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# Temporal changes in plant available phosphorus in a long term experiment growing winter wheat (*Triticum aestivum*) on Rothamsted, England.

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Environment and Natural Resources

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# Abstract

Phosphorus is a macronutrient essential for all life, but it is a limited resource and it's unwise use can cause detrimental algal growth. Therefore, an effective agricultural use of phosphorus, where large amounts of food is produced from the applied phosphorus, is important.

Phosphorus binds strongly in soils, and plants need active mechanisms for it's uptake. There is no general agreement on the seasonality of phosphorus binding, as there is done little research on the field, and the results are diverging.

On the long-term experiment “Exhaustion Land” (established 1856) in Rothamsted, England, soil and plant samples were harvested 10 times from March to August. The plants were weighed and phosphorus analysed, and the soil samples were analysed for plant available phosphorus by Olsen P and DGTs exposed for 6, 24 and 72 hours.

The results showed total plant available phosphorus changing but little trough the growing season, but the most available part (measured by DGTs exposed for 6 hours) increases early on, for then being depleted below the limit of detection.

This is regarded as the plants transforming more heavily plant available phosphorus to more easily available phosphorus before uptake. The plants transform heavily plant available to easily available earlier on in the season than their main uptake of phosphorus. The soil buffers the heavily plant available fraction geochemically, and fast enough for the heavily available phosphorus not to be depleted over the course of a single growing season.

The geochemical buffering of P could influence breeding for low P environment, and a general knowledge of P seasonability and plant uptake could advise the farmes on when to fertilise.



# Samandrag

Fosfor er eit næringsstoff naudsynt for alt liv, men det er ein avgrensa ressurs som på avvege kan føra til uynskja algebløming. Difor er det viktig med god fosforbruk i landbruket, der det kjem mykje mat frå den tilførde fosforgjødsla.

Fosfor bitt seg sterkt i jorda, og plantane må bruka krefter på å taka det opp. Det er ikkje semje om korleis fosforbindinga endrar seg gjennom vekstsesongen, då det er gjort lite forsking på feltet, og resultata har vore varierande.

På forsøksfeltet "Exhaustion Land" (starta i 1856) på Rothamsted i England vart det hausta jordprøver og kveiteplantar 10 gongar millom mars og august. Plantane vart vegde og målt fosfor i, og jordprøvene blei analysert for plantetilgjengeleg fosfor ved landbrukstesten Olsen P metode og DGTar eksponerte i 6, 24 og 72 timar.

Resultata viste at total plantetilgjengeleg fosfor endrar seg lite gjennom vekstsesongen, men at den mest plantetilgjengelege delen (målt med DGT eksponert i 6 timar) vert større tidleg i vekstsesongen, for so å verta umåleleg liten.

Dette er tolka som at plantane gjer tungt plantetilgjengeleg fosfor om til lett plantetilgjengeleg fosfor, for so å taka det opp. Plantane gjer tungt plantetilgjengeleg fosfor om til lett plantetilgjengeleg tidlegare i sesongen enn hovudopptaket deira av fosfor. Jorda etterfyller det tungt plantetilgjengelege fosforet geokjemisk, og fort nok til at tungt plantetilgjengeleg fosfor ikkje vert utarma i laupet av ein enkel vekstsesong.

Kjennskap til den geokjemiske fosforbufferen kan hjelpa foredlarar i planteal for fosforfatig jord, og kunnskap om planteopptak og endringar gjennom sesongen kan vera til nytte i gjødslingsrådgjevinga.

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# Preface

Any master degree at the Norwegian University of Life Sciences is to be ended with a thesis building on a independent study conducted by the student. This is the end result of my 60 ETC master thesis work, and by extension an end result of five years studying environment and natural resources at the university's Department of Environmental Science.

The thesis is on phosphorus, the cycling of which is a major part of why I chose a specialisation in soil sciences. The rumour of an impending breakdown of phosphorus supply had reached outside of the sciences, making the topic interesting for social and political reasons as well as the purely soil scientific ones. The problem of phosphorus supply is of course – and luckily - more complicated than reaches outside ears, just as soil phosphorus behaviour is. If there had not been more to soil than meets the eye, it would have not been such an interesting subject. The work on this thesis has given me a deeper understanding on the theoretical aspects of phosphorus in soils and the environment, as well as lots of practical experience in lab and field work.

In England in was part of the Department of Sustainable Soil and Grassland Systems at Rothamsted, a group which welcomed me with open arms. I would especially like to thank technichans Javier Hernandez and Sarah Dunham and professor Steve McGrath for their knowledge and helpfulness.

At the Department of Environmental Science I would like to thank the soil science group for being welcoming to me as a student and giving good advice. My supervisors professor Åsgeir R. Almås and professor Tore Krogstad deserve special mention for their help and guidance, as well as for doing most of the paperwork involved with the project, thus giving me the time to focus on it's scientific content.

At Ås and at home, I have told friends and family about my master thesis, phosphorus and soil sciences. I thank you for listening and your patience, even though it might have gotten boring to hear about it time and again (and again). Explaining my field to laypeople have helped my understanding of it.

The master thesis is part of the larger Agropro project, and has received funding from YARA as well. I am much obliged.

Åsmund Mikalsen Kvifte

13th of May, 2016

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# Table of contents

1 Introduction....1
1.1 P resource depletion....1
1.2 Eutrophication....1
1.3 Increased yield per area....2
1.4 P reactions in soil....3
1.5 Plant P uptake....4
1.6 The effect of P fertiliser....5
1.7 Seasonal variability of P availability....6
1.8 Hypotheses....6
1.9 Experimental set up....6
2 Materials and methods....7
2.1 Site description....7
2.2 Soil and plant sampling....7
2.3 Soil pH measurements....8
2.4 Plant digestion....8
2.5 Total nitrogen analysis....8
2.6 Olsen P....8
2.7 Diffusive Gradients in Thin Films (DGT) P....9
2.8 Statistics....9
2.9 Citation software....9
3 Results....10
3.1 Yield data....10
3.2 Soil pH....11
3.3 Total nitrogen analysis....11
3.4 Olsen P results....12
3.5 P concentrations of the DGTs....14
3.6 DGT-P concentrations....15
3.7 P Development in DGT-gels....15
4 Discussion....16
4.1 P pools measured by DGT....16
4.2 Changes in 6 hour Pgel....16
4.3 Changes in 72 hour Pgel....16
4.4Changes in 24 hour Pgel....17
4.5 Buffering and development of Olsen P....17
4.6 Exposure times for DGTs....18
5 Conclusions....19
6 References....20
Appendix 1: Reference material 1, SRM 1547, Peach leaves....27
Appendix 2: Reference material 2, internal grass standard....29
Appendix 3: Tables of plant dry matter and P and soil P from March to August....30

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# 1 Introduction

Phosphorus (P) was the 13th element to be discovered, being first isolated by Henning Brand in 1669, though also independently discovered by others (Weeks 1932). It was among the first elements to be identified as a necessary plant nutrient (von Liebig 1840), and it is essential for all life. In the environment, it occurs mainly as phosphates, which has likely been the dominant form since the formation of the Earth (Griffith *et al.* 1977).

On the primitive Earth, P was likely barely available (Griffith *et al.* 1977), and it's biological importance and ubiquitousness is likely testament to it being biologically uniquely useful (Schlesinger & Bernhardt 2013). It fulfils many functions, among them acting as an energy carrier (most importantly in ATP) and an information carrier (in DNA, RNA), roles for which P likely is the best suited element in a cell environment (Westheimer 1987). It is also a main constituent of vertebrate bone tissue and crustacean crusts (Griffith *et al.* 1977).

Today, the availability of P is still an important question, it being the second most common growth limiting macronutrient, with nitrogen being the most common (Schachtman *et al.* 1998). In addition, there are causes of concern both for the origin and the fate of phosphates.

## 1.1 P resource depletion

As the ambient levels of P in most rocks are too low to sustain optimal plant growth (Hinsinger 2001) , additional P fertilization is required. A P gas, phosphane, is formed under methanogenic conditions (Gassmann & Glindemann 1993), but the gas is not reported to occur over the µg/m³ level, and is thus seldom important (Glindemann *et al.* 2005). The atmospheric deposition of P are estimated to average roughly 50 g P/ha/year (Meybeck 1982), while plants typically need 1 g P/kg dry matter or more to thrive (Aasen 1997). Thus, atmospheric contributions are negligible in most areas.

Therefore, P fertilizer is important to ensure optimal yields. This P comes mainly from phosphate rocks, especially the mineral apatite (Smil 2000). Recently, it has been debated if the supplies of P rocks are sufficient to feed the world: for example, Van Vuuren *et al.* (2010) and Cordell *et al.* (2009) has voiced concerns about a possible depletion of the world P resources in the near future. Van Kauwenbergh (2010) believed this was a misunderstanding stemming from P resources being underestimated by the United States Geological Survey, which has since increased their estimate to levels agreeing with Van Kauwenbergh – $60 - 70 \cdot 10^9$ tons $P_2O_5$ (Jasinki 2016). Edixhoven *et al.* (2014) criticized the whole debate, claiming it was based on data too uncertain to conclude either way.

