaction between treatment and harvest, $\chi^2_{(16)}=25.5$, $p = 0.0613$ (Table 4.5). There was no statistically significant effect of treatment on the concentration of organic P in soil, $\chi^2_{(8)}=8.68$, $p = 0.370$, but there was a statistically significant effect of time, $\chi^2_{(8)}=26.1$, $p < 0.001$ (Table 4.5). p-values for multiple comparisons received a Bonferroni adjustment. Values are marginal means ± one standard error of the mean. The difference in organic P concentration between baseline sampling (156 ± 1.30 mg kg^{-1}) and stem elongation (166 ± 3.03 mg kg^{-1}, $p = 0.845$) was not statistically significant but the concentration of organic P in soil was statistically significantly higher during anthesis (171 ± 2.66 mg kg^{-1}) compared to the baseline condition ($p = 0.008$). There were no other statistically significant comparisons.

Table 4.5: Organic P concentration (mg kg^{-1}) in soils receiving different fertiliser treatments. Soils were sampled before fertiliser applications (baseline), during stem elongation and during anthesis. Repeated measures analysis was conducted in RStudio using the GLMER function in the lme4 package, with ID as a random factor and sampling time, treatment and their interaction as factors. The interaction effect between treatment and time was not statistically significant ($p = 0.0613$) and the effect of treatment on organic P was not statistically significant ($p = 0.370$).The concentration of organic P was statistically significantly higher during anthesis than at the baseline sampling point ($p = 0.008$). There were no other statistically significant comparisons. Data are mean ± one standard error of the mean (SEM). Treatment details can be found in Table 4.1. n = 9.

	Mean $P_{organic}$ ± SEM (mg kg^{-1})		
Treatment	Baseline	Stem elongation	Anthesis
Zero	154 ± 1.85	178 ± 3.50	167 ± 6.08
Half rate Pi	169 ± 5.33	166 ± 7.03	185 ± 11.2
Full rate Pi	153 ± 3.93	178 ± 3.67	175 ± 6.93
Full rate Ni	154 ± 2.89	152 ± 8.31	164 ± 6.64
Inorganic	152 ± 2.75	161 ± 16.8	165 ± 7.50
PS substitution	152 ± 4.27	161 ± 4.96	178 ± 6.39
PS only	159 ± 3.52	175 ± 2.14	178 ± 3.90
DC substitution	156 ± 1.46	154 ± 11.3	172 ± 5.71
DC only	151 ± 4.61	173 ± 6.53	151 ± 13.3

4.3.6 Soil available nitrogen

A generalised linear mixed-effects model was run to test for the effect of treatment and harvest on NO_3-N concentration in soil, using the glmer function in the R package lme4 (Bates et al., 2015), as assumptions of the two-way mixed ANOVA were violated and could not be met by data transformation. The model was run with a Gaussian distribution and log link function. Treatment, harvest and their interaction were included as factors. Core ID was included as a random factor. The two-way interaction effect between treatment and harvest was statistically significant, $\chi^2_{(16)} = 447$, $p < 0.001$. Simple main effects were tested for the effect of treatment at each sampling point using the glm function in the stats package in R (R Core Team, 2018) (Table 4.6). There was no statistically significant difference in NO_3-N concentration between treatments before the application of fertilisers ($X^2_{(8)}$ = 13.2, p = 0.105). There was a statistically significant effect of treatment on NO_3-N concentration during stem elongation ($X^2_{(8)}$ = 58.0, $p < 0.001$) and anthesis ($X^2_{(8)}$ = 24.7, p = 0.002). Adding nitrogen fertilisers, regardless of form, increased NO_3-N concentration during stem elongation compared to the zero input control, but this was only significant for the PS only, full rate Ni, DC substitution and DC only treatments (Table 4.6). By anthesis, NO_3-N concentration was statistically significantly lower in the inorganic treatment than the zero-input control (p = 0.0114). There were no other statistically significant differences between treatments during anthesis. The simple main effect of sampling point for each treatment was tested using the glmer function in R but treatment was removed as a factor (Table 4.6). There was an increase in NO_3-N concentration over time for all treatments except the full rate Pi treatment. In treatments receiving N from organic or inorganic sources, NO_3-N concentration generally peaked at stem elongation before returning to baseline levels. For the zero input and half rate Pi treatment, NO_3-N concentration peaked during anthesis.

A mixed two-way ANOVA was run to determine the effect of treatment, harvest and their interaction on NH_4-N concentration in soil. Data received a square root transformation in order to conform to test assumptions. Following transformation, data was normally distributed in every cell of the design (Shapiro-Wilk's, $p > 0.05$), there was homogeneity of variances at each harvest (Levene's test, $p > 0.05$) and homogeneity of covariances (Box's test, p = 0.407). The assumption of sphericity was met for the two-way interaction (Mauchly's test, $\chi^2_{(2)}$ = 1.80, p = 0.407). The interaction effect was statistically significant, $F_{(16,138)}$ = 1.94, p = 0.021, η^2 = 0.184. A test of simple main effects showed the effect of treatment was only statistically significant during anthesis (Table 4.7). The concentration of NH_4-N was statistically significantly higher in the zero-input treatment than the inorganic (p = 0.017) and PS substitution (p = 0.004) treatment. There were no other statistically significant comparisons. The simple main effect of sampling point for each treatment was determined by one-way repeated measures ANOVA (Table 4.7). Except for the zero input and DC only treatment, NH_4-N concentration was statistically significantly lower during anthesis compared to the baseline. The difference in NH_4-N concentration between the baseline and stem elongation sampling point was not statistically significant for any treatment except the DC substitution treatment where NH_4-N concentration at stem elongation increased significantly compared to the baseline.

Table 4.6: Nitrate-N (NO_3-N) concentration (mg kg^{-1}) in soils receiving different fertiliser treatments. Soils were sampled before fertiliser applications (baseline), during stem elongation and during anthesis. Repeated measures analysis was run on RStudio using the GLMER function in the lme4 package. Values are means ± one standard error of the mean (SEM). Statistics at the end of rows are for the effect of time for each treatment. In a row, values followed by the same lowercase letter are not statistically significantly different ($p > 0.05$). Statistics at the bottom of columns are for the effect of treatment at each time point. In a column, values sharing the same uppercase letter are not statistically significantly different ($p > 0.05$). Statistically significant values are highlighted bold. Treatments are described in Table 4.1. n = 9.

	Mean NO_3-N ± 1 SEM (mg kg^{-1})					
Treatment	Baseline	Stem elongation	Anthesis	X^2	Degrees freedom	p
Zero	2.12 ± 0.255^{a}	2.91 ± 0.289^{abAB}	3.49 ± 0.408^{bB}	13.5	2	**0.001**
Half rate Pi	2.02 ± 0.162^{a}	2.19 ± 0.156^{aA}	2.68 ± 0.331^{bAB}	11.6	2	**0.003**
Full rate Pi	2.43 ± 0.177	2.59 ± 0.300^{A}	2.70 ± 0.384^{AB}	1.40	2	0.497
Full rate Ni	2.05 ± 0.228^{a}	10.4 ± 2.20^{bC}	1.82 ± 0.210^{aAB}	73.1	2	**<0.001**
Inorganic	2.31 ± 0.349^{a}	5.29 ± 1.33^{bABC}	1.47 ± 0.177^{cA}	35.2	2	**<0.001**
PS substitution	1.87 ± 0.227^{a}	3.82 ± 0.447^{bABC}	1.84 ± 0.234^{aAB}	41.1	2	**<0.001**
PS only	1.41 ± 0.181^{a}	8.92 ± 1.61^{bC}	2.35 ± 0.407^{aAB}	48.9	2	**<0.001**
DC substitution	1.99 ± 0.210^{a}	8.27 ± 2.26^{bC}	2.12 ± 0.350^{aAB}	74.5	2	**<0.001**
DC only	2.19 ± 0.403^{a}	7.96 ± 3.07^{bBC}	2.17 ± 0.266^{aAB}	47.3	2	**<0.001**
X^2	13.2	58.0	24.7			
Degrees freedom	8	8	8			
p	0.105	**<0.001**	**0.002**			

Table 4.7: Ammonium-N (NH_4-N) concentration (mg kg^{-1}) in soils receiving different fertiliser treatments. Soils were sampled before fertiliser applications (baseline), during stem elongation and during anthesis. Repeated measures analysis was run by mixed two-way ANOVA on square root transformed data. A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated ($p < 0.05$). Statistics at the end of rows are for the effect of time for each treatment. In a row, values followed by the same lowercase letter are not statistically significantly different ($p > 0.05$). Statistics at the bottom of columns are for the effect of treatment at each time point. In a column, values followed by the same uppercase letter are not statistically significantly different ($p > 0.05$). Values are untransformed means ± one standard error of the mean (SEM). Statistically significant values are highlighted bold. Treatments are described in Table 4.1. n = 9.

	Mean NH_4-N ± 1 SEM (mg kg^{-1})							
Treatment	Baseline	Stem elongation	Anthesis	Sphericity assumed?	F	Degrees freedom	p	η²
Zero	0.722 ± 0.078	0.714 ± 0.060	0.611 ± 0.086^{B}	✓	0.906	2,16	0.424	0.102
Half rate Pi	0.810 ± 0.075^{a}	0.664 ± 0.040^{a}	0.442 ± 0.039^{bAB}	✓	11.9	2,16	**0.001**	0.598
Full rate Pi	0.591 ± 0.041^{a}	0.766 ± 0.075^{ab}	0.438 ± 0.041^{bAB}	✓	9.12	2,16	**0.002**	0.533
Full rate Ni	0.759 ± 0.099^{a}	0.887 ± 0.070^{a}	0.404 ± 0.045^{bAB}	✓	12.2	2,16	**0.001**	0.604
Inorganic	0.750 ± 0.079^{a}	0.835 ± 0.062^{a}	0.320 ± 0.031^{bA}	✓	22.7	2,14	**<0.001**	0.764
PS substitution	0.640 ± 0.053^{a}	0.780 ± 0.065^{a}	0.314 ± 0.051^{bA}	✓	20.7	2,16	**<0.001**	0.721
PS only	0.714 ± 0.081^{a}	0.888 ± 0.074^{a}	0.422 ± 0.069^{bAB}	✓	15.2	2,16	**<0.001**	0.655
DC substitution	0.716 ± 0.059^{a}	0.820 ± 0.042^{b}	0.362 ± 0.035^{bAB}	✓	33.2	2,16	**<0.001**	0.806
DC only	0.673 ± 0.117^{ab}	0.869 ± 0.068^{b}	0.353 ± 0.033^{aAB}	✓	11.1	2,12	**0.002**	0.650
F	0.805	1.68	2.88					
Degrees freedom	8,69	8,69	8,69					
p	0.600	0.120	**0.008**					
η²	0.085	0.163	0.250					

4.3.7 Soil pH

The effect of treatment, time and their interaction on soil pH was determined by mixed two-way ANOVA. There was one outlier with a studentised residual of minus 7.10 which was removed from the analysis. Residuals were normally distributed in each cell of the design (Shapiro-Wilk's, $p > 0.05$), there was homogeneity of variances at each sampling point (Levene's test, $p > 0.05$) and homogeneity of covariances (Box's test, $p = 0.970$). The assumption of sphericity was met for the interaction between treatment and sampling point (Mauchly's test of sphericity, $X^2_{(2)} = 0.053$, $p = 0.762$). There was no statistically significant interaction between treatment and time on pH, $F_{(16,118)} = 0.980$, $p = 0.483$, $\eta^2 = 0.117$. Marginal means are presented in Table 4.8. The main effect of time showed no statistically significant difference in mean pH at different time points $F_{(2,118)} = 2.87$, $p = 0.061$, $\eta^2 = 0.046$. The main effect of treatment showed that there was no statistically significant difference in pH between fertiliser treatments, $F_{(1,59)} = 1.05$, $p = 0.411$, $\eta^2 = 0.124$.

Table 4.8: pH in soils receiving different fertiliser treatment. Soils were sampled before fertiliser applications (baseline), during stem elongation and during anthesis. Repeated measures analysis was conducted by two-way mixed ANOVA on raw data. The interaction effect and main effect of treatment and time were not statistically significant (p = 0.482, p = 0.411 and p = 0.061, respectively). Values are means ± one standard error of the mean (SEM) or marginal means ± one SEM. Treatment details can be found in Table 4.1. n = 9.

	Mean pH ± SEM			
Treatment	Baseline	Stem elongation	Anthesis	Marginal mean
Zero	7.31 ± 0.0541	7.14 ± 0.0547	7.25 ± 0.0455	7.32 ± 0.038
Half rate Pi	7.35 ± 0.0556	7.26 ± 0.0376	7.18 ± 0.0607	7.27 ± 0.038
Full rate Pi	7.28 ± 0.788	7.19 ± 0.0580	7.27 ± 0.0718	7.23 ± 0.040
Full rate Ni	7.09 ± 0.694	7.13 ± 0.0582	7.35 ± 0.0561	7.19 ± 0.035
Inorganic	7.24 ± 0.111	7.23 ± 0.0499	7.32 ± 0.0396	7.25 ± 0.040
PS substitution	7.39 ± 0.0811	7.20 ± 0.0793	7.34 ± 0.0458	7.32 ± 0.038
PS only	7.26 ± 0.0473	7.24 ± 0.0731	7.35 ± 0.0688	7.28 ± 0.038
DC substitution	7.29 ± 0.0589	7.19 ± 0.0582	7.30 ± 0.0507	7.26 ± 0.040
DC only	7.16 ± 0.109	7.13 ± 0.0932	7.29 ± 0.0900	7.22 ± 0.043
Marginal mean	7.26 ± 0.027	7.21 ± 0.023	7.29 ± 0.022	

4.3.8 Nutrient leaching losses

Results of the leaching study conducted after harvest were analysed by one-way ANOVA or non-parametric alternatives. There was no statistically significant difference in the mean volume of leachate collected from each treatment, $F_{(8,69)} = 1.02$, p = 0.429, $\eta^2 = 0.106$, which was used to calculate nutrient loading.