In addition to the total reserve size, it's uneven distribution raises geopolitical concerns (Cooper *et al.* 2011, Gilbert 2009): Over 70 % of the resources are located either in Morocco or Western Sahara (Jasinki 2016), which is occupied by Morocco, an occupation further complicating the geopolitical situation (Cooper *et al.* 2011).

There is also a considerable energy cost associated with the mining and production of P fertiliser (Goldstein *et al.* 1993).

## 1.2 Eutrophication

Anthropogenic forcing of the biogeochemical P cycle is regarded as one of the foremost environmental issues (Rockström *et al.* 2009), where the emissions of P to waterways, lakes and estuaries are major concerns (Carpenter & Bennett 2011).

In a famous experiment, Schindler (1974) split lakes with membranes, fertilized them with combinations of nitrogen, phosphorus and carbon, and found P to limit increased algal growth and eutrophication (Figure 1). This is because parts of the plankton community in lakes are able to compensate for a lack of nitrogen by nitrogen fixation, atmospheric gas exchange will supply carbon, while there is no way for the limnic community to increase the P status (Schindler 1978, Hutchinson 1973).

*Figure 1: Schindler's (1974) experiment. The grey part has recieved full nutrient loading, while the darker part has recieved full nutrient loading sans P. The difference stems from green algae.*

Anthropogenic eutrophication has been recognised as a problem since at least the mid-20th century by those affected, but the scientific understanding of the reasons problem arose first in the 1970s (Schindler 2006). Eutrophication causes a loss in water quality, owing to increased algal growth, often of algae harmful to man (Schindler 2012). When the algae die, their decomposition consumes the available oxygen, which kills fish present (Schindler *et al.* 2008).

The solution to the problem is to reduce P loading (Schindler *et al.* 2008), although there may be a significant delay between input reduction and improvement of water quality due to internal remobilisation of sedimented P (Bergström *et al.* 2015, Burger *et al.* 2007).

Today, agriculture is the major source of waterway P in many countries, among them Norway and the UK, due to an over-application of P fertiliser (Ulén *et al.* 2007). Applying more fertiliser than was taken out was regarded as a way to build up reserves, but too large P reserves in the soil will cause galloping losses of P in surface run-off (Johnston & Dawson 2005). Therefore, reducing P input from agriculture is a key to alleviating the problem.

## 1.3 Increased yield per area

The resource reserve and pollution situation might encourage a reduced use of P fertiliser, but increased populations demand more food production, thus more P fertiliser. By 2030, the world population is expected to rise to 8.5 billions, a 15 % increase since 2015 (United Nations 2015). Food consumption is expected to have a faster rise as people eat more and change their diets to include more animal based products (Kuyper & Struik 2014).

At the same time, urban areas are expected to double, to 1.1 % of Earth's ice-free land surface (Seto *et al.* 2011), an expansion which many places displace agricultural lands (Det kongelige landbruks- og matdepartementet 2015, (Jiang *et al.* 2013).

The cultivation of new agricultural land carries a large environmental premium (Garnett *et al.* 2013), and the intensified use of existing agricultural land may also carry heavy costs (Smith *et al.* 2016). Therefore it is important to intensify agriculture in an environmentally sound way (Campbell *et al.* 2014), which will include an efficient application of fertiliser to agricultural lands (Syers *et al.* 2008).

## 1.4 P reactions in soil

P is not a very rare element, even if soil solution concentrations are low: It is the 11th most abundant element in the lithosphere (Smil 2000). The scarcity of P is caused by phosphate reacting with cationic soil constituents, or it being incorporated into organic molecules (Hinsinger 2001).

Degryse *et al.* (2009) gave a schematic outline of the partition of elements in soil (figure 2). The element in solution can complex with other soil solution constituent or associate itself to solid particles. The solid and complexed constituents are either labile or inert on a given time scale, and the pools will equilibrate between each other: there will be an equilibrium between inert solid element and labile solid element and between labile solid element and free dissolved element.

Thus, a depletion of the free dissolved pool will cause a mobilisation from the labile solid phase, which in turn causes a labilisation of the inert solid phase.

Syers *et al.* (2008) and Johnston *et al.* (2014) applies similar concepts for (inorganic) phosphorus, theoretically splitting it into four pools after plant availability (figure 3): beginning with a directly plant available soil P pool, and then progressively less labile. These pools do not precisely predict how the P is bound, as plant availability will be a function of binding energies, which will differ internally in fractions bound to the same soil constituents (Syers *et al.* 2008).

There has been thorough research on the fractions binding P in soil (Smil 2000). P binding in soil was first described in 1850 (Way 1850), and research has continued to the present day (Condron & Newman 2011).

The most important inorganic fractions studied has been aluminium, iron, and calcium, all binding P due to their highly positive charge (Havlin *et al.* 2005). Calcium is regarded being the dominant P binder in neutral to alkaline soils while aluminium and iron will dominate P binding in acid soil (Tan 2011). The amount of P bound to these fractions is commonly assessed by sequential extraction (Condron & Newman 2011). While useful, sequential extractions are time consuming, multi-step procedures measuring fractions which may re-distribute themselves during extraction, thereby increasing both probability of operator error and general variability (Young *et al.* 2005).

The ultimate end result of P binding by soil constituent can be precipitation of secondary P minerals (Hinsinger 2001). Lindsay (1979) and Lindsay *et al.* (1989) are the standard works on the minerals formed and their solubility and behaviour. In

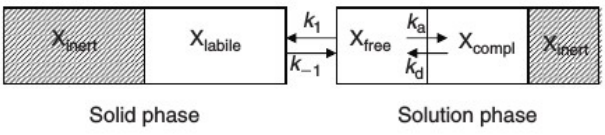



*Figure 2: Schematic presentation of elemental speciation in soil. Species occur either as inert or active species, and can react to adjacent pools. From Degryse et al. (2009)*

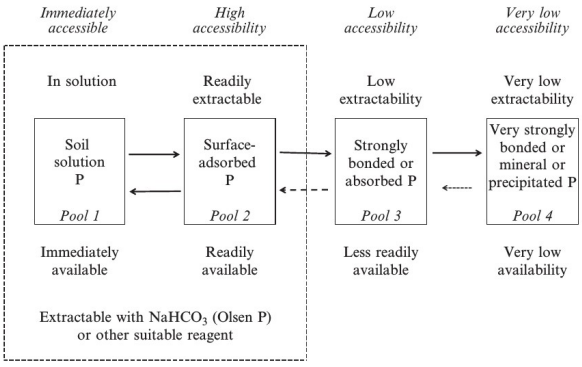



*Figure 3: Model of operational P pools. The pools are defined by how strong reagent is needed to extract the P within. There is internal movement in the pools, so P added to the accessibe pools will relocate to the less accessible pools. A depletion of the available pools will mobilise more P from the less accessible pools. From Johnston et al. (2014).*

soils, calcium-phosphate minerals have been directly observed as well as, less frequently, aluminium- and iron-phosphates (Hinsinger 2001). Still, Syers *et al.* (2008) argue precipitation and mineralogy to be of less importance for plant nutrition, as uptake is dependent of binding strength rather than binding agent.

A varying amount (5 – 95 % according to Smil (2000)) of the P in soils are organically bound, with phytates being the most important compounds (Gyaneshwar *et al.* 2002). Organic P is generally quite unstable in soils, with turnover times of weeks to years (Smil 2000).

Part of the organically bound P will be present in micro-organisms; typically 2 – 10 % of total soil P will be microbially bound (Richardson & Simpson 2011). Micro-biota is the chief regulator of transformation between inorganic and organic forms of P (Khan *et al.* 2009), and will mainly immobilise and mineralise P in their immediate surroundings. As micro-biota are spread throughout the soil, more concentrated hot spots of P will cause less microbial activity than if P is spread evenly through the soil (McLaughlin *et al.* 1988b). The transformation of organic P will also depend on carbon supply (Nziguheba *et al.* 1998), and plants may be able to increase P supply by manipulating carbon exudation from their roots (Richardson & Simpson 2011).

## 1.5 Plant P uptake

For a plant to take up any nutrient, there has to be contact between the plant root and the nutrient molecule to be taken up (Brady & Weil 2010). P is taken up as $HPO_4^{2-}$ and $H_2PO_4^-$, likely co-uptaken with cations (Schachtman *et al.* 1998). The uptake will deplete the immediate surroundings of the root, and continuous resupply is necessary for further uptake (Shen *et al.* 2011). The soil can resupply itself by diffusion and mass flow, with diffusion by far being the dominant process (Hinsinger 2001). In addition, the plant will actively explore the soil and solubilise P (Yuan *et al.* 2015).

The uptake of phosphate ions have to be active, as the concentration of P inside the plant often is 3 orders of magnitude larger than ambient (mM inside, µM outside) (Raghothama 1999). There are multiple systems in the plant taking up P, and the uptake will depend on these as well as the rooting of the plant (Hinsinger 2001). Plants may also cooperate with mycorrhizae, where the fungi explores the soil for nutrients while the plant supplies the fungi with organic carbon (Schachtman *et al.* 1998).