4.3.8.1 Soluble reactive phosphorus leaching losses

The effect of treatment on the concentration of SRP in leachate (mg L^{-1}) was determined by one-way ANOVA. One outlier with a studentised residual of 3.10 was removed from the analysis. Data were square root transformed to achieve normality in each cell of the design (Shapiro-Wilk's, p > 0.05). There was homogeneity of variances following the transformation (Levene's test, $p = 0.282$). There was a statistically significant effect of treatment on the concentration of SRP in leachate, $F_{(8,\ 78)} = 8.86$, $p < 0.001$, $\eta^2 = 0.507$. p-values for multiple comparisons were Bonferroni-adjusted (Table 4.9). The mean SRP concentration in leachate was statistically significantly higher in the full rate Pi treatment compared to the inorganic treatment receiving inorganic P and inorganic N. Although the mean SRP concentration was higher in leachate from the inorganic treatment than the PS or DC substitution treatments, this difference was not statistically significant ($p = 1.00$ for both treatments) (Table 4.9).

The effect of treatment on the total SRP load (mg) in leachate was determined by multiplying the concentration of SRP in leachate by the volume of leachate collected (Table 4.9). Residuals were positively skewed in two cells of the design (Shapiro-Wilk's, $p < 0.05$) but the assumption of normality was met following square root transformation of the data. There was homogeneity of variances (Levene's test, $p = 0.566$) and there were no cases with studentised residuals greater than 3.00. The effect of treatment on SRP load in leachate was statistically significant, $F_{(8,79)} = 4.87$, $p < 0.001$, $\eta^2 = 0.358$. There was no statistically significant difference in SRP load between the zero-input control and the inorganic ($p = 1.00$), PS substitution ($p = 0.555$) or DC substitution ($p = 1.00$) treatment. Substituting inorganic P with PS or DC had no statistically significant effect on the total amount of SRP in leachate (inorganic compared to PS or DC substitution, $p = 1.00$ in both cases).

Table 4.9: Nutrient leaching losses from cores receiving different fertiliser treatments (Table 4.1). Statistically significant differences between treatments were tested on square root transformed data by Welch's one-way ANOVA with Games-Howell post-hoc for ammonium-nitrogen (NH_4-N) and soluble reactive phosphorus (SRP) concentration and load. The effect of treatment on nitrate-N (NO_3-N) concentration and load was determined by one-way ANOVA on log-transformed data. Untransformed means and standard errors are presented for NH_4-N and SRP. Pairwise comparisons received a Bonferroni adjustment. In a column values followed by the same letter are not statistically significantly different ($p > 0.05$). n = 9.

Treatment	Volume (ml)	NH_4-N (mg L^{-1})	NH_4-N (mg)	NO_3-N (mg L^{-1})	NO_3-N (mg)	SRP (mg L^{-1})	SRP (mg)
Zero	76.5 ± 11.1	1.92 ± 0.345^{c}	119 ± 21.8^{c}	17.1 ± 3.80^{ab}	1320 ± 398	0.170 ± 0.016^{bcd}	12.6 ± 1.62^{ab}
Half rate Pi	79.1 ± 12.4	0.510 ± 0.103^{ab}	36.3 ± 7.15^{ab}	16.4 ± 2.82^{b}	1644 ± 406	0.238 ± 0.035^{cd}	17.0 ± 2.99^{ab}
Full rate Pi	81.9 ± 7.42	1.16 ± 0.282^{b}	94.6 ± 24.8^{bcd}	19.8 ± 5.36^{b}	1280 ± 397	0.310 ± 0.041^{d}	26.2 ± 4.21^{b}
Full rate Ni	81.9 ± 7.42	0.447 ± 0.107^{ab}	38.6 ± 10.4^{ab}	5.80 ± 2.27^{ab}	557 ± 235	0.112 ± 0.019^{ab}	9.981 ± 2.28^{a}
Inorganic	100 ± 6.30	0.386 ± 0.073^{ab}	37.8 ± 7.25^{abd}	4.54 ± 2.13^{ab}	460 ± 223	0.144 ± 0.024^{abc}	14.4 ± 2.54^{ab}
PS substitution	85.1 ± 6.67	0.205 ± 0.034^{a}	16.6 ± 2.52^{a}	14.3 ± 4.37^{ab}	1110 ± 313	0.102 ± 0.026^{ab}	9.39 ± 3.01^{a}
PS only	82.8 ± 7.42	0.355 ± 0.057^{ab}	29.7 ± 5.97^{ab}	11.2 ± 4.95^{ab}	935 ± 434	0.088 ± 0.013^{a}	7.10 ± 0.975^{a}
DC substitution	102 ± 7.90	0.898 ± 0.295^{ab}	64.2 ± 14.0^{bcd}	4.40 ± 1.64^{a}	434 ± 167	0.140 ± 0.028^{abc}	13.3 ± 1.97^{ab}
DC only	95.6 ± 12.1	0.305 ± 0.099^{ab}	27.7 ± 7.75^{ab}	6.80 ± 2.13^{ab}	708 ± 284	0.067 ± 0.018^{a}	7.91 ± 1.85^{a}

4.3.8.2 Ammonium-N leaching losses

A one-way ANOVA was run to determine the effect of treatment on the concentration of NH_4-N in leachate (mg L^{-1}). There was one outlier with a studentised residual of 6.49 which was removed from the analysis. Residuals were not normality distributed in one cell of the design (Shapiro-Wilk's, $p = 0.002$), so a square root transformation was applied. Following the transformation, the assumption of normality was met for every cell of the design (Shapiro-Wilk's, $p > 0.05$) but the assumption of homogeneity of variances was violated (Levene's test, $p = 0.007$). Therefore, a Welch's test with Games-Howell post-hoc test was conducted on square root transformed data to determine the effect of treatment on the concentration of NH_4-N in leachate.

There was a statistically significant effect of treatment on the concentration of NH_4-N in leachate (Welch's $F_{(8,\ 27.1)} = 7.76$, $p < 0.001$). The concentration of NH_4-N in leachate was highest in the zero-input control and lowest from the PS substitution treatment (Table 4.9). The concentration of NH_4-N in leachate was significantly lower in the PS substitution treatment than the full rate Pi (Games-Howell, $p = 0.024$) and zero input (Games-Howell, $p = 0.001$) treatments. There were no statistically significant comparisons between other treatments.

The concentration of NH_4-N in leachate was multiplied by the volume of leachate collected to obtain the total amount of NH_4-N lost from each core (mg). There were three outliers with studentised residuals greater than 3.00 which were removed from the analysis. The assumption of normality was violated in one cell of the design (Shapiro-Wilk's, $p = 0.043$) but was met following square root transformation of the data. However homogeneity of variance's was violated (Levene's test, $p = 0.031$) so a Welch's test with Games-Howell post-hoc test was conducted on square root transformed data. There was a statistically significant effect of treatment on the total amount of NH_4-N lost in leachate (Welch's $F_{(8,\ 26.1)} = 6.80$, $p < 0.001$). Total leaching losses of NH_4-N (mg) were highest from the zero-input control (Table 4.9). Compared to treatments receiving their full allocation of available N and total P, NH_4 (mg) leaching losses were significantly higher from the zero input control compared to the PS substitution (Games-Howell, $p = 0.005$) and inorganic (Games-Howell, $p = 0.047$) treatments but there was no statistically significant difference between the zero and the DC substitution (Games-Howell, $p = 0.506$) treatment. Total NH_4-N losses were statistically significantly higher from the DC substitution treatment compared to the PS substitution treatment (Games-Howell, $p = 0.048$).

4.3.8.3 Nitrate-N leaching losses

The effect of treatment on the concentration of NO_3-N (mg L^{-1}) and load (mg) in leachate was determined by one-way ANOVA on log-transformed data. There were two outliers with studentised residuals greater than 3.00 which were removed from the analysis for each parameter. There was a statistically significant effect of treatment on the concentration of NO_3-N in leachate (mg L^{-1}), $F_{(8,76)} = 4.04$, $p = 0.001$, $\eta^2 = 0.325$. The concentration of NO_3-N was lowest in treatments that received inorganic N (Table 4.9) and highest in treatments that only received N from pig slurry or no N. The only statistically significant differences in NO_3-N leachate concentration occurred between the DC substitution treatment and the half rate ($p = 0.027$) and full rate Pi treatments ($p = 0.047$).

There was a statistically significant effect of treatment on the load of NO_3-N in leachate (mg), $F_{(8,\ 76)} = 2.49$, $p = 0.020$, $\eta^2 = 0.229$. As with NO_3-N concentration, adding N as NH_4NO_3 reduced the amount of nitrate-N in leachate, but pairwise comparisons showed no statistically significant difference between any combinations of treatments (p > 0.005).

4.4 Discussion

The primary aim of this study was to determine how substituting inorganic phosphorus fertilisers with two organic alternatives, pig slurry and digested cake, affects short-term soil P cycling in soil. The study tested the prediction that integrating fertiliser sources would result in a higher concentration of labile P in soil, measured as Olsen's and water-soluble P, compared to application of inorganic fertilisers alone. Contrary to the study's predictions, there was no statistically significant difference in Olsen's P concentration between the inorganic treatment and either substitution treatment during stem elongation or during anthesis. These findings corroborate those of chapter three but are in disagreement with results of field trials sampled decades after initiation of fertilisation (Ahmed et al., 2019; Hu et al., 2018; Xin et al., 2017) which show increases in soil available P following combined fertiliser applications.

Short-term studies suggest that the increase in Olsen's P reported after combined fertiliser applications may not emerge immediately. Zhao et al. (2016) reported a trend towards increasing available P concentration in a combined compared to an inorganic treatment after two years of application, but the difference between treatments was not statistically significant. Results of repeated measures analysis reported here suggest a similar trend to Zhao et al., (2016); although the difference in Olsen's P concentration between treatments receiving their full allocation of N and P was not statistically significantly different, treatments differed in their ability to increase P phytoavailability over time. Olsen's P concentration statistically significantly increased between each sampling point for the pig slurry substitution treatment. By contrast, the only statistically significant increase in Olsen’s P concentration occurred between the baseline and stem elongation sampling for the digested cake substitution and inorganic treatment. These results suggest that the mechanisms leading to increased P phytoavailability under combined fertilisation may occur slowly through gradual accumulation of soil available P.

The ability of treatments to increase Olsen’s P concentration in a season may influence the amount of P added with the next fertiliser application (Agriculture and Horticulture Development Board, 2017). It is therefore important to consider absolute values of Olsen’s P after the major period of P uptake. During anthesis, Olsen’s P concentration decreased in the order PS substitution, inorganic, DC substitution. Based on British fertiliser recommendations (RB209, AHDB, 2020) and assuming negligible losses between anthesis and soil sampling for the following season, the P application rate for the inorganic and PS substitution treatment would be lower than for the DC substitution treatment (AHDB, 2020). This is despite there being no statistically significant difference in Olsen’s P concentration between these treatments during anthesis. The results therefore suggest that it may be beneficial to sample soils receiving combined fertiliser applications more frequently than the three to five years suggested in RB209. More frequent sampling would ensure P is not over-applied to soils fertilised with treatments which are more effective at increasing Olsen’s P concentration.

Differences in P cycling were expected between soils fertilised with pig slurry and digested cake, based on our current understanding of the effect of these amendments on the size of the labile P pool. The most noticeable difference between the pig slurry and digested cake treatments was the effect of adding inorganic P. Pig slurry increased the concentration of available P, whether it was applied alone or in combination with inorganic P. In contrast, digested cake only increased Olsen's P concentration when applied with inorganic P. This finding supports previous research which shows AD products are less effective at increasing soil available P than inorganic alternatives applied at the same rate (Alleoni et al., 2008; Brandt et al., 2004; Elliott et al., 2005, 2002) but suggests low P phytoavailability after the application of digested cake can be alleviated by the addition of inorganic P. This is unlikely to be due to interactive effects of co-applying organic and inorganic fertilisers, as the concentration of Olsen's P in the DC substitution treatment was still below the level of the inorganic treatment. The results suggest digested cake could be used on soils where the starting P status is already high to provide other nutrients, such as N, without increasing P to levels that increase P leaching losses.

P_{water} was analysed throughout the growing cycle to estimate the effect of integrated fertiliser applications on P leaching risk (Leinweber et al., 1999; Pote et al., 1996; Schoumans & Groenendijk, 2000). Although there was no statistically significant difference in the concentration of P_{water} between treatments at any time point, of treatments receiving their full allocation of N and P only the inorganic and PS substitution treatments increased the concentration of P_{water} compared to the baseline level. Unlike the inorganic and pig slurry substitution treatment, digested cake did not increase the concentration of P_{water} compared to the baseline when applied alone or in combination with monocalcium phosphate. The results of P_{water} analysis therefore indicate that the inorganic and PS substitution treatments increased P leaching risk during the growing cycle compared to the baseline level while fertilisation with DC alone or in combination with inorganic P did not result in the same level of P leaching risk. P_{water} results corroborate

Olsen's P data, which shows that the inorganic and PS substitution treatment were more effective at increasing P phytoavailability than digested cake.

P leaching losses were determined after harvest. The most noticeable difference in the concentration of SRP in leachate was caused by the addition of N, which decreased the concentration of SRP in leachate compared to the application of inorganic P alone. This was likely due to the positive effects of N on plant productivity described in chapter five leading to increased uptake of P from the soil reserve. As expected from P_{water} and Olsen's P data, there was no statistically significant difference in the concentration or load of P in leachate between the inorganic, PS substitution and DC substitution treatments. The concentration and load of soluble reactive P was higher than expected from soils amended with the DC substitution, given the lower concentration of P_{water} and Olsen's P in soil during anthesis compared to the inorganic and pig slurry substitution treatment. It is possible that inorganic P was immobilised due to the addition of high levels of carbon in the digested cake and re-mineralised at a period in the crop's growing cycle when P uptake was low (Guppy et al., 2005a; Rose et al., 2007). This asynchrony between crop demand and soil supply could explain why concentrations and loads of P in leachate are disproportionately high from the digested cake substitution treatment, compared to the levels of P_{water} and Olsen's P recorded during anthesis. P immobilisation may also explain why soil P_{water} concentration did not increase under the DC substitution treatment. The results suggest that in the short term, fertiliser sources could be combined without increasing the risk of P leaching losses from agricultural soils.

This study also reported the effect of organic fertiliser substitution on the concentration of NO_3-N and NH_4-N in soil, to gain a comprehensive view of the effect of combined fertiliser applications on phytoavailability of key nutrients in soil. In the zero-input treatment, the concentration of NO_3-N increased over time and peaked during anthesis, suggesting mineralisation of organic N exceeded crop uptake. The concentration of NO_3-N in N fertilised treatments typically peaked during stem elongation, before returning to the baseline level. During stem elongation the concentration of NO_3-N in soil decreased in the order DC substitution, inorganic, PS substitution. These differences were not statistically significant. However, of treatments receiving their full allocation of N and P only the DC substitution treatment statistically significantly increased NO_3-N concentration compared to the zero-input control during stem elongation. It is possible that inorganic N added with DC was immobilised after its addition before being mineralised later in the crop growing cycle. Immobilisation of fertiliser-N prior to the stem elongation sampling point could reduce the potential for N uptake by the crop during this rapid period of growth and result in higher levels of NO_3-N in soil. Fertiliser-N immobilisation has been reported in soils receiving integrated nutrient management (Gentile et al., 2008, 2013, 2009) due to activation of the soil microbial community following carbon inputs. N immobilisation would also be consistent with trends in P phytoavailability recorded in the same treatment. Similar phenomenon could be expected here, given high inputs of total carbon and organic matter with the digested cake amendment. By anthesis, there was no statistically significant difference in the concentration of NO_3-N between the zero-input control and both organic substitution treatments but the concentration of NO_3-N in the zero-input control was statistically significantly higher than that of the inorganic treatment, likely due to differences in plant N uptake described in chapter five**.**

While N is a critical plant nutrient, the mis- and overapplication of nitrogenous fertilisers is a major cause of aquatic eutrophication (Bobbink et al., 1988;

Bobbink & Willems, 1987; Vitousek et al., 1997). Agriculture is responsible for 50-80% of the total N load reaching aquatic environments (European Environment Agency, 2005). In this study, substitution of inorganic fertilisers with organic alternatives had no statistically significant effect on NO_3-N leaching losses when measured as concentration or load, compared to treatments receiving only inorganic fertilisers. However, adding fertilisers regardless of form or nutrient effectively reduced the concentration of NH_4-N in leachate compared to the zero-input control. NH_4-N load in leachate was significantly lower from soils receiving the PS substitution than the DC substitution, suggesting sustained release of NH_4-N in the latter treatment after the major period of N uptake in wheat.

4.5 Conclusions

In this study, partial substitution of a crop's P requirement with pig slurry or digested cake led to no statistically significant difference in P phytoavailability or leaching losses compared to the application of inorganic fertilisers. The data presented here therefore suggests that inorganic P can be substituted with organic alternatives without significantly impacting the availability of plant nutrients or the environmental risks associated with fertiliser applications in the short term. More frequent sampling within seasons would provide greater insight into P dynamics over the crop cycle. Over multiple seasons, responding to changes in crop available P caused by previous fertiliser management may show when farmers could expect to reduce or eliminate their requirement for phosphorus. The data suggests that the PS substitution treatment could be the most effective at increasing crop available P over multiple growing seasons and reducing farmers' requirements for rock-derived phosphate.

Chapter 5 Wheat productivity and phosphorus uptake following integrated fertiliser management on a Cambisol with a low starting P status

5.1 Introduction

During the Green Revolution, agricultural productivity was increased through the use of inorganic fertilisers, new crop varieties, better pest control and technological advancements (Tilman et al., 2002, 2001). Production of grains including wheat, barley, maize, rice and oats increased almost three-fold between 1960 and 2009 (Charles et al., 2010), largely as a result of agricultural intensification. Forecasts predict that by 2050 global food production will need to increase by 48.6% compared to 2013 levels (FAO, 2017a) to meet the food and energy demands of a growing population with shifting dietary habits (Keyzer et al., 2005; Pelletier & Tyedmers, 2010).

Cereals provide an important source of calories and protein, especially in low- and middle-income countries where this food group supplies 50% of daily calorific intake (FAO, 2017a). Global cereal production will need to increase from 2.07 billion tonnes in 2005-2007 to 3.01 billion tonnes in 2050 in order to match demand, an increase of 45.4% (Alexandratos & Bruinsma, 2012). The majority of this increase will need to be achieved through higher yields rather than land expansion (FAO, 2017a) but the potential for growth using methods that were successful during the Green Revolution is lowered because both nitrogen (N) and phosphorus (P) fertiliser production depend on finite resources in the form of natural gas (Chen et al., 2018) and phosphate rock (Cordell et al., 2009), respectively. Understanding how alternative fertiliser practices affect wheat yields is therefore crucial from a human health perspective.

Integrating organic inputs into farm nutrient cycles could help to reduce farmers' requirements for chemical fertilisers derived from finite resources while maintaining yield. Numerous studies have asked how supplementing inorganic fertiliser sources with organic inputs affects productivity. Yields are typically higher when the organic amendment represents an additional nutrients source, compared to when inorganic fertilisers are applied alone (Bandyopadhyay et al., 2010; Chivenge et al., 2009; Kanchikerimath & Singh, 2001; Martínez et al., 2017; Zhao et al., 2009). Meta-analyses have shown that the combined use of organic residues and inorganic N fertilisers leads to higher yields than the application of organic residues or inorganic N fertilisers alone (Chivenge et al., 2011; Wei et al., 2016) but conclude that the increase in yield is due to higher N inputs in combined treatments rather than interactive effects (Chivenge et al., 2011).

The results of substitution design studies, where at least N application rates are matched between inorganic and combined treatments, are more varied and have found that integrated nutrient management can stabilise (Liu et al., 2009; Xie et al., 2016; Zhao et al., 2016), decrease (Gong et al., 2009; Xie et al., 2016; Xin et al., 2017) and increase (Ge et al., 2010; M. Liu et al., 2009; Pincus et al., 2016; Vanlauwe et al., 2011) yields compared to the use of inorganic fertilisers alone. The yield response varies depending on the organic input (Liu et al., 2009; Vanlauwe et al., 2011; Zhao et al., 2016), the year (Zhao et al., 2016), farmer participation (Pincus et al., 2016), percent substitution (Pincus et al., 2016; Xie et al., 2016) and N input rate (Pincus et al., 2016). Therefore, while it appears that the integrated use of organic and inorganic fertilisers has the potential to achieve similar or higher yields than the application of chemical fertilisers alone, farmers may require additional knowledge of their organic input and system to ensure the success of this management strategy.

Incubation and greenhouse experiments have shown that integrating the use of organic and inorganic fertilisers can increase P phytoavailability compared to their application separately (Ahmed et al., 2019; Garg & Bahl, 2008; Halajnia et al., 2009; Leinweber et al., 1999; Mao et al., 2015; Reddy et al., 2005; Toor & Bahl, 1997; Xin et al., 2017). The effect of integrated nutrient management on soil nutrient cycling and the concomitant effect on crop P uptake has been investigated by several authors. In a greenhouse study, the percentage of P applied that was taken up by crops three weeks after fertilisation was greater when soil was mulched than when it was unmulched (Othieno, 1973). Bolan et al., (1994) reported greater P uptake by ryegrass maintained in a greenhouse when soils were amended with organic acids and inorganic P compared to when inorganic P was applied alone, although the differences were marginal and in some cases P uptake decreased in the presence of organic acids (OAs). In a field study where the rate of P application was higher in integrated than organic and inorganic treatments the percentage of applied P recovered in wheat and rice biomass was comparable between the integrated and inorganic treatment, although total P uptake was higher in the integrated treatment (Mao et al., 2015). Xin et al., (2017) also reported higher total P uptake and concentration in plants receiving organic and inorganic P, compared to plants only receiving inorganic P, when P application rates were equal. P uptake and concentration matched the trends in soil available P concentration, suggesting increased P phytoavailability under integrated nutrient management directly increased plant P uptake (Xin et al., 2017).

The aim of this study was to determine how substitution of inorganic P with organic alternatives affects wheat productivity and nutrient uptake, compared to the application of inorganic P alone. Digested cake (DC), a product of the waste water treatment process, and pig slurry (PS) were applied in combination with inorganic P. DC has been shown to produce yields comparable to mineral fertilisers (Alburquerque et al., 2012; Losak et al., 2014; Vanden Nest et al., 2015; Vaneeckhaute et al., 2016) while application of pig slurry typically results in lower yields than mineral fertilisers (Gong et al., 2009; Li, 2013; Martínez et al., 2017). This study tested the hypothesis that integrating the use of organic and inorganic fertilisers affects P dynamics in soil, leading to increases in P phytoavailability and uptake and yield compared to the sole application of inorganic fertilisers. The hypothesis was tested in a greenhouse study using intact soil cores over one growth cycle of winter wheat, *Triticum aestivum* L. 'Skyfall'.

5.2 Methods

5.2.1 Greenhouse experiment

Wheat plants were maintained following the experimental procedure outlined in chapter four. Briefly, intact soil cores were collected from an arable field at Leeds University Farm. The soil is a well-drained, loamy, calcareous brown earth from the Aberford series of Calcric Endoleptic Cambisols (Cranfield University, 2018) underlain by dolomitic limestone of the Cadeby formation (British Geological Survey, 2018). 25 cm deep cores were collected in 10.4 x 28.0 cm polyvinylchloride (PVC) pipes (*internal diameter x height*). After collection, cores were amended to 23 cm depth by removing soil from the bottom of the core before being transferred to a greenhouse (20^{o}C, 16-hour photoperiod). Cores were arranged in a randomised block design with nine treatments and nine replicates per treatment. Cores were sown with two pre-germinated winter wheat seeds, *T. aestivum* L., ‘Skyfall’. Skyfall is a group one milling wheat and the most popular winter wheat variety in the United Kingdom (UK) (RAGT, 2017). The second seedling to emerge in each pot was removed immediately, leaving one plant per pot.

Fertiliser treatments (**Table 4.1**) were applied five weeks after sowing which coincided with the final stages of tillering. Treatments are described in detail in chapter four**.** Two organic fertilisers, digested cake from waste water treatment and pig slurry from a commercial pig unit, were used to substitute inorganic P and N. Inorganic P and N were supplied with monocalcium phosphate ($Ca(H_2PO_4)_2$) and ammonium nitrate (NH_4NO_3), respectively. The digested cake treatment had to be supplemented with NH_4NO_3, as the amount of available N supplied by the amendment was very low. Several controls were also included to determine the effect of individual nutrients on productivity.

Cores were maintained at 60% water-filled pore space by watering with deionised water every day until after stem elongation when they were watered every other day. The mass of each core was recorded before each watering event. The mass of water added at each watering event was calculated by subtracting the mass of the core before watering (g) from the target mass of the core at 60% water-filled pore space. This gave and estimation of evapotranspiration since the previous watering (Allen et al., 1998; Hartmann, 2016; Verstraeten et al., 2008). The mass of water added at each watering event was summed to give an estimation of cumulative evapotranspiration (g), or the total amount of water provided to the plant, to each core at set timepoints. Cumulative evapotranspiration (g) was calculated one week after fertilisation and at stem elongation, anthesis and harvest. Each calculation is for all watering events until that time point meaning that the values are nested. Cumulative evapotranspiration was used as a proxy for biomass accumulation during the growing cycle as evapotranspiration has been shown to be correlated with biomass (Djaman et al., 2013; Payero et al., 2009; Payero et al., 2008). This measurement could be affected by growth of the plant. Average fresh weight at the end of the experiment was 9.46 ± 0.497 g (mean ± standard error of the mean, S.E.M.) and the average mass of fresh soil in each core was 3030 ± 93.3 g (mean ± S.E.M.). The plant therefore accounted for less than 0.5% of total biomass of the plant and soil combined, suggesting the any effect on watering weight was negligible.