Roots differ between plants and strains, and they therefore have different uptake efficiency (Heppell *et al.* 2015). The nutrient status of the plant will influence it's hormone production (Vance *et al.* 2003), and plants are known to prioritise root growth under P stress (Brouwer 1983), and are more easily infected with mycorrhizae (Gyaneshwar *et al.* 2002). In addition, plants are known to change their root architecture and anatomy under P stress (Brown *et al.* 2012a):

Plants will develop more roots in the relatively P rich topsoil (Lynch & Brown 2001), develop root types requiring with less photosynthetic burden per root area (Miller *et al.* 2003) and produce longer roots (Eissenstat 1992). Still, translocating metabolites to roots may hinder overground growth, and translocating the roots to the topsoil makes the plant more susceptible to both drought and waterlogging (Brown *et al.* 2012a).

A photosynthetically cheap way to increase root area, is the development of root hairs. (Heppell *et al.* 2015). Root hairs are important for P uptake (Raghothama 2005), especially in P deficient or heavier soils. Barley and Rovira (1970) demonstrated that root systems with root hairs absorbed 78 % more P than mutants without in clays, but this difference disappeared under hydroponic cultivation. Although longer root hairs increase P content in barley (*Hordeum Vulgare L)*, yield is regulated by their presence rather than length (Brown *et al.* 2012b). In wheat (*Triticum aestivum L*) root hairs are able to overcome mild P deficiencies, but not severe (Yuan *et al.* 2015).

In addition to modifying root architecture to

optimise P uptake, the roots transform their environments chemically to make P more available. This is done by exuding organic anions, protons or molecules targeting organic, mineral P or the molecules binding P (Hinsinger 2001).

In general, organic acids or anions are more effective P solubisers than $H^+$ alone (Staunton & Leprince 1996). Organic anions compete with P on ion exchangers, and more negative anions will be better at solubising P (Guppy *et al.* 2005). Still, when added at realistic soil concentrations (10 – 100 μM), the lifetime of such compounds are only a 2 – 3 hours (Jones 1998), leading Brown *et al.* (2012a) to question whether anionic exudates are effective in providing sufficient P.

Phosphatases are a broad group of enzymes exuded by plant roots and micro-organisms to mineralise organic P (Eivazi & Tabatabai 1977). Some evidence points to microbial phosphatase being the most efficient (Tarafdar *et al.* 2001), and wheat has been shown to be ineffective in mineralising phytate, while being able to utilise some forms of organic P as efficiently as inorganic fertiliser (Richardson *et al.* 2000). Phosphatases can thus be determining how much P a plant will be able to utilise, especially in highly organic or P starved soils (Richardson & Simpson 2011).

## 1.6 The effect of P fertiliser

Most of the P utilised by plants is residual P from earlier seasons (McLaughlin *et al.* 1988c), as the greater part of freshly applied P ends up in pools not directly plant available (Johnston 2001). The effect of P fertiliser is thus not an entirely straightforward matter, and it has been assessed in a number of ways (Syers *et al.* 2008).

Johnston *et al.* (2014) discusses three methods to assess the efficiency of applied fertiliser:

1) The direct method use radiolabeled P fertiliser. This is a costly method, and as the radioisotopes of P are short-lived ($^{32}P$, the cheapest isotope, has a half life of 14.3 days, $^{33}P$ of 25 days), the method is not usable for more than one season, and even the single season may have to be arrested (as in for example McLaughlin *et al.* (1988a)).

2) The difference method takes the difference in yield or total P content between a fertilised field and an unfertilised one. This gives the response of a single crop to an application of fertiliser, a response which will be greater if there is less residual P in the soil.

3) The balance method divides P removed from the field by P applied to the field. If the ratio is above 1, the soil is enriched in P, and P is mined from the soil if it is below 1.

These methods will give different efficiencies, because they are asking different questions. The balance method answers if the soil is depleted or enriched in P, the difference method if further enrichment will cause an increase in yield, and radioisotopic methods are able to discern where the plant takes it's P from.

Traditionally, single extraction methods has been used to find how much plant available P is present in soil (Mason *et al.* 2010). Olsen *et al*'s (1954) method is widely used internationally in both agricultural and research work (Carter & Gregorich 2007), and it is the standard method for agricultural P tests in the UK (DEFRA 2010). In Scandinavia the ammonium-lactate method is used to determine the levels of multiple nutrients in agricultural soils, among them P (Egner *et al.* 1960). Recently, Diffusive gradients in thin Films (DGT) methods have been proposed as a better alternative, as the technique mimics the diffusive parts of plant uptake (Kruse *et al.* 2015).

Critical P is the level of P in a soil where a further enrichment would not lead to increased yield, but a depletion would lead to decreased yield (Mallarino & Blackmer 1992). Thus, at critical P or above the "difference method" (method 2) would give a P efficiency of 0 %. At critical P or above it would therefore be advisable to rely solely on the balance method to calculate fertiliser need. Critical P level is dependent on both the crop grown, farming practises and soil and climatic conditions (Johnston *et al.*

2013). In the UK, critical P is typically 16 – 25 mg/l Olsen P for grain (DEFRA 2010). The values are experimentally determined, and the variability of practises and years makes it inadvisable to advise a more precise number (Johnston *et al.* 2013).

## 1.7 Seasonal variability of P availability

P Plant availability is known to change during the year, as a result of both climate and biologically induced processes. Even so, there is a limited amount of published literature on how P availability change: Most of the literature has been on grasslands, and the results are somewhat contradictionary (Styles & Coxon 2007). This is attributed to the inherit variability of the fields environment as well as standard P tests drowning out seasonal differences (Pote *et al.* 1999). For farmers, it would be a boon with methods independent of season, as they then can sample when there is time rather than at a specific and set time. For scientific work, it could be detrimental as a more precise view of P status can be needed.

Studies examining grassland have found $NaCO_3$-extractable P to be higher in summer than winter (Tate *et al.* 1991), plant available P to be higher in winter than summer (Styles & Coxon 2007, Sharpley 1985), and lower in spring than autumn (Blakemore 1966). Pote *et al.* (1999) found water extractable P to be higher in autumn than spring, but not samples extracted by the standard P test Mehlich III. In cropland (Garbouchev 1966) and forests (Haines & Cleveland 1981) P has also been found to be lower in spring than autumn, while Lamb and Rehm (2002) found P in a maise-soybean rotation to either be higher in spring than autumn or there to be no consistent differences.

This hints at the seasonal effect on P availability depends both on the plants grown and of climatic and/or soil factors.

## 1.8 Hypotheses

This study will test the following in wheat (*Triticum aestivum L. cv Crusoe)*:

1. Plant induced mobilisation of P will increase it's availability in the early part of the growing season.
2. Plant uptake of P will deplete the soil of P.
3. After plant uptake of P is over, it will increase towards a winter maximum.
4. DGT techniques will be a better agricultural soil test than Olsen P

## 1.9 Experimental set up

To test these hypotheses, a field trial was carried out on a long-term experimental field at Rothamsted, England. The experiment grew winter wheat of the cultivar Crusoe. Soils and plant matter were harvested ten times during the growing season. The plants were weighed and analysed for quantifying plant uptake, while plant available soil P was measured by the Olsen *et al.* (1954) method and with Diffusive Gradients in Thin Films (DGT) (DGT research 2015), with exposure times of 6, 24 and 72 hours.

# 2 Materials and methods

## 2.1 Site description

The field work was carried out at the "Exhaustion Land" long term experiment at Rothamsted, England (51.82 N, 0.37 W). Normal yearly precipitation is 704 mm (1971 - 2000), average temperature 9.6 ºC (Rothamsted Research 2016).

The soil is a naturally well drained silty clay loam containing 20 % clay, 52 % silt and 28 % sand. The soil is of an acidic origin, but has been chalked since time immemorial. The FAO World Reference Base for Soil Resources classify is as a Chromic Luvisol (Young *et al.* 2005).

The experiments at the "Exhaustion Land" was established in 1856 by Lawes and Gilbert, and different fertilizer treatments were tested until 1901, when an experiment for studying the long term effects of earlier fertilization was initiated (the titular exhaustion). Therefore, no nutrients were added until 1939. From 1940 N was applied (Johnston & Poulton 1977).

Starting 1986, the "Exhaustion Land" was split and five plots were divided into four subplots each and dressings of 0, 100, 200 or 300 kg $P_2O_5 \cdot ha^{-1} \cdot year^{-1}$ (giving a dressing of 0, 44, 87 and 131 kg P respectively) were added until 1992. From 1992 until 1999, no P was added, but afterwards, 20 kg $P \cdot ha^{-1}$ was applied yearly to the plots receiving fertilizer 1986 – 1992. The plots have received basal N and K to avoid those elements being limiting factors (Johnston *et al.* 2014). The dressing of 131 kg $P \cdot year^{-1} \cdot ha^{-1}$ is named 1, 87 kg $P \cdot year^{-1} \cdot ha^{-1}$ is 2, 44 kg $P \cdot year^{-1} \cdot ha^{-1}$ is 3 and 0 kg $P \cdot year^{-1} \cdot ha^{-1}$ is 4.

## 2.2 Soil and plant sampling

12 plots of the experiment, three of each P level, were sampled ten times each during the 2015 growing season from March to August, fortnightly late April to mid July (table 1).

Each sampling harvested a 0.25 m² square per plot.

*Table 1: Sampling dates*

| Harvest | Sampling date | Days since last sampling |
|---|---|---|
| 1 | 4th of March | - |
| 2 | 27th of March | 23 |
| 3 | 23rd of April | 27 |
| 4 | 6th of May | 13 |
| 5 | 21st of May | 15 |
| 6 | 4th of June | 14 |
| 7 | 16th of June | 12 |
| 8 | 1st of July | 15 |
| 9 | 13th of July | 12 |
| 10 | 5th of August | 23 |

Areas on the very edge of the field were not chosen, to avoid edge effects. The plants were cut roughly 1 – 2 cm above soil surface, and stored in a plastic bag.

Growth stage was determined in situ according to Zadoks' scale (Zadoks *et al.* 1974) , although this was not done consistently. If a plant sample was required to determine growth, it was on taken on the same plot but outside of the 0.25 m² area harvested for samples.

After plants were harvested, 5 soil cores 0 – 23 cm from different parts of the harvested area were collected with a soil auger.

After collection, both soil and plant samples were stored in a cold room awaiting processing.

During harvests 1 – 4, plants were rinsed in tap water to remove soil, then soaked in Reverse Osmosis (RO) water. After soaking, the plants were rinsed in RO water, and placed in premarked and weighed paper bags and oven dried to constant weight at 80 ºC to measure dry weight. From harvest 5 onwards, the soaking step was skipped, and from harvest 6 onwards the plants were split into ears, stems and leaves, which were oven dried at 80 ºC in separate premarked and weighed paper bags or metal trays. After drying, the bag or tray were reweighed, and thus plant weight determined. Plants were then stored in paper bags, before being milled and a representative subsample transferred into a sterilin vial for digestion.

Roughly 10 g of soil matter were weighed into

small metal foil trays, weighed and oven dried overnight at 105 ºC. The next day, soil dry weight was found, and moisture content calculated (equation 1):

$$Moisture\ content = \frac{W_{wet} - W_{dry}}{W_{wet}} \qquad (1)$$

where W is weight.

## 2.3 Soil pH measurements

Early and late April, soil pH was measured in the 10 g samples dried for soil moisture content using a Jenway pH meter 3310 with a VWR flat tip, double junction pH electrode, buffered at pH 4 and 7. The soil was transferred to a vial, and 25 ml of RO water was added. The vial was shaken, left to rest for half an hour, and shaken again. It was then shaken once more, before being measured. The pH meter was read when pH had stabilized, or after 30 seconds, whichever was shortest. The electrode was rinsed in 18.0 MΩ·cm$^{-1}$ water between each measurement.

Every 10 samples, the calibration of the pH electrode was controlled against the pH 7 buffer. A drift of more than ± 0.15 would have resulted in a recalibration. A soil standard was measured as the last measurement late April.

## 2.4 Plant digestion

Roughly 0.250 g of milled plant material were weighed into a test tube. Every 10th sample was repeated, and for each batch of 54 tubes (21 for the last batch) there were two blank samples and two samples of a peach leaf standard (appendix 1). Two times, at the start and towards the end of weighing, there were also two samples of an internal grass reference material used at Rothamsted (appendix 2).

The plants were then digested as per the nitric-perchloric method in Zhao *et al.* (1994) : To each test tube, 5 ml of 85 % $HNO_3$ s.g. 1.42 – 15 % 60 % $HClO_4$ was added. The tubes were swirled by hand and left to predigest for at least 2 hours. They were swirled again at least once during these two hours, to ensure all plant matter was in contact with the acid.

*Table 2: Temperature regime for plant digestion*

| Time (minutes) | Temperature (ºC) |
|---|---|
| 185 | 25 |
| 220 | 60 |
| 80 | 100 |
| 70 | 120 |
| 145 | 175 |
| 300 | 50 |

Afterwards, the test tubes were placed in a cool Carbolite heating block, which was then heated as according to the heating pattern in table 2.

This pattern was carried out overnight. If the test tubes contained < 0.5 ml liquid the next day, the process proceeded. If not, they were reheated until dry enough.

Next, 5 ml of 25 % $HNO_3$ was added to each test tube, the tubes were then whirlmixed and reheated at 80 ºC for 60 minutes.

18.0 MΩ·cm$^{-1}$ water was then added until there were roughly 18 ml liquid in each tube. The tubes were then rewarmed to 80 ºC for further 30 minutes before being taken off the Carbolite block and left to cool. Cool samples were thinned to 25 ml, and transferred to a labelled sterilin vial and submitted to ICP analysis on a Perkin Elmer Optima ICP-OES.

## 2.5 Total nitrogen analysis

For analysing total nitrogen, 0.150 ± 0.001 g of plant dry matter was weighed into weighing trays, and analysed on a LECO TRUMAC instrument.

## 2.6 Olsen P

After storage in cold room from harvest and until early august, air dried soil was sieved through a 2 mm sieve, following Olsen *et al.* (1954) method: 5 g of soil was extracted by shaking with 100 ml 0.5 M $CaCO_3$ for 30 minutes (giving a ratio of 1/20 soil/liquid), and then filtered. Afterwards, a molybdate reagent was added to make the P colourimetrically detectable, and concentration was measured by colourimetric constant flow analysis on a Skalar San Plus Colourimetric continuous flow

Analyser.

## 2.7 Diffusive Gradients in Thin Films (DGT) P

Soil stored in a cold room since harvest was placed in a plastic tray, each plot each harvest in a separate tray, and wetted with 18.0 MΩ·cm⁻¹ water. Three DGTs were immediately placed in each plastic tray and exposed to soil, and the trays were covered in plastic foil to avoid loss of moisture.

The DGTs were composed of three layers. Facing the soil solution was a membrane, inside of the membrane a inert hydrogel and an iron oxide ion exchanger inside of the gel. The membrane will exclude large species from diffusing into the DGT, and the hydrogel layer creates a concentration gradient between the soil solution and the iron oxide, which functions as an infinite sink for P until saturation. Holding these three layers in place were a plastic holster.

Exposure of the DGTs was ended by removing them from the soil and rinsing them with 18.0 MΩ·cm⁻¹ water. The first DGT from each tray was removed after 6 hours (5:59 ± 0:05:3), the second after 24 hours (23:59 ± 0:02:4) and the third after 72 hours (71:59 ± 0:03:1). 5 DGTs were left unexposed to be used as blanks.

The DGTs were then dismantled using a screwdriver. The ion exchange gel was then removed and put in a test tube by tweezers which had been soaked in a dilute $HNO_3$ solution for at least 4 hours.

10 ml 1.6 M $HNO_3$ was added by pipette to each tube, and the tubes were submitted for ICP-MS analysis on a Perkin Elmer NexION 300X ICP-MS..

P concentration ($P_{DGT}$) in soil as measured by the DGT for 24 hours was calculated as according to equation 2 (Zhang & Davison 1995):

$$P_{DGT} = \frac{P_{ICP} \cdot (V_{gel} + V_{acid})}{f_e \cdot \Delta g} \cdot (D \cdot A \cdot t)^{-1} \quad (2)$$

where $P_{dgt}$ (mg/l) is P concentration in the DGT. $P_{ICP}$ (µg/l) is P concentration as measured on ICP MS. $V_{gel}$ (0.16 ml)and $V_{acid}$ (10 ml) is volume of gel and nitric acid respectively. $F_e$ (80 %) is the apparent extraction efficiency as recommended from the DGT manual (DGT research 2015). Δg (0.1 cm) is the thickness of the membrane and gel of the DGT, i.e. the distance between soil solution and ion exchanger. D ($5.57 \cdot 10^{-6}$ cm²/s) is the diffusivity of P, and A (3.14 cm²) is the exposed area. t is time in seconds.

In addition, the mass of P adsorbed in each gel ($P_{gel}$) was calculated (equation 3) to allow comparison between different exposure times. This was done for all three exposure times.

$$P_{gel} = \frac{P_{ICP} \cdot (V_{gel} + V_{acid})}{f_e} \quad (3)$$

with the same units as above.

## 2.8 Statistics

R (R Core Team 2016) has been the program of choice for the statistical analyses of this study, with the «Rkward» interface (Rödiger *et al.* 2012). On the DGT data, Dean and Dixon (1951) test was used to remove outliers. One outlier was found, in the 24 hours exposure treatment: Harvest 5, treatment 3.

Results are considered significant if $P < 0.05$, and are given with ± 1 standard deviation.

## 2.9 Citation software

Jabref was used as citation management software (JabRef Development Team 2016).