Cores were harvested 24 weeks after sowing, equivalent to 19 weeks after fertilisation. Aboveground parts were removed with scissors and separated into shoot plus leaves and grains. Number of ears per plant was counted. Chaff was removed from grain by hand. Parts were weighed before and after drying at 40°C for 48 hours. Dried grains were counted by hand before being ground into a powder for analysis of total N and P uptake by the one-step method (general methods, 2.9). Protein content was estimated by multiplying N concentration (mg g^{-1}) by 5.7 (De Silva et al., 2018; RAGT, 2017; Tkachuk, 1996).

5.2.2 Data analysis

IBM SPSS Statistics 21 and RStudio were used for data analysis. Tests were conducted to confirm that assumptions were met before analysis. Non-parametric tests or generalised linear models (GLM) were used when assumptions were violated and could not be met by data transformation.

5.3 Results

5.3.1 Summary

Main results are summarised in Figure 5.1 as percentage difference compared to the zero-input control. The overall effect of treatment on grain dry weight was statistically significant ($p < 0.001$). Although there was no statistically significant difference in grain dry weight between the inorganic treatment and either substitution treatment ($p = 1.00$ for both treatments), only the inorganic treatment increased grain dry weight compared to the zero-input control ($p = 0.002$). There was no statistically significant effect of treatment on the concentration of nitrogen ($p = 0.307$) or phosphorus ($p = 0.089$) in grain. The total amount of P and N in grain was affected by treatment ($p < 0.001$ for both nutrients). The total amount of N in grain was statistically significantly higher in the inorganic treatment than the DC substitution ($p = 0.008$) and PS substitution treatments ($p = 0.006$). There was no statistically significant difference in total P uptake by plants between the inorganic, DC substitution and PS substitution treatment.

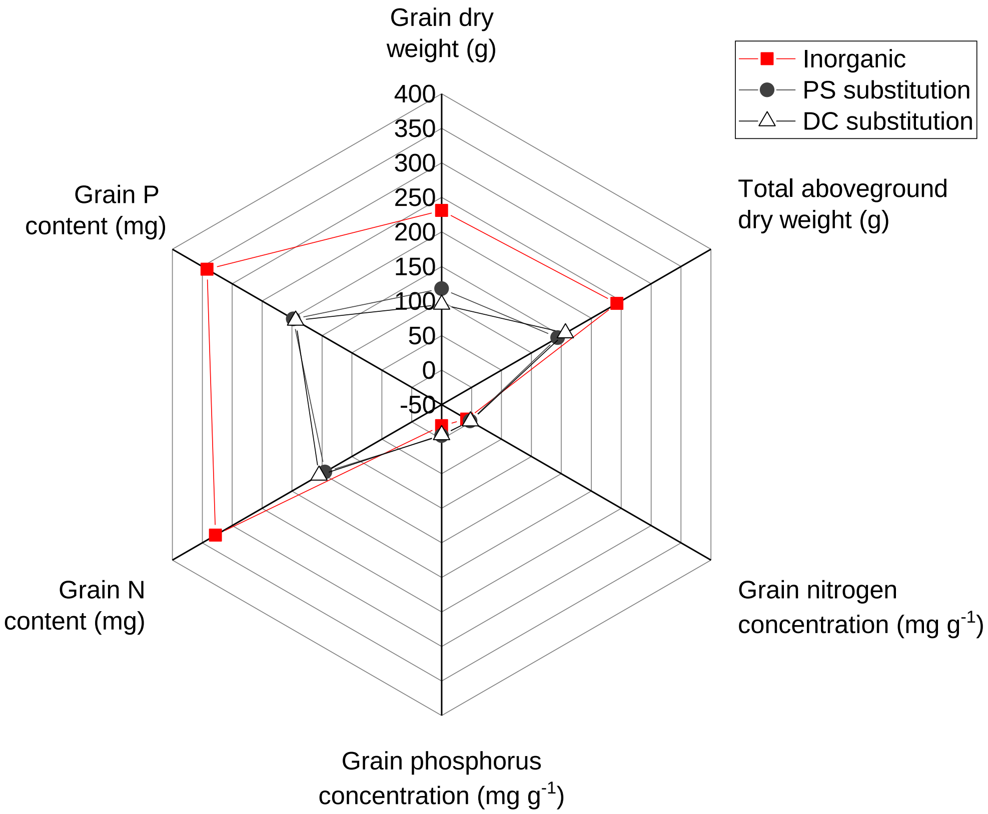

Figure 5.1: Summary of main findings. Results are expressed as percent change compared to the zero-input control. Only the inorganic and substitution treatments are included for ease of interpretation. n = 9.

5.3.2 Biomass accumulation over the growing cycle

Biomass was estimated through cumulative evapotranspiration (CET) measurements. The effect of treatment on CET was analysed at four time points: 1, 4 (stem elongation sampling), 10 (anthesis sampling) and 19 (harvest) weeks after fertilisation (Table 5.1). Analysis was conducted using the Kruskal-Wallis H test given violations of normality (Shapiro-Wilk's, $p > 0.05$) and homogeneity of variances (Levene's test, $p < 0.05$) which could not be met by data transformation. Post-hoc analysis was run using Dunn's procedure and p-values for multiple comparisons received a Bonferroni adjustment. Distributions were similar between groups for all time points, as assessed by inspection of a boxplot.

There was no statistically significant difference in median CET between treatments one week after fertilisation, $X^2_{(8)} = 5.61$, $p = 0.691$. There was a statistically significant difference in median CET between treatments 4 weeks after fertilisation which corresponds with the stem elongation sampling point, $X^2_{(8)} = 18.0$, $p = 0.021$; however pairwise comparisons based on Bonferroni-adjusted p-values showed no statistically significant difference in CET between treatments (Table 5.1). The effect of treatment on median CET was also statistically significant during anthesis, $X^2_{(8)} = 53.6$, $p < 0.001$, and at harvest, $X^2_{(8)} = 54.0$, $p < 0.001$. During anthesis, CET of treatments receiving their full allocation of P and N decreased in the order inorganic, pig slurry substitution, digested cake substitution but these differences were not statistically significant (Table 5.1). The same pattern in terms of significance was recorded at harvest, although CET now decreased in the order inorganic, DC substitution, PS substitution. During anthesis and at harvest, CET was statistically significantly lower in the zero input control compared to the inorganic, PS substitution and DC substitution treatments (Table 5.1).

Linear regression showed that CET during stem elongation, anthesis and at harvest statistically significantly predicted plant total dry weight at harvest although more of the variation in total dry weight was explained by CET during harvest than during anthesis or stem elongation (Table 5.2).

Table 5.1: Cumulative evapotranspiration (grams) of wheat plants according to fertiliser treatment at different time points during the crop cycle. Values are medians and 95% confidence intervals of the median, reported to three significant figures. The overall effect of treatment on cumulative evapotranspiration was determined by Kruskal-Wallis H Test. Pairwise comparisons were analysed using Dunn's procedure with a Bonferroni adjustment. In a column, values followed by the same letter are not statistically significantly different ($p > 0.05$). Results are reported to three significant figures. Treatments are described in Table 4.1. n = 9.

Treatment	1 week after fertilisation	Stem elongation[e]	Anthesis	Harvest
Zero	208 (94, 245)	815 (650, 938)	2880 (2430, 3190)^{a}	4320 (3430, 4580)^{a}
Half rate Pi	173 (106, 191)	732 (676, 956	3090 (2320, 3300)^{ab}	4290 (3580, 4620)^{ab}
Full rate Pi	123 (78, 356)	822 (705, 1050)	2300 (1720, 2670)^{abc}	4560 (3380, 5760)^{abc}
Full rate Ni	206 (106, 242)	999 (870, 1650)	3410 (3210, 5000)^{d}	7120 (6300, 8890)^{d}
Inorganic	205 (137, 358)	1230 (862, 1600)	5250 (4430, 6120)^{d}	7520 (6750, 9090)^{d}
PS substitution	184 (107, 273)	1060 (788, 1360)	4740 (4000, 5200)^{d}	6640 (5380, 7460)^{bcd}
PS only	125 (107, 273)	784 (690, 1330)	3880 (3580, 4430)^{abcd}	5610 (4960, 6430)^{abcd}
DC substitution	162 (107, 182)	993 (785, 1200)	4260 (4070, 5160)^{d}	6480 (5830, 7560)^{cd}
DC only	146 (30, 334)	847 (373, 1530)	4500 (3330, 6250)^{abcd}	6640 (4390, 8500)^{bcd}

[e] Pairwise comparisons showed no statistically significant difference in cumulative evapotranspiration between treatments during stem elongation despite the overall effect of treatment being statistically significant ($p = 0.021$)

Table 5.2: Linear regression outcomes and equations for the prediction of wheat total dry weight at harvest from cumulative evapotranspiration at four points during the growing cycle. Abbreviations: TDW, total dry weight; CET, cumulative evapotranspiration; F statistic, effect size. n = 9.

Time point	Degrees freedom	F statistic	p-value	Adjusted R^2	Regression equation
One week after fertilisation	1,75	1.96	0.166	0.012	TDW = 7.79 + (0.008 x CET)
Stem elongation	1, 75	29.0	<0.001	0.269	TDW = 2.15 + (0.007 x CET)
Anthesis	1, 75	231	<0.001	0.752	TDW = -2.76 + (0.004 x CET)
Harvest	1,75	204	<0.001	0.801	TDW = -4.37 + (0.002 x CET)

5.3.3 Aboveground dry weight

5.3.3.1 Grain weight

The effect of treatment on grain dry weight was assessed by Kruskal-Wallis *H* test, as residuals were not normally distributed in two cells of the design (Shapiro-Wilk's, $p < 0.05$) and this test assumption could not be met by data transformation. There were no outliers with studentised residuals greater than ± 3.00. Visual inspection of a boxplot showed distributions of grain dry weight were similar between treatments. There was a statistically significant effect of treatment on grain dry weight, ($\chi^2_{(8)} = 40.0$, $p < 0.001$) (Table 5.3). Highest median grain dry weight was achieved in the inorganic and the full rate Ni treatments. Median grain dry weight was statistically significantly lower in the zero input, half rate Pi and full rate Pi treatments than the inorganic and full rate Ni treatments (Table 5.3). Adding Pi to Ni led to a small but statistically insignificant increase in median grain dry weight (full rate Ni compared to inorganic, $p = 1.00$). Median grain dry weight for treatments receiving their full allocation of N and P decreased in the order inorganic, PS substitution, DC substitution, although the differences between treatments were not statistically significant.

5.3.3.2 Shoot and total aboveground dry weight

A one-way ANOVA was run to determine the effect of treatment on shoot dry weight. A square root transformation was applied to ensure data met the assumption of homogeneity of variances (Levene's test, $p = 0.108$). Residuals were normally distributed (Shapiro-Wilk's, $p > 0.05$) and there were no cases with studentised residuals greater than ± 3.00. There was a statistically significant effect of treatment on shoot dry weight, $F_{(1,8)} = 79.6$, $p < 0.001$, $\eta^2 = 0.902$. Tests for multiple comparisons were Bonferroni corrected (Table 5.3). Shoot dry weight was only increased compared to the zero-input control when N was added, regardless of form. There was no statistically significant effect of adding inorganic P when all the N budget came from NH_4NO_3 (inorganic compared to full rate Ni, p = 1.000). The digested cake and pig slurry substitutions performed similarly to each other (p = 1.000). Adding inorganic P had no statistically significant effect on shoot dry weight when digested cake was the organic amendment (p = 1.000), but significantly increased shoot dry weight when added with pig slurry (p = 0.034). Although shoot dry weight was numerically lower in the DC substitution treatment compared to the inorganic treatment, this difference was not statistically significant (p = 0.374). However, mean shoot dry weight of the PS substitution treatment was significantly lower than the inorganic treatment (p = 0.017).

A square root transformation was also applied to achieve homogeneity of variances for total aboveground dry weight (Levene's, p = 0.50). Residuals were normally distributed and there were no cases with studentised residuals greater than ± 3.00. Results of one-way ANOVA on square root transformed data showed that there was a statistically significant effect of treatment on total aboveground dry weight, $F_{(1,8)} = 45.6$, $p < 0.001$, $\eta^2 = 0.845$. Tests for multiple comparisons were Bonferroni corrected (Table 5.3). Compared to the zero-input control, adding P alone (full rate Pi) had no statistically significant effect on aboveground dry weight (p = 1.00) while adding N alone (full rate Ni) led to a statistically significant increase in total aboveground dry weight ($p < 0.001$). Total aboveground dry weight was statistically significantly lower in both the DC substitution (p = 0.012) and the PS substitution (p = 0.003) treatments, compared to the inorganic treatment.

Table 5.3: Dry weight of wheat plants at harvest, separated into grain, shoot and total aboveground dry weight. The effect of treatment on grain dry weight was assessed by Kruskal-Wallis H test and Dunn's procedure for post-hoc testing. Pairwise comparisons received a Bonferonni adjustment. The effect of treatment on shoot and total aboveground dry weight was assessed by one-way ANOVA on square root transformed data. Values are medians and 95% confidence intervals (CI) of the median or untransformed means ± one standard error of the mean (SEM), depending on the analysis conducted. In a column, values followed by the same letter are not statistically significantly different ($p > 0.05$). Treatments are described in Table 4.1. n = 9.

Treatment	Median grain dry weight (95% CI) (g)	Mean shoot dry weight ± one SEM (g)	Mean total aboveground dry weight ± one SEM (g)
Zero	1.69 (0.619, 2.37)^{a}	3.02 ± 0.247^{ab}	4.39 ± 0.344^{a}
Half rate Pi	1.22 (0.429, 2.64)^{a}	2.96 ± 0.093^{a}	4.36 ± 0.316^{a}
Full rate Pi	1.92 (1.23, 2.72)^{a}	3.25 ± 0.198^{ab}	5.15 ± 0.369^{a}
Full rate Ni	4.60 (3.45, 7.58)^{b}	7.86 ± 0.335^{ef}	13.2 ± 0.899^{cd}
Inorganic	5.59 (1.27, 8.11)^{b}	8.85 ± 0.277^{f}	15.1 ± 0.486^{d}
PS substitution	3.69 (1.17, 5.34)^{ab}	7.23 ± 0.214^{de}	10.7 ± 0.677^{bc}
PS only	2.71 (0.770, 3.66)^{ab}	5.91 ± 0.266^{c}	8.28 ± 0.662^{b}
DC substitution	3.29 (1.99, 6.02)^{ab}	7.68 ± 0.318^{ef}	11.3 ± 0.931^{cd}
DC only	3.55 (2.71, 6.30)^{ab}	7.64 ± 0.374^{ef}	11.7 ± 0.504^{c}

5.3.4 Grain characteristics

The effect of treatment on the number of grains per plant, equivalent to number of grains per pot, was assessed by Kruskal-Wallis H test. There were two outliers with studentised residuals greater than ± 3.00 which were removed from the analysis. Data was not normally distributed in two cells of the design (Shapiro-Wilk's, $p > 0.05$) and normality could not be achieved by transformation. Medians and confidence intervals are presented in Table 5.4. Pairwise comparisons were run with Dunn's procedure and p-values for multiple comparisons received a Bonferroni adjustment. Distributions were not similar between groups as assessed by visual inspection of a boxplot. Mean rank values are therefore presented in the text. There was a statistically significant effect of treatment on the number of grains per plant, $X^2_{(8)} = 36.9$, $p < 0.001$. Only the inorganic (mean rank = 59.3, $p = 0.008$) and full rate Ni (mean rank = 58.9, $p = 0.012$) treatments increased the number of grains above the level of the zero-input control (mean rank = 20.4). There was no statistically significant difference in the number of grains between the inorganic treatment, the PS substitution treatment (mean rank = 43.9) and the DC substitution treatment (mean rank = 43.3).

The effect of treatment on number of ears per plant was assessed by a Kruskal-Wallis H Test as the assumptions of normality and homogeneity of variances were violated (Shapiro-Wilk's, $p < 0.05$ and Levene's test, $p = 0.024$, respectively). Assumptions could not be met by data transformation. There were no outliers with studentised residuals greater than ± 3.00. Medians and confidence intervals are presented in Table 5.4. Pairwise comparisons were run using Dunn's procedure with a Bonferroni adjustment. Distributions were not similar between groups as assessed by visual inspection of a boxplot. Therefore mean rank values are presented in the text. There was a statistically significant effect of treatment on median number of ears per plant, $X^2_{(8)} = 35.1$, $p < 0.001$. Plants receiving the half and full rate Pi treatments had statistically significantly lower number of ears per plant (mean rank = 12.2 and 22.8, respectively) than treatments receiving the full rate Ni treatment (mean rank = 57.5) ($p = 0.001$ and $p = 0.042$, respectively) and the inorganic treatment (mean rank = 59.1) ($p < 0.001$ and $p = 0.015$, respectively) (Table 5.4). There were no other statistically significant comparisons between treatments.

Mean weight per grain was assessed by one-way ANOVA. There were two outliers with studentised residuals greater than ± 3.00 which were removed from the analysis. The assumption of homogeneity of variances was met (Levene's test, $p = 0.267$) and normality was achieved in each cell of the design (Shapiro-Wilk's test, $p > 0.05$). There was no statistically significant effect of treatment on mean weight per grain, $F_{(8,63)} = 1.95$, $p = 0.068$, $\eta^2 = 0.198$.

Table 5.4: Effect of treatment on wheat grain characteristics. The effect of treatment on number of grains and ears was assessed by Kruskal-Wallis H test with Dunn's procedure post-hoc analysis. Pairwise comparisons received a Bonferonni adjustement. Values are medians and 95% confidence intervals (CI) of the median. The effect of treatment on mean weight per grain was determined by one-way ANOVA. Pairwise comparisons received a Bonferonni adjustment. Values are means ± one standard error of the mean (SEM). In a column, values followed by the same letter are not statistically significantly different ($p > 0.05$). Treatments are described in Table 4.1. n = 9.

Treatment	Number of grains (Median, 95% CI)	Number of ears (Median, 95% CI)	Weight per grain (mg) (Mean ± one SEM)
Zero	55.0 (43.0, 86.0)^{a}	3.00 (3.00, 6.00)^{ab}	30.6 ± 2.25
Half rate Pi	44.0 (0.00, 71.1)^{a}	2.50 (0.00, 4.00)^{a}	33.4 ± 1.80
Full rate Pi	52.0 (43.0, 86.0)^{a}	3.00 (3.00, 4.00)^{a}	30.9 ± 1.32
Full rate Ni	127 (98.0, 207)^{b}	7.00 (5.00, 8.00)^{b}	37.2 ± 11.9
Inorganic	172 (42.0, 209)^{b}	7.00 (3.00, 9.00)^{b}	33.9 ± 1.29
PS substitution	121 (43.0, 167)^{ab}	6.00 (3.00, 8.00)^{ab}	30.6 ± 2.25
PS only	90.0 (31.0, 115)^{ab}	5.00 (3.00, 5.00)^{ab}	29.6 ± 1.43
DC substitution	104 (62.0, 172)^{ab}	5.00 (2.00, 8.00)^{ab}	31.3 ± 2.26
DC only	105 (83.0, 193)^{ab}	6.00 (4.00, 6.00)^{ab}	34.0 ± 1.83

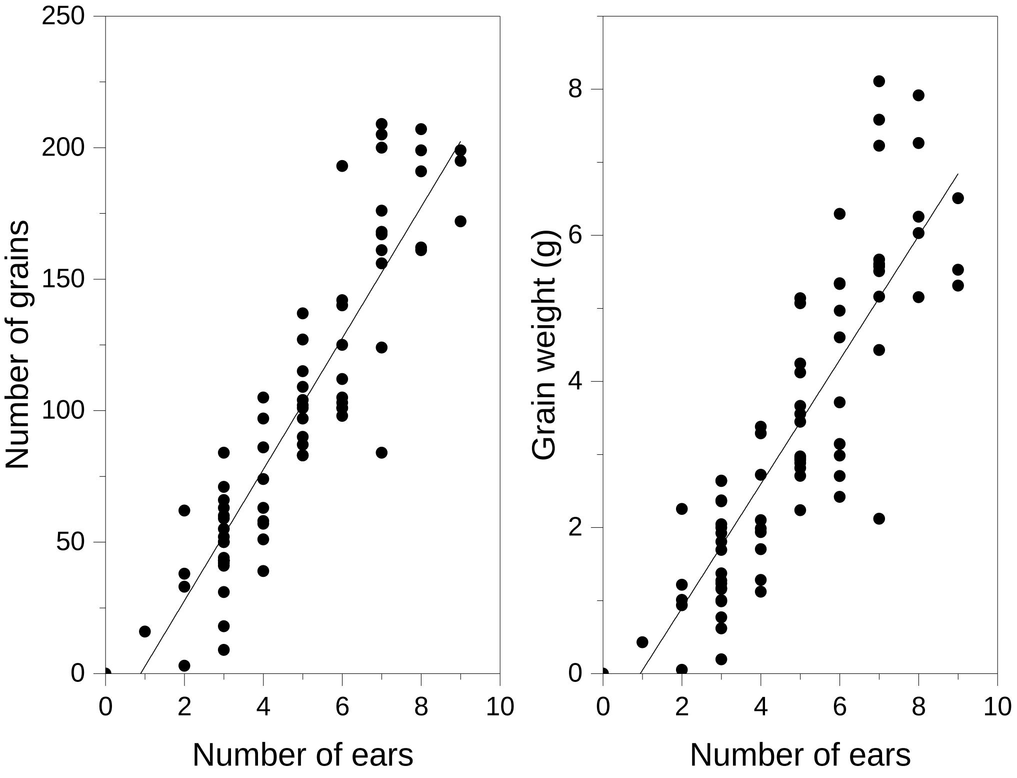

Figure 5.2: Relationship between number of ears per plant and number of grains, and number of ears per plant and total grain dry weight (g). The relationship between number of ears and number of grains ($p < 0.001$, adjusted $R^2 = 82.1$), and number of ears and grain dry weight was statistically significant ($p < 0.001$, adjusted $R^2 = 72.8$). n = 9.

A scatterplot suggested a strong, positive linear relationship between number of ears and total grain weight and number of ears and number of grains (Figure 5.2). The significance of the relationship was tested by linear regression. Number of ears accounted for 82.1% of the variation in number of grains. Number of ears per plant statistically significantly predicted grain weight, $F_{(1,73)} = 341$, $p < 0.001$ (Equation 5.1). The regression equation predicts that number of grains increases by 25 with each extra ear. Number of ears accounted for 72.8% of the variation in total grain weight. Number of ears per plant statistically significantly predicted grain weight, $F_{(1,73)} = 199$, $p < 0.001$. The regression equation predicts that total grain weight increases by 0.856 g for each extra ear (Equation 5.2).

Equation 5.1: Regression equation for prediction of number of grains from number of ears.

$$Number\ of\ grains = -22.0 + (24.9 \times number\ of\ ears)$$

Equation 5.2: Regression equation for prediction of grain weight from number of ears.

$$Grain\ weight\ = -0.871 + (0.856\ \times number\ of\ ears)$$

5.3.5 Grain nutrient content

The effect of treatment on grain nutrient content and concentration was assessed by one-way ANOVA. Where test assumptions could not be met by data transformation, robust or non-parametric methods were employed. The assumption of homogeneity of variances was violated for P concentration (Levene's test, $p < 0.001$) so data was analysed by Welch's robust ANOVA. The assumption of normality was violated for total P uptake in one cell of the design (Shapiro-Wilk's, $p = 0.028$) so a Kruskal-Wallis H test was run. For total N uptake, the assumption of homogeneity of variances was only met following a square root transformation (Shapiro-Wilk's, $p > 0.05$) so analysis was run on this data. Untransformed means and standard errors are presented in Table 5.5. Tests for multiple comparisons received a Bonferroni adjustment.

There was no statistically significant effect of treatment on the concentration of N or P in grain; $F_{(8.65)} = 1.21$, $p = 0.307$, $\eta^2 = 0.130$ and Welch's $F_{(8,65)} = 2.73$, $p = 0.089$, respectively. There was a statistically significant effect of treatment of the total N uptake in grain, $F_{(8,64)} = 11.0$, $p < 0.001$, $\eta^2 = 0.579$. Total N uptake (mg) in grain was statistically significantly higher in the inorganic treatment than the DC substitution ($p = 0.008$) and PS substitution treatments ($p = 0.006$). The effect of treatment on total grain P content was statistically significant ($X^2_{(8)} = 36.8$, $p < 0.001$) but there was no statistically significant difference in total P uptake between the inorganic, DC substitution or PS substitution treatment.

Protein concentration was comparable to the mass fraction expected by industry (11.9%) (RAGT, 2017). There was no statistically significant effect of treatment on protein concentration ($F_{(8,65)} = 1.21$, $p = 0.307$, $\eta^2 = 0.130$) although there was a trend towards increased protein content with the addition of organic fertilisers.

Table 5.5: Wheat grain nitrogen (N) and phosphorus (P) uptake and concentration and protein content (%) after the application of different fertiliser treatments. N concentration ([N]), total N uptake, protein content and P concentration ([P)] were analysed using one-way ANOVA. Values are means ± one standard error of the mean (SEM). Total P uptake was analysed by Kruskal-Wallis H test. Values are medians and 95% confidence intervals (CI) of the median. Pairwise comparisons received a Bonferroni adjustment in all cases. In a column, values followed by the same letter are not statistically significantly different (p > 0.05). Treatments are described in Table 4.1. n = 9.