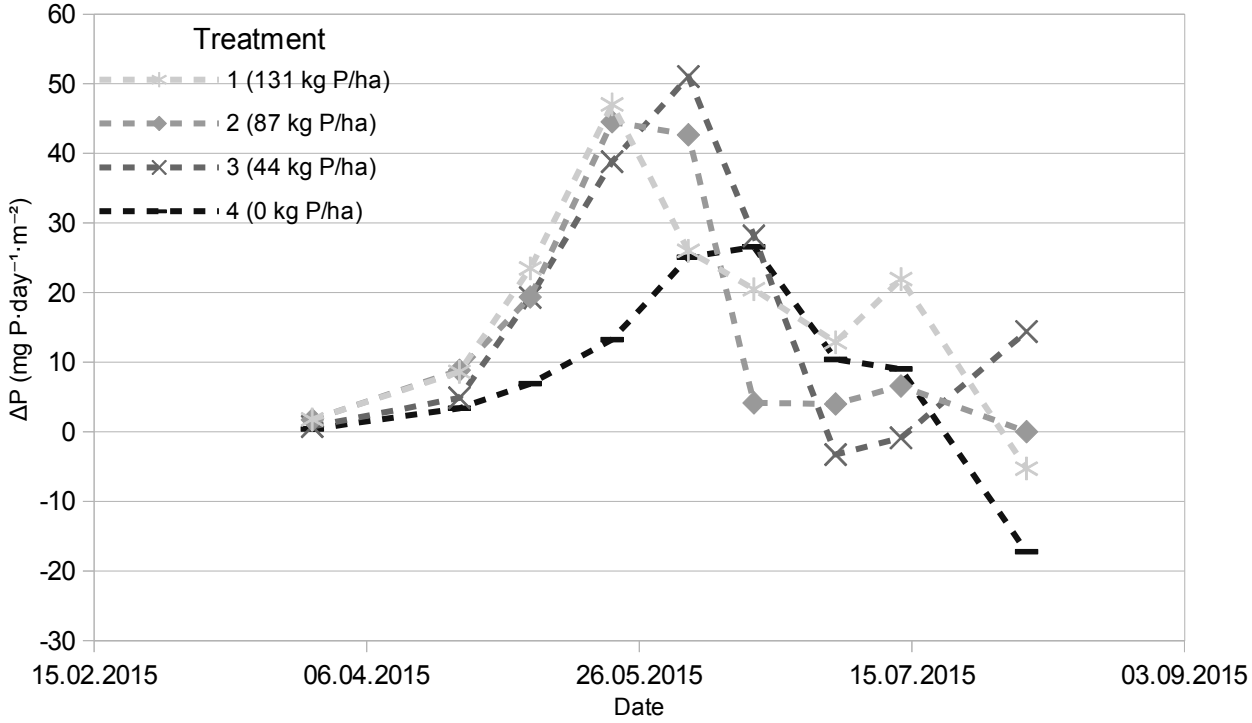



*Figure 4: Average daily changes in above-ground P content of wheat between harvests on the Exhaustion land 2015. Treatment 1 got 131 kg P·ha⁻¹·yr⁻¹ from 1986 to 1992. Treatment 2 got 87, treatment 3 got 44 P·ha⁻¹·yr⁻¹. Treatment 4 has not been fertilised with P since 1901. Between 1993 and 2000, no P was applied, but from 2000, 1.5 kg P·ha⁻¹·yr⁻¹ has been applied to treatments 1, 2 and 3. All treatments received basal fertilisation with other nutrients. Points are average of 3 measurements. Standard derivations not shown due to readability, but are given in table 3.*

# 3 Results

## 3.1 Yield data

P uptake seems to follow bell curves (figure 4), peaking in late May to early June. As this is above-ground P content, the uptake might in reality peak somewhat earlier and then redistribute from root to shoot (Römer & Schilling 1986). The uptake curve seems to be both retarded and widened with less P in the soil, although variability is great. The P uptake of treatment 1 and 2 are peaking from the 6th of May until 4th of June (table 3). Treatment 3 starts peaking at the same time, but continues for another two weeks until the 16th of June. Treatment 4 starts peaking after the 21st of May, but has a lower peak lasting until 16th of June.

*Table 3: Average daily P uptakes (mg·day⁻¹·m⁻²). Each value is the average of 3 measurements ± 1 standard deviation.*

| Period | Daily P uptake (mg·day⁻¹·m⁻²) Treatment | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 4.3 – 27.3 | 1.8 ± 0.62 | 1.7 ± 1.1 | 0.7 ± 0.30 | 0.3 ± 0.23 |
| – 23.4 | 8 ± 2.0 | 8.8 ± 2.1 | 4.9 ± 0,57 | 3.4 ± 0.92 |
| – 6.5 | 23 ± 7.0 | 19 ± 2.3 | 20 ± 10 | 7 ± 2.2 |
| – 21.5 | 47 ± 7.6 | 40 ± 12 | 40 ± 14 | 13 ± 6.4 |
| – 4.6 | 30 ± 30 | 42 ± 6 | 50 ± 19 | 30 ± 15 |
| – 16.6 | 20 ± 18 | 0 ± 15 | 30 ± 25 | 30 ± 36 |
| – 1.7 | 10 ± 23 | 0 ± 21 | 0 ± 14 | 10 ± 31 |
| – 13.7 | 20 ± 16 | 10 ± 30 | 0 ± 36 | 10 ± 11 |
| – 5.8 | -5 ± 11 | 0 ± 15 | 10 ± 34 | -17 ± 7.1 |

Plants grown on the higher P treatments seem to have the main part of their P uptake slightly earlier than plants with less an abundant P supply. A somewhat retarded peaking as a response to lower P treatments is known from for example *Brassica campestris* (Jiao *et al.* 2015), while Leigh and Johnston (1986) did not find P deficient barley (*Hordeum vulgare L*) to peak at all in 1981, and deficient and non-deficient barley to peak at the same

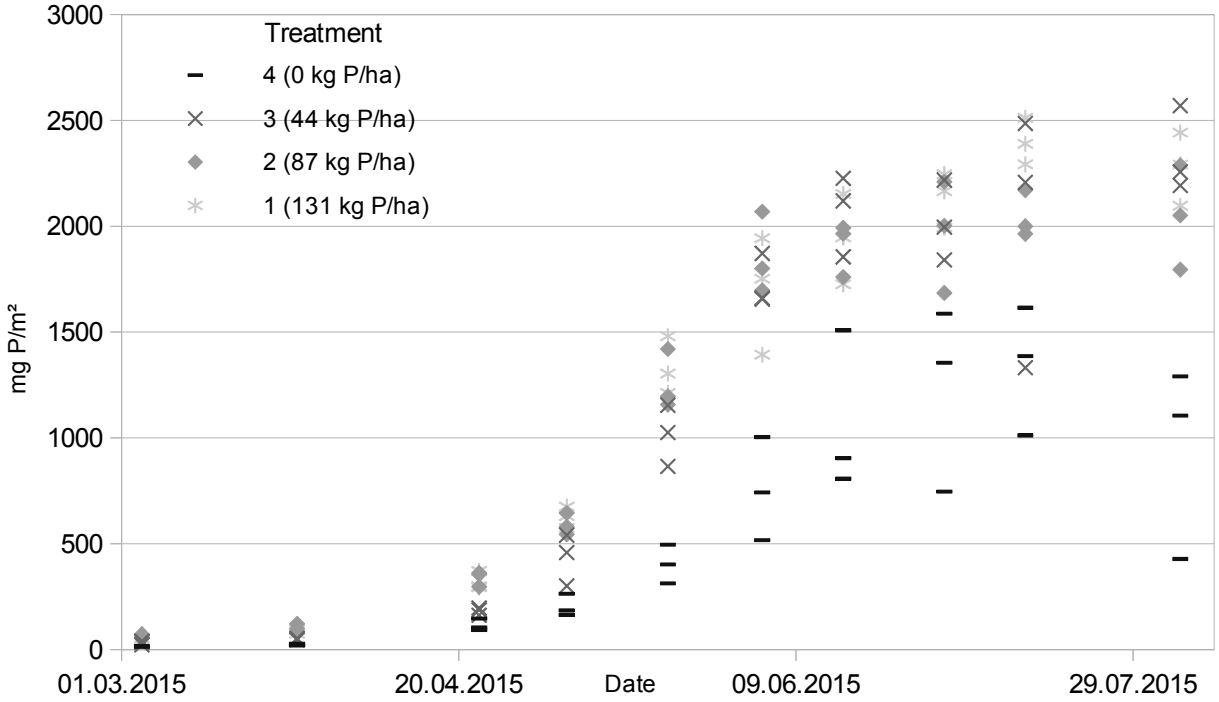



*Figure 5: Above-ground plant P content of wheat on the Exhaustion Land 2015. Treatment 1 got 131 kg P·ha⁻¹·yr⁻¹ from 1986 to 1992. Treatment 2 got 87, treatment 3 got 44 P·ha⁻¹·yr⁻¹. Treatment 4 has not been fertilised with P since 1901. Between 1993 and 2000, no P was applied, but from 2000, 1.5 kg P·ha⁻¹·yr⁻¹ has been applied to treatments 1, 2 and 3. All treatments recieved basal fertilisation with other nutrients.*

time in 1980. Peak P uptake is likely mainly dependent on other factors, but abundant P supplies seems to encourage it to happen slightly earlier in the season.

Total above-ground plant P (figure 5) reaches a plateau after the peak uptake is over and is relatively stable from the 16th of June and onwards. Total plant dry weights (figure 6) seem to have plateaued by 13th of July. Thus, plant P content plateaued roughly a month before plant dry weights did. Afterwards, both plant P and dry matter were redistributed from leaves and stems to the ears, in addition to a P dilution taking place in all three above-ground plant parts (data not shown).

All three fertilised regimes had similar final yields, which was to be expected, as Olsen P was above the established critical level for the "Exhaustion Land", which is 12 ± 1.9 based on the seasons 1986 – 2008 (Poulton *et al.* 2013).