Treatment	Mean [N] ± one SEM (mg g^{-1})	Mean total N uptake ± one SEM (mg)	Mean protein content ± one SEM (%)	Mean [P] ± one SEM (mg g^{-1})	Median total P uptake (95% CI) (mg)
Zero input	20.0 ± 1.64	26.4 ± 5.17^{a}	11.4 ± 0.936	4.50 ± 0.165	5.04 (2.96, 10.5)^{a}
Half rate Pi	17.1 ± 0.84	25.7 ± 5.55^{a}	9.76 ± 0.479	3.95 ± 0.232	5.72 (1.91, 8.97)^{a}
Full rate Pi	17.0 ± 1.26	30.7 ± 1.91^{ab}	9.70 ± 0.716	4.30 ± 0.146	7.35 (4.90, 10.4)^{a}
Full rate Ni	16.6 ± 1.95	78.1 ± 12.9^{cd}	9.44 ± 1.11	3.18 ± 0.381	17.1 (10.5, 26.2)^{ab}
Inorganic	18.4 ± 1.15	113 ± 6.48^{d}	10.5 ± 0.654	3.62 ± 0.287	22.3 (13.3, 30.6)^{b}
PS substitution	19.5 ± 1.07	64.8 ± 11.4^{bc}	11.1 ± 0.609	4.27 ± 0.120	15.0 (5.42, 23.6)^{ab}
PS only	20.9 ± 1.58	53.7 ± 8.09^{abc}	11.9 ± 0.898	4.25 ± 0.252	12.8 (3.16, 14.6)^{ab}
DC substitution	19.6 ± 1.42	67.3 ± 9.92^{bc}	11.2 ± 0.812	4.22 ± 0.367	14.8 (8.46, 17.8)^{ab}
DC only	20.6 ± 1.81	83.3 ± 10.1^{cd}	11.7 ± 1.03	3.89 ± 0.211	14.5 (9.95, 23.4)^{ab}

5.3.6 Nutrient availability, uptake and plant productivity

The effect of nutrient availability during stem elongation and anthesis on plant nutrient content and productivity was determined using data presented in chapter four**.** Soil nutrient concentrations that were statistically significantly affected by treatment were entered into regression models, to determine if treatment-driven effects on soil nutrient status affected total dry weight. Factors affected by treatment and therefore included in models were the concentration of nitrate-N (NO_3-N) during stem elongation and the concentration of NO_3-N, ammonium N (NH_4-N) and Olsen's P during anthesis. A hierarchical multiple regression (HMR) approach was adopted, to understand the contribution of each nutrient to final biomass. Concentrations at stem elongation and during anthesis were considered in separate models as independence of errors is an assumption of HMR.

5.3.6.1 Soil nutrient status and plant productivity

Linear models were fitted to test for the relationship between NO_3-N concentration during stem elongation and total dry weight using the lm function in the R package lme4 (Bates et al., 2015). Residuals were not normally distributed (Shapiro-Wilk's test, $p < 0.001$) and this assumption could not be met by data transformation. Therefore, a generalised linear model (GLM) with gamma distribution and log link function was run on square root transformed NO_3-N data, using the glm function in the stats package of RStudio (R Core Team, 2018). There was independence of errors (Durbin Watson statistic = 2.06, $p = 0.884$) and residuals were normally distributed (Shapiro-Wilk's, $p = 0.08$). There was a weak but statistically significant positive relationship between the concentration of NO_3-N in soil during stem elongation and total aboveground dry weight at harvest, $X^2_{(1)} = 1.67$, Cragg & Uhler's $R^2 = 0.10$, $p = 0.01$ (Equation 5.3).

Equation 5.3: Regression equation for the prediction of total dry weight from NO_3-N concentration during stem elongation. Note that NO_3-N concentration was square root transformed to improve model fit.

$$Total\ dry\ weight = \left(1.72\ \times\ \sqrt{NO_3 - N}\right) + 5.31$$

A HMR was run to determine the contribution of NO_3-N, NH_4-N and Olsen's P concentration during anthesis to total dry weight. The function run_model in the package AutoModel in RStudio was used to run HMR (Lishinkski, 2015). Factors were added sequentially, resulting in three models which included the following predictors: (i) model one, Olsen' P; (ii) model two, Olsen's P and NH_4-N; and (iii) model three, Olsen's P, NH_4-N and NO_3-N. There was independence of errors (Durbin Watson statistic = 1.93, p = 0.391), the variance inflation factor was less than 10 for each predictor, there was no evidence of multicollinearity, there were no standardised residuals greater than three and no Cook's distance values greater than 0.2. The addition of NH_4-N and NO_3-N to the prediction of total dry weight statistically significantly increased the F value, but there was no statistically significant increase in the F value when only Olsen's P was included in the model (Table 5.6).

Table 5.6: Results of hierarchical multiple regression analyses to determine the relationship between nutrient availability during anthesis and total dry weight at harvest. Three models were run. Model one included Olsen's P concentration, model two included Olsen's P and ammonium-N (NH_4-N) concentrations and model three included Olsen's P, NH_4-N and nitrate-N (NO_3-N). n = 9.

Model	Predictor	Coefficient	Adjusted R^2	F value change	p-value for F change	Overall p-value
One	Olsen's P	-0.104	0.0205	2.55	0.114	1.00
Two	Olsen's P NH_4-N	-0.102 -9.62	0.1658	13.7	<0.001	<0.001
Three	Olsen's P NH_4-N NO_3-N	-0.0382 -0.924 -2.29	0.4129	31.3	<0.001	<0.001

Similar analysis was run to determine the effect of nutrient concentrations at different time points on grain dry weight. However, there were issues with autocorrelation which limited interpretation of results so this analysis was not included. There was a statistically significant relationship between total dry weight and grain dry weight, $r_{\text{Spearman's}}(75) = 0.925$, $p < 0.001$, which suggests that the factors determining total dry weight could also be important for determining grain weight. Because grain nutrient concentrations were statistically significantly similar between treatments and differences only emerged as a function of grain dry weight (Table 5.5), it was decided that running regression analysis on grain nutrient content would only reflect differences in grain dry weight and would therefore be inappropriate.

5.4 Discussion

The aim of this study was to determine how the substitution of inorganic P with pig slurry or digested cake affects wheat productivity and P uptake compared to the use of inorganic fertilisers only. Cumulative evapotranspiration (CET) measurements were used to estimate biomass during plant growth and showed trends in biomass accumulation emerging early in development. During stem elongation and anthesis, CET was higher from the inorganic treatment compared to either substitution treatment although the difference was not statistically significant. The same trend was reported in grain yield at harvest; wheat grain yield decreased in the order inorganic, pig slurry substitution, digested cake substitution. However, there was no statistically significant difference in grain dry weight between treatments receiving their full allocation of N and P. This is contrary to the hypothesis that integrating the use of organic and inorganic fertilisers would increase yield compared to the sole application of inorganic N and P but supports findings of previous research which show that substituting inorganic inputs with organic alternatives maintains maize (Bedada et al., 2014), wheat (Zhao et al., 2016) and rice (Xie et al., 2016) yields in the first season of experimentation. These findings suggest that inorganic P requirements could be reduced without negatively affecting wheat grain yield in the short term.

The study was designed to allow the role of different nutrients in determining grain dry weight to be assessed. Adding inorganic P resulted in marginal increases in grain dry weight, while adding inorganic N alone or in combination with inorganic P statistically significantly increased grain dry weight compared to the zero-input control. These results suggest that N was the most important limiting nutrient in the soil used here. Adding N increased the number of ears per plant and there was a statistically significant, positive relationship between number of ears and number of grains. The weight of individual grains was similar between treatments.

Therefore, the data indicates that adding N, regardless of form, increased grain dry weight by promoting ear formation and therefore grain number.

Further support for the importance of N in biomass determination was provided from results of linear and hierarchical multiple regression. These methods were used to determine how the concentration of nutrients at different growth stages affected total dry weight at harvest. Only nutrients shown to be affected by treatment were included in the analysis: NO_3-N during stem elongation and NO_3-N, NH_4-N and Olsen’s P during anthesis. There was a weak but statistically significant positive relationship between the concentration of NO_3-N in soil during stem elongation and total aboveground dry weight. At anthesis, NH_4-N and NO_3-N both statistically significantly increased the F value of the HMR model and were negatively related to total aboveground dry weight. Results of HMR therefore indicate that plants with the greatest total aboveground dry weight come from soils with the lowest concentration of NH_4-N and NO_3-N during anthesis. Together, this suggests high NO_3-N availability during stem elongation promoted the growth of bigger plants with greater demand for N, resulting in a lower concentration of available N in soil during anthesis. Adding Olsen’s P concentration during anthesis to the model led to no statistically significant change in the F value, supporting the conclusion that P is not significantly affecting wheat productivity in this soil.

Despite giving some indication of the effect of nutrient concentration on final biomass, there are limitations of using a mid-season measurement of available N and P as a predictor of final biomass. Unlike in incubation studies where nutrient cycles are driven by inherent soil biological and chemical properties, the inclusion of a plant will have a large influence on nutrient concentrations at the time of sampling due to uptake. The value at a given sampling point will therefore reflect what has already been consumed by the plant, as well as what is available for future uptake and growth. Consequently, the concentration of available nutrients may be low in a treatment that promotes plant growth and vice versa. Sampling time should therefore be carefully considered when planning studies and interpreting results. Studies may be complemented by incubation experiments that show changes in nutrient concentration over time without plant uptake or enhanced by more frequent sampling to highlight peaks in N and P availability.

Although yields were comparable between treatments receiving their full allocation of N and P, the DC and PS substitution treatments resulted in a 41.1 and 33.4% decrease, respectively, in grain dry weight compared to the inorganic control. Results of HMR and available nutrient concentrations at each sampling point can be used to suggest reasons for lower yields following integration of inorganic fertilisers with pig slurry or digested cake. Despite receiving an equal quantity of readily available N in fertilisers, NO_3-N concentration was lower in the PS substitution treatment during stem elongation than the inorganic or DC substitution treatments. The loss of N as NH_3 following the application of pig slurry has been raised as an issue in previous studies (Martínez et al., 2017) and could explain the lower concentration of NO_3-N in soils fertilised with PS reported here (chapter four, 4.3.6). HMR results suggest the lower concentration of NO_3-N during stem elongation may have limited biomass accumulation. Total N applied in the PS substitution treatment was 250 kg ha^{-1}, which is the organic manure N field limit in nitrate vulnerable zones (NVZ) (Defra, 2013). Supplying a higher proportion of N as inorganic fertiliser has been shown to maintain yields at a level that is closer to the inorganic control (Li, 2013; Xie et al., 2016). It may be beneficial to reduce the size of the P substitution for organic inputs with a low phosphorus-to-nitrogen ratio. As a result, the organic amendment would supply less N allowing for more N to be applied from inorganic sources such as NH_4NO_3.

However, applying a greater proportion of nitrogen from inorganic sources may not always be effective at increasing yield. Grain yield in the DC substitution treatment was low, despite 99% of readily available nitrogen being supplied by NH_4NO_3. The possibility of N immobilisation in the DC substitution treatment between fertilisation and the stem elongation sampling point was discussed in chapter four (4.4). N deficiency has been shown to limit the number of tiller buds and tiller growth (Birch & Long, 1990; Longnecker et al., 1993; Power & Alessi, 1978), which itself is positively correlated with number of ears and yield (Bulman & Hunt, 1988; Power & Alessi, 1978). N deficiency has also been shown to decrease grain number, especially if the deficiency is large and/or lasts for a long time (Jeuffroy & Bouchard, 1999). The median number of ears and grains was lower in the DC substitution treatment than the inorganic and PS substitution treatment, which matches symptoms of N deficiency outlined above. More frequent sampling between fertilisation and stem elongation would provide further information about the intensity and duration of the deficiency and therefore the role of N immobilisation in determining dry weight in the DC substitution treatment.

Previous studies have shown that integrating the use of organic and inorganic fertilisers increases P phytoavailability (Ahmed et al., 2019; Garg & Bahl, 2008; Halajnia et al., 2009; Leinweber et al., 1999; Mao et al., 2015; Reddy et al., 2005; Toor & Bahl, 1997; Xin et al., 2017) and the concentration and uptake of P into plant tissue, compared to the application of inorganic fertilisers alone (Bolan et al., 1994; Mao et al., 2015; Othieno, 1973; Xin et al., 2017). In this study, there was no statistically significant effect of treatment on the concentration of Olsen's

P in soil during the major period of nutrient uptake in wheat (chapter four). As expected based on soil analysis, the concentration of P in grain was similar between treatments. Total P uptake into grain was highest in the inorganic treatment, followed by the pig slurry substitution and the digested cake substitution but this can be attributed to differences in grain weight rather than P phytoavailability. Protein content was similar between treatments and close to the industry expected value of 11.9% (RAGT, 2017). Under British fertiliser guidance (RB209, AHDB, 2020), organic amendments should be applied based on their total rather than available nutrient content. The results show that nutrient content of plants was not compromised in either substitution treatment despite estimated phosphorus phytoavailability of 50% for both pig slurry and digested cake in RB209. Equally, grain P concentration was similar between plants receiving only 30 kg ha^{-1} P_2O_5 from monocalcium phosphate, digested cake or pig slurry suggesting phosphorus phytoavailability was similar in the inorganic and organic amendments. This suggests that applications based on nutrient phytoavailability would be unnecessary from a plant perspective and could result in the overapplication of nutrients, with increased risk of environmental pollution.

5.5 Conclusions

Substituting 50% of the P budget with pig slurry or digested cake had no statistically significant effect on wheat grain yield or P uptake into grain compared to when monocalcium phosphate supplied all P. The data presented here suggests that inorganic P requirements could be reduced with small effects on yield in the short term, but that N nutrition should be carefully managed in order to maximise phosphorus use efficiency.

Chapter 6 Discussion

6.1 Background and aims

Inorganic fertilisers increase crop yields, but their production depends on finite resources and is associated with environmental degradation. Inorganic nitrogen (N) production consumes fossil fuels (Chen et al., 2018) and emits carbon dioxide (Rafiqul et al., 2005) while inorganic phosphorus (P) fertilisers are derived from finite rock reserves (Smil, 2000) whose extraction leads to nonpoint-source P pollution (Kuo & Muñoz-Carpena, 2009). There is concern surrounding the size of P rock reserves with some authors predicting peak P production will occur within 15 years of this thesis (Cordell et al., 2009) and more recent estimates predicting P reserves could be exhausted by 2040 (Blackwell et al., 2019). Concerns over P scarcity are heightened due to the uneven distribution of P reserves across the globe; 85 % of P rock is found in three countries which could be a source of geopolitical tension (Elser & Bennett, 2011). Currently, global P flows can be considered linear (Elser & Bennett, 2011), with mined rock being applied as fertiliser and lost directly from soil or indirectly from human or animal waste following the consumption of products fed or fertilised with inorganic P. Integrating animal and human waste into farm nutrient budgets re-circulates P that would otherwise be lost from the system and could reduce farmers' requirements for rock-derived P.