Overall, at the final harvest treatment 3 had the highest ear dry weights with an average of 2200 ± 200 g dry matter/m² and all three plots yielding above 2000 g/m². Treatment 1 and 2 had similar albeit slightly lower yields of 2100 ± 170 (1) and 1900 ± 230 (2) g dry matter/m². Although the whole ear weights more than than grain alone, these high yields show P was not a limiting factor, and there were few other factors limiting growth either.

## 3.2 Soil pH

Soil pH ranged from 6.3 to 7.3, which is within recommended pH ranges for wheat, and thus not expected to be a hinder for plant growth and development (YARA 2014).

## 3.3 Total nitrogen analysis

Nitrogen levels were as normal for plants, roughly 1.5 % at harvest (Aasen 1997), and are thus used solely as a check of the other plant data. No mistakes was detected by peculiar nitrogen contents.

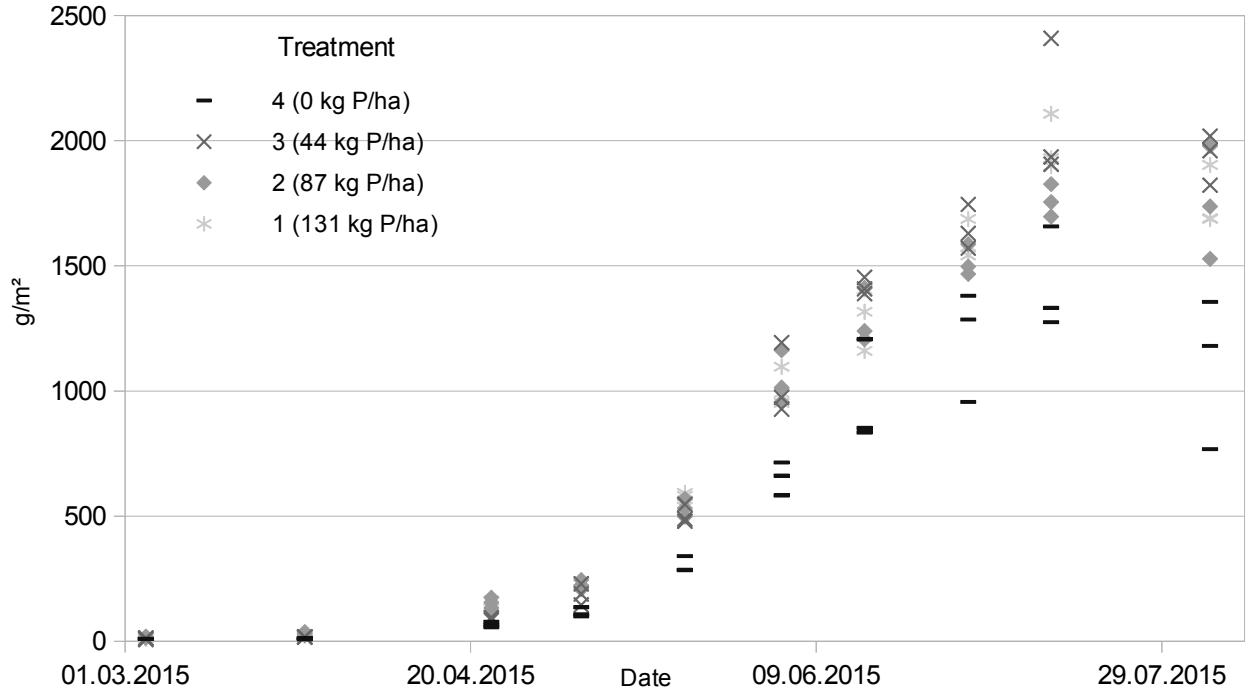



*Figure 6: Dry weights of above-ground wheat plants on the Exhaustion Land 2015. Treatment 1 got 131 kg P·ha⁻¹·yr⁻¹ from 1986 to 1992. Treatment 2 got 87, treatment 3 got 44 P·ha⁻¹·yr⁻¹. Treatment 4 has not been fertilised with P since 1901. Between 1993 and 2000, no P was applied, but from 2000, 1.5 kg P·ha⁻¹·yr⁻¹ has been applied to treatments 1, 2 and 3. All treatments recieved basal fertilisation with other nutrients.*

## 3.4 Olsen P results

Different fertilization regimes have caused different Olsen P levels, but there were no significant differences in Olsen P during the growing season (figure 7). By TukeyHSD-tests, there were not found any difference between treatments 1 and 2, while all other treatments differed by any reasonable measure. In the two higher treatments, a small dip is visible during May, with a rise until mid July afterwards. Despite neither dip nor rise being significant, they are interesting as the they correspond well to a similar rise in $P_{gel}$.

The Olsen P contents range from 6.1 ± 0.61 to 34 ± 4.6, with treatment 4 being below recommended Olsen P concentrations for grain while treatment 3 is within those ranges (16 – 25 mg/l (DEFRA 2010)) and treatments 1 and 2 are well above. On the "Exhaustion Land" and similar soils growing wheat, Olsen P is thus likely to be independent of season for a wide range of different soil P concentrations.

*Table 4: Olsen P, Plant P and their comparative sizes at the 5th of August 2015*

| Treatment | Olsen P (mg/m²) | Plant P (mg/m²) | Plant P/Olsen P (%) |
|---|---|---|---|
| 1 | 9090 ± 30 | 2300 ± 170 | 25 ± 1,9 |
| 2 | 8200 ± 660 | 2000 ± 250 | 25 ± 4,7 |
| 3 | 5500 ± 200 | 2300 ± 200 | 42 ± 1,1 |
| 4 | 1800 ± 450 | 900 ± 450 | 50 ± 22 |

The soil down to a depth of 23 cm weights 299 kg/m² (Johnston & Poulton 1977). Comparing the Olsen P per m² down to 23 cm depth at a given harvest, with the P in the plants (figure 8), gives the fraction of Olsen P taken up in a single season. The comparative sizes of Olsen P and plant P at the final harvest is shown in table 4.

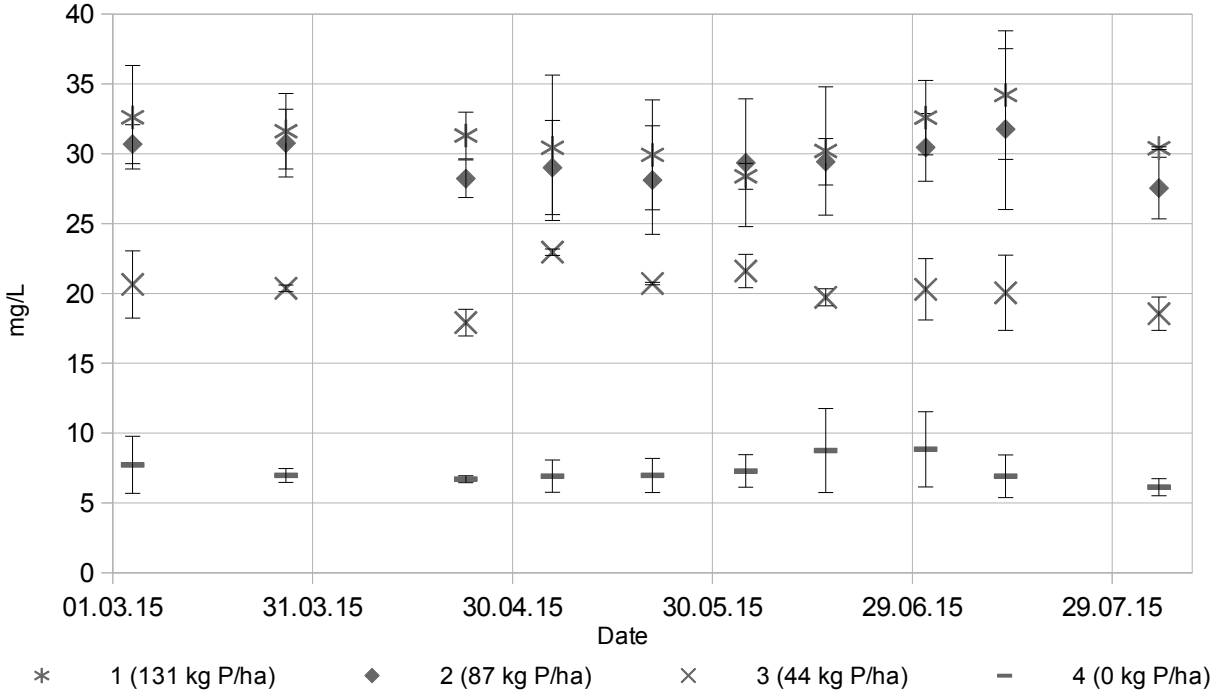



*Figure 7: Olsen P on the Exhaustion Land 2015. Treatments are fertiliser regimes 1986 – 1992. Between 1993 and 2000, no P was applied, but from 2000, 1.5 kg P·ha⁻¹·yr⁻¹ has been applied to treatments 1, 2 and 3. Points are averages of 3 ± 1 SD.*

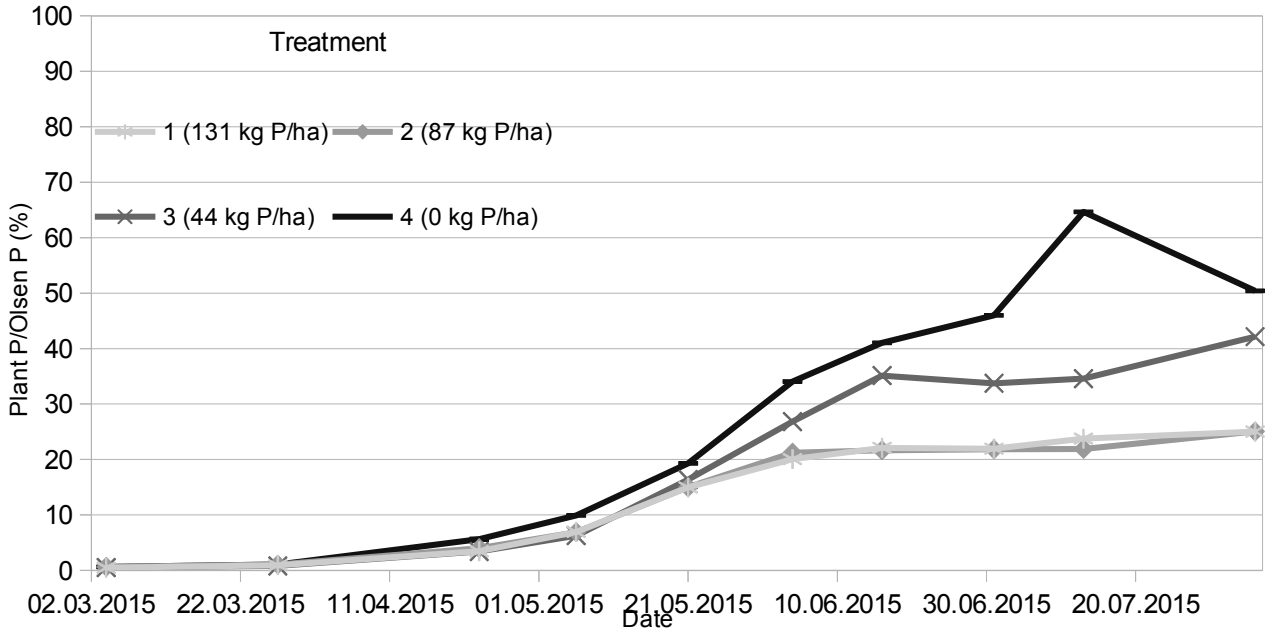
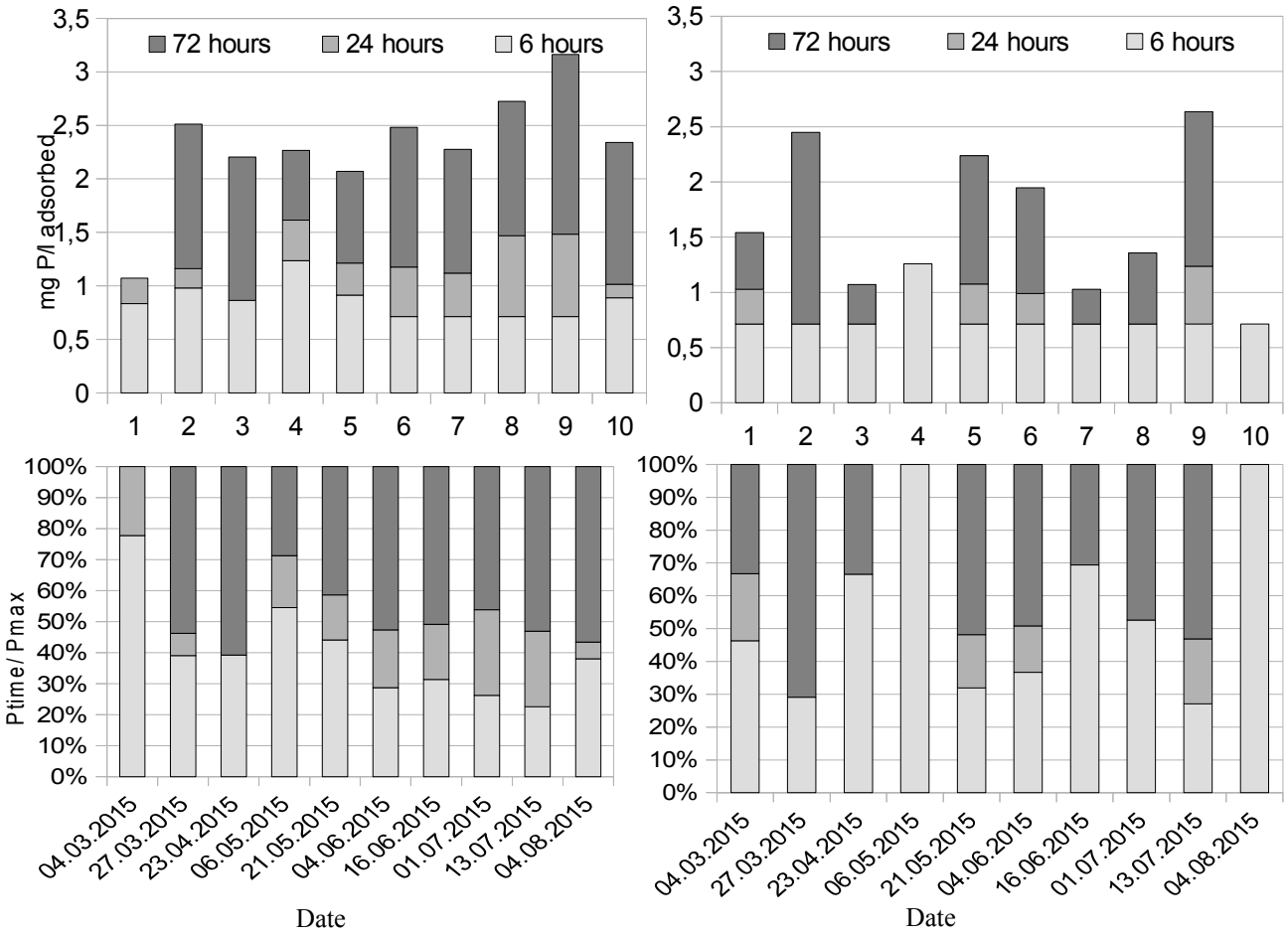



*Figure 8: Above ground plant P/ha as a percentage of Olsen P/ha on a given harvest on the Exhaustion Land 2015. Treatment 1 got 131 kg P·ha⁻¹·yr⁻¹ from 1986 to 1992. Treatment 2 got 87, treatment 3 got 44 P·ha⁻¹·yr⁻¹. Treatment 4 has not been fertilised with P since 1901. Between 1993 and 2000, no P was applied, but from 2000, 1.5 kg P·ha⁻¹·yr⁻¹ has been applied to treatments 1, 2 and 3. All treatments recieved basal fertilisation with other nutrients. Points are averages of 3 Plant P (whole plant summed together) and 3 Olsen P measurements.*
*Soil weight to a depth of 23 cm is 299 kg/m² (Johnston & Poulton 1977). Olsen P does not change significantly during the growing season, so the development is due to plant uptake.*




*9a, treatments 1 and 2 together* *9b, treatment 3*

*Figure 9: Development of DGT-P on the Exhaustion Land 2015. 9a is treatments 1 and 2, 9b is treatment 3 and 9c is treatment 4. Harvests are numbered in sequence with harvest 1 being the harvest 4h of March and onwards untill harvest 10 the 5th of August. The upper collumns are concentrations in the DGTs exposed for 6,24 and 72, while the bottom is the internal fractionation of those three fractions. When the concentration after a shorter exposure time is lagrer than the concentration after a longer exposure, the higher concentration is given*

During a single growing season, wheat was able to utilise 25 – 50 % of what is regarded as plant available P, depending on nutrient status of the soil.

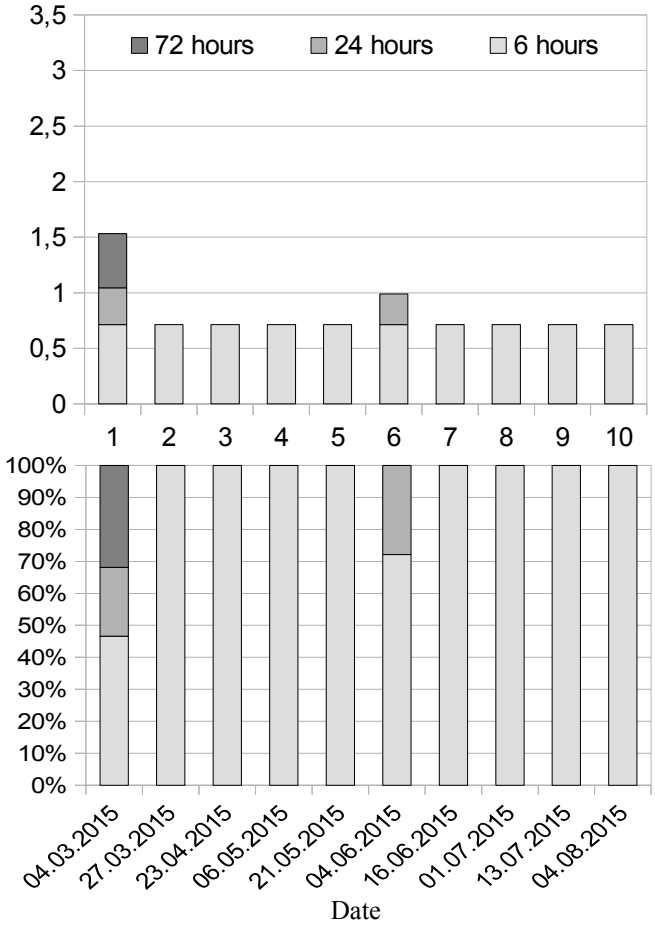
The amount P taken away in harvest will decrease when the plant available P decreases below it's critical level, and yield levels begin to suffer. With decreasing plant available P, and thus decreasing Olsen P, plant P decreases as well, though not as sharply. This leads to a larger portion of Olsen P being utilised and carried away by each yield at lower P levels.