Organic and inorganic inputs differ in their composition and therefore have contrasting effects on soil nutrient cycling. While the chemical composition of inorganic fertilisers is clearly defined, organic inputs contain a wide range of macronutrients, micronutrients, heavy metals and pharmaceuticals which can affect crop productivity and nutrient cycling. The integration of waste products into agriculture could therefore affect the phytoavailability of nutrients to plants and microorganisms as well as the environmental impact of fertilisation. It has been shown that organic inputs increase the risk of P losses in leachate and runoff compared to inorganic alternatives (Audette et al., 2016; Vanden Nest et al., 2015; Yan et al., 2018) while N losses in leachate and gas are typically higher from inorganically treated soils (Kramer et al., 2006; Küstermann et al., 2010; Marie et al., 2015; Tuomisto et al., 2012). However, nutrient losses caused by organic fertilisers can be similar or exceed nutrient losses caused by inorganic fertilisers when results are reported on a per product basis (Mondelaers et al., 2009; Skinner et al., 2014; Tuomisto et al., 2012). This suggests that the yield gap between organic and inorganic farming systems must be closed in order for the environmental benefits of organic amendments to be realised (Tuomisto et al., 2012).

This thesis focuses on the integrated use of organic and inorganic fertilisers in cereal production. In countries where the use of fertilisers is limited by supply, quality or financial constraints, this practice is advocated as a means to provide the full complement of essential plant nutrients (Gentile et al., 2009). Farmers are encouraged to combine the use of organic and inorganic fertilisers in countries such as the United Kingdom and China to minimise the environmental costs associated with agriculture (Defra, 2010; Shuqin & Fang, 2018). The latter approach could encourage farmers to reduce their use of fertilisers produced using techniques which consume finite resources and emit pollutants (Nemecek et al., 2011).

Current studies suggest that substituting inorganic fertilisers with organic alternatives can increase P phytoavailability (Ahmed et al., 2019; Hu et al., 2018; Reddy et al., 2005), crop P uptake (Mao et al., 2015; Othieno, 1973; ten Hoeve et al., 2018) and P leaching (Leinweber et al., 1999) compared to the sole application of inorganic P. A number of mechanisms leading to increased P phytoavailability under integrated fertiliser management have been suggested including: (i) decreased P sorption/increased P desorption (Jiao et al., 2007; Reddy et al., 2005); (ii) increased production of alkaline phosphatase by soil microorganisms (Chen et al., 2017; Hu et al., 2018; Liu et al., 2010); and (iii) higher concentrations of soil exchangeable calcium (Ca-ex) leading to the production of phytoavailable calcium phosphate minerals (Hu et al., 2018). In certain cases, higher concentrations of available P may be explained by larger inputs of total P in combined compared to inorganic treatments (Chen et al., 2017; Liu et al., 2010) although the same trends have also been reported when P application rates are equal (Hu et al., 2018; Reddy et al., 2005).

Many previously published studies report findings of a single soil sampling occasion that takes place after harvest, decades after the initiation of integrated fertilisation or from experiments with unmatched P application rates (Bolan et al., 1994; Li et al., 2017; Mokolobate & Haynes, 2003; Sun et al., 2015). The aim of this thesis was to determine the short-term effect of integrated fertiliser applications on P phytoavailability, crop yields and P leaching when P application rates are equal between treatments. The approach taken provides results with useful applications because: (i) P is taking over as the nutrient limiting fertiliser application rates across Europe (Sigurnjak et al., 2017); and (ii) understanding the immediate effects of integrated nutrient management provides insight into the possible consequences of the transition period as farmers become less reliant on inorganic P.

6.2 Main findings

A summary of main findings and their relation to the project’s initial aims and objectives is provided in Table 6.1.

Table 6.1: Project aims, objectives, hypotheses and predictions are compared with project findings. Abbreviations: PS, pig slurry; DC, digested cake.

Chapter	Aim and objectives	Hypotheses and predictions	Main findings
Three	To determine how combining organic and inorganic fertilisers affects P phytoavailability and wheat productivity in the short term. The main questions were: 1. How does substituting inorganic fertilisers with PS affect P phytoavailability during the major period of P uptake in wheat? 2. Do shifts in P phytoavailability affect wheat productivity? 3. Does the ratio of organic to inorganic P affect P potential P leaching losses?	Substituting inorganic P with PS will affect P phytoavailability compared to the application of inorganic fertilisers applied alone. P phytoavailability, potential leaching losses and uptake will be higher in the treatment receiving PS and inorganic P than the treatment receiving only inorganic P.	1. Substituting inorganic P with pig slurry had no statistically significant effect on P phytoavailability or potential P leaching losses compared to the application of inorganic P alone. 2. Wheat aboveground dry weight was statistically significantly lower in both treatments receiving pig slurry after eight weeks growth.
Four	To determine how substituting inorganic P with organic alternatives affects P phytoavailability in the short term. The main questions were: 1. How does substituting inorganic fertilisers with organic alternatives affect P phytoavailability during and after the major period of P uptake in wheat? 2. How important is the organic amendment being incorporated in determining the magnitude and direction of the response in terms of P dynamics? 3. How does substitution of inorganic fertilisers with organic inputs affect P leaching losses compared to the application of inorganic P alone? Is the	Substituting inorganic P with PS or DC will affect P dynamics compared to the application of inorganic fertilisers applied alone. P phytoavailability and losses will be higher in both substitution treatments compared to the inorganic treatment, but the effect will be greater for PS than DC.	1. There was no statistically significant difference in P phytoavailability between the inorganic or either substitution treatments during stem elongation or anthesis. 2. Out of the inorganic and both substitution treatments, only the PS substitution treatment significantly increased the concentration of Olsen's P above the level of the zero-input control during anthesis. 3. PS increased Olsen's P concentration compared to the baseline when applied alone. DC only increased Olsen's P concentration compared to the baseline when applied with inorganic P. 4. The concentration of available P in soil increased between stem elongation and

	response the same for both organic amendments?		anthesis for the PS substitution treatment, while $P_{\text{Olsen's}}$ peaked during stem elongation and then plateaued for the inorganic and DC substitution treatments. 5. There was no statistically significant difference in P leaching losses between the inorganic treatment and either substitution treatment.
Five	To determine how shifts in nutrient availability caused by substitution of inorganic P with DC and PS affect wheat productivity and nutrient uptake, compared to the application of inorganic P alone. The main questions were: 1. How does grain yield respond to substitution of inorganic P with DC and PS? 2. Is the nutrient content of grain affected by substitution of inorganic P with DC and PS? 3. Can changes in soil nutrient availability during stem elongation or anthesis explain yield trends?	Phosphorus phytoavailability will be increased when inorganic P is substituted with DC or PS, increasing P uptake and grain yield compared to the application of inorganic P alone.	1. Grain yield decreased in the order inorganic, PS substitution, DC substitution, but there was no statistically significant difference between treatments. 2. There was no statistically significant difference in grain N or P concentration between treatments. 3. N was the key nutrient in determining productivity. There was a small, statistically insignificant effect of adding P on grain yield. 4. It was not possible to determine the effect of nutrient availability on grain dry weight due to data failing to meet test assumptions.

6.2.1 Empirical chapters

The aim of chapter three, the first empirical chapter, was to determine how partial substitution of inorganic P with pig slurry (PS) affects soil P phytoavailability, uptake and leaching risk during the major period of nutrient uptake in wheat. P phytoavailability, measured as Olsen's P ($P_{Olsen's}$) concentration, was similar between treatments. Adding P increased P leaching risk, regardless of the ratio of organic:inorganic P, although leaching was estimated from measures of water-soluble phosphorus (P_{water}) rather than being directly quantified. The most noticeable effect of substitution was on biomass; adding pig slurry alone or in combination with inorganic fertilisers significantly ($p < 0.001$) decreased yield compared to the application of inorganic fertilisers alone. However this was unlikely to be due to differences in P phytoavailability, as Olsen's P concentration was similar between treatments. These findings oppose those of previous studies which show increased P phytoavailability, leaching risk (Leinweber et al., 1999), P uptake (Bolan et al., 1994; Mao et al., 2015; Othieno, 1973; Xin et al., 2017) and yield (Bedada et al., 2014; Ge et al., 2010; Pincus et al., 2016; Zhao et al., 2016) when fertiliser sources are combined compared to the sole application of inorganic P. The data suggests that the form of N added and immobilisation/remineralisation reactions, which have been described in previous studies (Gentile et al., 2013, 2009), may be responsible for the decrease in yield reported in this study. The results of chapter three led to four main questions for future research:

1. Do the same patterns in available P concentration occur on a soil with a low starting P status?
2. How do changes in the concentration of available P and N over time affect wheat productivity and nutrient uptake?

3. How do different organic amendments behave in combination with inorganic fertilisers?
4. Do observed decreases in total aboveground dry weight at the end of stem elongation affect grain yield in substitution treatments?

To answer the questions presented by the results of chapter three, a study was undertaken in which inorganic fertilisers were substituted with digested cake (DC) or pig slurry. Results are reported in chapter four, the second empirical chapter. Plants were grown to harvest and cores were repeatedly sampled during the study which allowed soil nutrient dynamics to be monitored at major periods of wheat development. The inputs were chosen because results of current studies suggest that the amendments differ in their ability to increase soil available P concentration and because of the low N:P ratio of DC compared with PS; DC had to be supplemented with ammonium nitrate (NH_4NO_3) while PS provided all the plant's readily available N.

Results of chapter four largely corroborate those of chapter three in terms of the effect of integrated fertiliser management on soil available P concentration. During stem elongation, the concentration of Olsen's P decreased in the order inorganic, pig slurry substitution, digested cake substitution but the difference between treatments was not statistically significant. This study extends upon chapter three by analysing soil available P concentrations during anthesis. At this time point, Olsen's P concentration decreased in the order PS substitution, inorganic, DC substitution although there was no statistically significant difference between these treatments. The results of this study therefore suggest that substituting inorganic P with PS and DC does not affect the concentration of phytoavailable P in soil compared to the sole application of inorganic P. This study challenges previous greenhouse and incubation studies which have shown the combined use of organic and inorganic fertilisers leads to short-term increases in available P concentrations compared to the sole application of inorganic fertilisers, but these studies often fail to account for the P contained in organic fertilisers (Bolan et al., 1994; Delgado et al., 2002; Mokolobate & Haynes, 2003; Othieno, 1973). The results presented here provide further support for the importance of ensuring treatments are matched for P application rate (Guppy et al., 2005a) when comparing available P concentrations in soil.

The analysis of between-subjects effects described above was less revealing than the effect of each treatment on Olsen's P concentration over time. There were two main findings of repeated measures analysis. Firstly, Olsen's P concentration only increased significantly compared to the baseline level when DC was applied in combination with inorganic P (DC substitution) and not when DC was applied alone (DC only), while PS applied alone (PS only) led to a statistically significant increase in Olsen's P concentration between the baseline and stem elongation sampling point. This finding supports research which has shown DC is less effective than inorganic fertilisers at increasing P phytoavailability (Alleoni et al., 2008; Brandt et al., 2004; Elliott et al., 2005, 2002), but also suggests this limitation could be overcome by the addition of inorganic P, a practice which may be critical in P-limited soils. Secondly, while Olsen's P concentration peaked during stem elongation for the inorganic and DC substitution treatment, the concentration increased significantly between stem elongation and anthesis for the PS substitution treatment. Considered together, these results suggest increases in available P concentration following integrated fertiliser management reported in long-term studies (Ahmed et al., 2019; Hu et al., 2018; Xin et al., 2017) may not develop immediately but come to fruition through gradual accumulation of the soil reserve.

The findings of chapter four show that treatments differed in their ability to increase P phytoavailability over time. Over one crop cycle, the PS substitution treatment increased mean Olsen’s P concentration 69.6% compared to the baseline level, while the DC substitution and inorganic treatments increased mean Olsen’s P concentration by 42.7% and 38.0%, respectively. In the United Kingdom, P inputs should not be made when the concentration of Olsen’s P exceeds 26 mg kg^{-1} (Agriculture and Horticulture Development Board, 2017). Assuming the percentage increase in the second year of application matched that reported in the first and that P application rates stayed the same, cores treated with the PS substitution, DC substitution and inorganic fertilisers could be expected to have a mean Olsen’s P concentration of 32.2, 21.0 and 24.6 mg/kg, respectively, at the end of the second year (Figure 6.1). In a “real-world” situation the farmer would only apply P in the DC substitution and inorganic treatments in the third year, while soils treated with the PS substitution would not receive any P. This suggests that repeated integrated fertiliser applications could increase the concentration of Olsen’s P in soil faster than the application of inorganic P alone, reducing the need for P fertilisation in later seasons. British fertiliser recommendations suggest sampling soils once every three to five years to ensure that the soil P index is maintained. The results presented here suggest that it may be beneficial to sample soils amended with organic and inorganic fertilisers more regularly than stated in the current recommendations to avoid over-applying P and increasing P leaching risk.

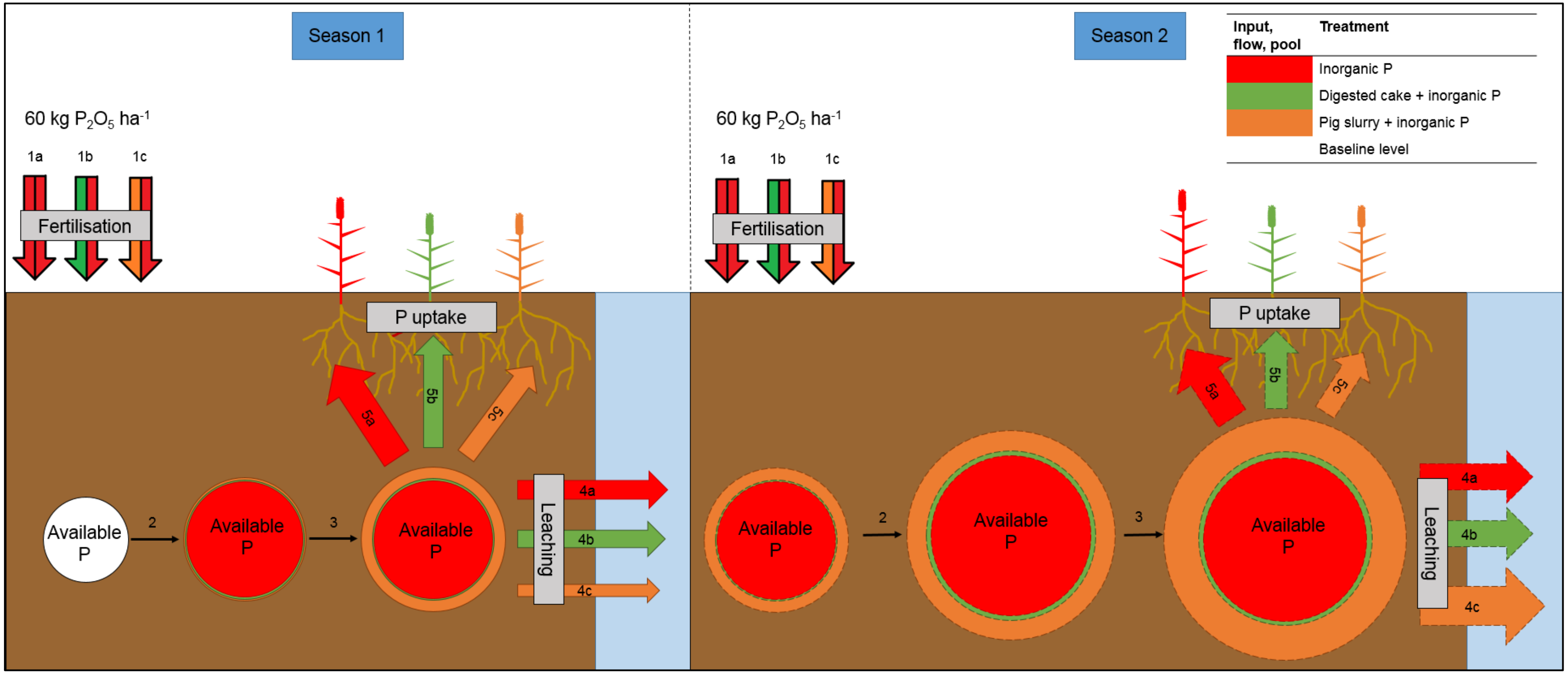

Figure 6.1: Summary of the effect of sole inorganic and integrated organic-inorganic fertiliser management in the first and second season of application. In season one, the concentration of available P during stem elongation compared to the baseline level (2) was increased by all treatments. There was no statistically significant effect of integrated fertiliser management (1b and 1c) on the concentration of soil available phosphorus (P), grain P content (5a – 5c) and P leaching (4a – 4c) compared to sole inorganic fertilisation (1a). The concentration of available P increased significantly between stem elongation and anthesis (3) for the pig slurry substitution only. Results in season two are predicted from trends in season one. Circles represent percentage increase in available P pools, rather than absolute P values, and are to scale. Total and grain biomass are represented by the size of plants.

In this study P leaching losses were measured directly following integrated nutrient management; previous studies have estimated P leaching by calculating unaccounted P but have not made direct measurements (Mao et al., 2015; Xin et al., 2017). As expected from Olsen's P and P_{water} results, there was no statistically significant effect of treatment on the concentration of soluble reactive phosphorus (SRP) in leachate collected at harvest. Cores were leached once at the end of the experiment to avoid confounding effects on the concentration of nutrients in soil. P_{water} was therefore measured throughout the growing period as a proxy for SRP leaching (Leinweber et al., 1999; Pote et al., 1996; Schoumans & Groenendijk, 2000). Although there was no statistically significant difference in P_{water} concentration between treatments at any time point, of treatments receiving their full allocation of N and P only the PS substitution and inorganic treatments increased the concentration of P_{water} compared to the baseline level. Therefore the results suggest that DC was less effective than pig slurry at increasing the concentration of both Olsen's P and P_{water} in soil. They also show that partial substitution of inorganic P with organic alternatives does not increase the risk of P leaching in the short term compared to the sole application of inorganic P.

The aim of chapter five was to determine how substitution of inorganic P with DC or PS affected wheat grain yield. In chapter three, results showed a 30% decrease in mean total aboveground dry weight when inorganic P was substituted with pig slurry. However, the conclusions that could be drawn from chapter three are limited because the experiment was terminated eight weeks after fertilisation, before the production of grain. The results of chapter five showed similar decreases in total aboveground dry weight at harvest; mean total aboveground dry weight was reduced by 29% and 25% for the pig slurry and digested cake substitution treatments, respectively, compared to the inorganic treatment.

Median grain dry weight decreased in the order inorganic, PS substitution, DC substitution but the difference between treatments was not statistically significant. However, only the inorganic and full rate Ni treatment statistically significantly increased grain dry weight compared to the zero-input control. The data suggests that N increased the number of ears and therefore the number of grains per plant, resulting in a higher median grain dry weight than if N was not applied. Regression analysis showed a weak but statistically significant positive relationship between the concentration of nitrate-N (NO_3-N) in soil during stem elongation and total dry weight at harvest. Using hierarchical multiple regression (HMR), it was shown that the concentration of ammonium-N (NH_4-N) and NO_3-N statistically significantly added to the F value of the model, with negative coefficients. The data therefore suggests that plants with greater demand or capacity for N uptake accumulated the most biomass.

Compared to N, the role of P in determining total aboveground dry weight was negligible. Median grain dry weight was 13.6% higher in the full rate Pi treatment compared to the zero-input treatment and 8.75% higher in inorganic treatment, which received inorganic N and P, than the treatment receiving the full rate of Ni only. However, these differences were not statistically significant suggesting P only had a small effect on yield. There was no statistically significant difference in the concentration or total amount of P in grain between the inorganic, PS substitution and DC substitution treatment which was expected given the similarity in Olsen's P concentration between treatments during stem elongation. The findings presented here challenge the few previously published papers investigating P uptake and content following combined fertiliser application which show that the integration of organic and inorganic fertilisers increases P uptake and concentration compared to the application of inorganic fertilisers alone

(Bolan et al., 1994; Mao et al., 2015; Othieno, 1973; Xin et al., 2017). It is possible that these patterns only develop after years of repeated applications (Xin et al., 2017) or when the total amount of P applied in the combined treatment exceeds that in the inorganic treatment (Mao et al., 2015; Othieno, 1973). The data therefore suggests that trends of higher P uptake under integrated fertiliser management may not emerge if P fertiliser application rates respond to changes in soil available P over time. The findings also show that grain protein content was unaffected by substitution of inorganic fertilisers with organic alternatives. Overall the findings indicate that grain nutrient content and quality could be unaffected by integrated fertiliser management in the short term.

The findings of this thesis provide strong support for the inclusion of plants in greenhouse studies investigating the effect of fertiliser management on soil nutrient status and leaching losses, particularly when studying nitrogen. Fertilisation strategies that increased productivity decreased the concentration of NO_3-N and NH_4-N in soil during anthesis and reduced the concentration and load of NH_4-N in leachate at harvest. For crops like winter wheat that are harvested in late summer (Agriculture and Horticulture Development Board, 2018), strategies that reduce the level of available nutrients in soil after crop removal are beneficial because most leaching takes place over winter (David et al., 1997; Greer & Pittelkow, 2018; Shepherd & Lord, 1996). If plants had not been included in the experimental design, it is possible N would have accumulated over the course of the experiment without being consumed by the plant and therefore N leaching would be expected to be higher from soils amended with N. The feedback between soil and plant is therefore crucial when determining the environmental impact of a fertiliser treatment.

6.2.2 Contribution to identified knowledge gaps

The introduction chapter found a lack of agronomically-relevant short-term studies investigating the effect of integrated fertiliser management on soil available P concentrations, crop P uptake, P leaching losses and yield. The data presented here shows that substituting 50% of the P budget with pig slurry or digested cake has no statistically significant effect on the soil available P concentration, P leaching losses, crop P uptake and grain yield in the first season compared to the sole application of inorganic fertilisers (Figure 6.1). Although available P concentrations were similar between treatments, trends in available P concentration over time differed; Olsen's P concentration was statistically significantly higher during anthesis compared to stem elongation for the pig slurry substitution treatment but there was no statistically significant difference between stem elongation and anthesis for the digested cake substitution and inorganic treatment. The results therefore show that the choice of organic fertiliser could have important consequences for P accumulation in the short term and affect the amount of fertiliser that needs to be applied in following seasons.

6.2.3 Implications and limitations of findings

This thesis focuses on the concentration of phytoavailable phosphorus in soil, a very small component of the wider global P cycle. Concerns over P largely focus on supply and leakiness, but these flows are intimately linked with soil available P. P phytoavailability concentration dictates P application rates (Agriculture and Horticulture Development Board, 2017) and is positively correlated with P leaching losses (Leinweber et al., 1999; Pote et al., 1996; Schoumans & Groenendijk, 2000). The data presented here suggests that integrating organic and inorganic P could reduce farmers' requirements for rock P while maintaining soil available P levels and P leaching losses, compared to the sole application of inorganic P. At a broad scale, adopting integrated fertiliser management could extend the lifetime of remaining P reserves and sustain yields without increasing the environmental cost of agriculture compared with inorganic fertilisation.

There are several limitations to the research presented in this thesis.

Firstly, the conclusions that can be drawn from this work are limited because experiments were conducted in a greenhouse. It is not possible to state that results obtained in a temperature and light-controlled environment are reproducible in the field. Temperature and drying-rewetting cycles have both been shown to affect phosphorus phytoavailability in soil (D. Sun et al., 2017). Higher average temperatures and regular watering in the greenhouse are therefore potential sources of difference between results obtained in controlled environments and the field.

Secondly, while this thesis focuses on and addresses the effect of recently-applied fertilisers on P phytoavailability it would benefit from the studies being repeated on the same soils over multiple seasons. This would allow the effect of both recent and repeated applications on phosphorus phytoavailability to be assessed. This information could be used to inform fertiliser guidance, especially related to the frequency of sampling in soils that have received integrated fertiliser applications.

Thirdly, the conclusions that can be drawn from this research are somewhat complicated by the inclusion of a plant. It is not possible to separate soil concentrations of N and P from plant uptake; is the concentration of available nitrogen lower because productivity is higher, or because the fertiliser added is less effective at increasing available nitrogen concentration? It would be possible to address this with radioactive labelling techniques, or with complimentary incubation studies in equal conditions but in the absence of a plant.

Fourthly, this study focuses on labile P concentration in the form of Olsen's P and water-soluble P. As a result, there is no information presented related to non-labile P forms. To elucidate the distribution of P across multiple pools a sequential extraction technique could be adopted. This would start to develop a mechanistic understanding of the effect of recently-applied fertilisers on P phytoavailability. Further mechanistic insights could be gained using P sorption isotherm techniques.

Finally, the research would benefit from inclusion of potassium in nutrient budgets. This would overcome doubts related to potassium limitation and productivity.

6.3 Future research questions

The results of this thesis generate several questions for future research.

1. Could the size of the substitution be optimised for yield and does this differ between organic inputs?
2. How do results from the greenhouse apply to the field?
3. Does integrated fertiliser management affect the amount of P fertiliser required in future seasons? After how many seasons is P no longer required? Do the increases in available P reported in long-term studies occur when P application rates respond to changes in concentration of available P in soil that result from fertilisation in the previous season?

These questions could be addressed in two studies. First, a greenhouse study following a similar design to the ones reported in this thesis could be used to determine the ratio of organic- to inorganic-P required to maximise yield under integrated fertiliser management. With this knowledge, a field study could be designed to answer questions two and three. Sampling during the growing season would allow for a more detailed understanding of how the integrated use of organic and inorganic fertilisers affects P cycling during the crop season. Quantitative PCR of microbial genes involved in inositol phosphate hydrolysis (*phoA, phoD, phoX)* and nitrification (*amoA, amoB, amoC, hao, nor*), analysis of soil calcium phosphates and monitoring of soil structure would provide more information on the mechanisms that lead to increased P phytoavailability reported in long-term studies and the time they take to develop. The results presented here suggest a field study of three to five years would be required to answer these questions.

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