## 3.5 P concentrations of the DGTs

To calculate a limit of quantification, 3 standards deviations was added to the average of the 5 blank DGTs, giving a limit of detection of 1.4 µg/gel for $P_{gel}$ or 94 µg/l for $P_{DGT}$ exposed for 24 hours.

Any value below this was set to 0.71 µg/gel or 47 µg/l, being half the limit of detection. 91 % of the DGTs exposed for 6 hours were below the limit of detection, 68 % of those exposed for 24 hours and 41 % of those exposed for 72 hours. No P was measured by the DGTs exposed for 6 hours in the unfertilised treatment (4), and this fraction was only detectable in treatment 3 on the 6th of May.

As the DGTs were measured on a ICP-MS rather colourimetrically, the P contents will include both organic and inorganic species (Menezes-Blackburn *et al.* 2016). This may cause some small organic




*9c, treatment 4*

molecules to be measured, but due to the rapid turn-over time of organic P, these species would likely mineralise and thus become available rapidly enough to be taken up while the roots explore the soil volume they are found in. As organic P will be less negative than mineral orthophosphate, it will have less affinity for the iron oxide gel and there will be less incentive for the molecule to diffuse into the DGT.

At some harvests, the DGTs were exposed for significantly longer or shorter times than at others, which could influence the $P_{gel}$ results, as they are not time corrected. 6 hour DGTs of 23$^{rd}$ of April were consistently exposed too long, but are still below the limit of detection. The other differences are a matter of minutes, making them negligible.

The DGTs iron oxide gels had volumes of 0.16 ml, and the largest amount of P found in any gel (sans the outlier) was 5 µg, well below the 6.7 µg/gel needed to saturate the 0.12 ml gels of Zhang *et al.* (1998). Thus, saturation of the iron oxide gel is unlikely to have happened, and the values calculated by equations 2 and 3 are likely to be valid.

## 3.6 DGT-P concentrations

As 68 % of DGTs exposed for 24 hours were below the limit of detection, $P_{DGT}$ is unsuitable for quantifying plant available P in the present study. When it is measurable, it gives results which are quite stable through the season. Speirs *et al.* (2013) do not report struggling against the limit of detection from measuring $P_{DGT}$ in 164 Australian soils of varying P status, nor do Six *et al.* (2012) from two Kenyan soils. The µg/l levels of $P_{DGT}$ might make the technique require more experienced operators than techniques operating on the mg/l concentrations.

## 3.7 P Development in DGT-gels

The P content of the gels were significant different between treatments at all three exposure times, but, by TukeyHSD-tests, no significant differences were found between treatments 1 and 2.

Figure 9 shows a peak in the 6 hour $P_{gel}$ at the harvest 6th of May, and TukeyHSD-tests showed this harvest to be higher than the harvests in June and July, as well as late April.

From the harvest 21st of May and onwards to the 13th of July, there seems to be an increasing trend for treatments 1 and 2, simultaneously with the increasing trend for Olsen P. Although the trends are weak and differences are not detectable by TukeyHSD, the apparent trend was detected by two different methods.

For the first harvest (4$^{th}$ of March) 72 hour $P_{gel}$ is undetectable, which is significantly lower than the other harvests This difference shall be noted, but not explained, as the experiment was not designed to explain such a minimum.

# 4 Discussion

## 4.1 P pools measured by DGT

The DGT technique will measure P available by diffusion and P desorbed by depletion of the soil solution (Christel *et al.* 2016). These will be plant available fractions, and experiments by Mason *et al.* (2013) and Six *et al.* (2012) have shown DGT to measure plant available fractions almost exclusively. This was done by adding radioactive P tracers, and comparing the relative abundance of the different P isotopes in DGTs and plant matter. The isotopic ratio of plant and DGT was found to be more similar than for plant and other soil tests. Therefore, it has recently been introduced as a standard agricultural soil P test in Australia (Kruse *et al.* 2015).

Still, as DGTs are passive samplers, they may be unable to measure P which can be mobilised by exudates or other rhizospherical transformations (Degryse *et al.* 2009). Once mobilised, the DGT will be able to measure it.

With a longer exposure time, the DGT will measure more strongly sorbed, but still desorbable, P. This will be **pool 1** and **2** of the four compartment model shown in figure 3 (Johnston *et al.* 2014). The 6 hour $P_{gel}$ is suggested to measure mostly **pool 1**, while the 24 and 72 hour $P_{gel}$ will measure **pool 2** as well. 72 hour $P_{gel}$ will measure a larger part of **pool 2**, as the longer exposure time allows a stronger depletion of the soil solution, inciting P with higher binding energies to desorb.

The internal fractionation between exposure times seems to change more during the growing season than total $P_{gel}$ concentrations. 6 hour $P_{gel}$ seems to be a larger fraction of total $P_{gel}$ in the period preceding maximum plant uptake than at other times, especially for treatments 1 and 2. Afterwards, the 6 hour fraction becomes a less dominant fraction until august in all fertilised treatments, though more in 1 and 2 than 3.

In treatments 1 and 2, the 24 hour fraction occupies roughly 20 % of total $P_{gel}$ from 6$^{th}$ of May to 13$^{th}$ of July. This constancy, combined with the depression of 6 hour $P_{gel}$, allows the 72 hour $P_{gel}$ to grow more dominant from May to harvest 9 the 13$^{th}$ July.

## 4.2 Changes in 6 hour $P_{gel}$

The most marked change is the peak in 6 hour $P_{gel}$ for the fertilised treatments at the 6th of May, visible in all fertilised treatments (figure 9a and b). This peak can be the result of biological mobilisation, as neither water content nor temperature had changed much since the previous harvest.

Whether this peak is caused by the wheat plants or by other organisms in the soil is not known, and it can be argued to be of less importance: All three treatments are within or above recommended Olsen P status for grains (DEFRA 2010), and plant are less dependent on symbiosis with other soil organisms with higher P status (Gyaneshwar *et al.* 2002). Soil micro-biota might compete with plants for P in the short term, but their turnover times in soils are rapid enough that the P may be made reavailable when the micro-biota dies (McLaughlin *et al.* 1988b).

Exudated low molecular weight organic acids will adsorb quickly to soil surfaces (Jones & Brassington 1998), increasing the negative charge of the surfaces. This would weaken the sorption of P to the same surface (Bowden *et al.* 1980), even if the total anion concentration is too low to saturate the anion exchanging surface (Guppy *et al.* 2005). The plant's P uptake would deplete the soil of P, causing a further incentive for P to desorb from the solid phase into the rhizosphere (Morel & Hinsinger 1999), where it will be taken up, causing a zone of depletion around the roots (Lewis & Quirk 1967).

An increased exudation coming prior (either in time, space or both) to increased uptake could thus explain both the peaking of 6 hour $P_{gel}$ and it's depletion afterwards.

## 4.3 Changes in 72 hour $P_{gel}$

Concurrently with the depression of the 6 hour $P_{gel}$, from the harvest 21$^{st}$ of May and until 13$^{th}$ of July, there seems to be increasing trends in both Olsen P

and 72 hour $P_{gel}$. Although they are to small to be significant by either method alone, two different methods giving similar trends makes it noteworthy.

Hinsinger and Gilkes (1996) reported a significant build up of NaOH-P bordering the P depleted part of the rhizosphere, likely caused by plant mobilised P ions diffusing both towards and away from the root (Hinsinger 2001, Kirk 1999, Nye 1983). Even so, this two way diffusion is unlikely to be the cause of the increasing trends in the latter half of the growing season, as plant mobilised P would likely be detected by 6 hour $P_{gel}$ as well.

As plants drain the soil for P, less available fractions react and become more labile (Bergström *et al.* 2015). This labilisation is predicted by the four compartment model (figure 3, (Johnston *et al.* 2014) and may be a process affected by a certain amount of inertia. Thus, while the depletion of immediately available P causes a resupply from the less available P, there is a significant lag time, both in it starting and it's ending. P**ool 2** resupplies **pool 1** and is in turn resupplied by **pool 3** and **4**. When **pool 1** no longer gets depleted, the resupply from **pool 2** ends, but the resupply of **pool 2** ends first somewhat later.

## 4.4 Changes in 24 hour $P_{gel}$

The behaviour of 24 hour $P_{gel}$ seem to lie somewhere in between the 6 hour $P_{gel}$ and the 72 hour $P_{gel}$. In treatment 1 and 2, it has a slight decrease after the 6$^{th}$ of May, just as 6 hour $P_{gel}$. but it seems to go up at the harvests 1$^{st}$ and 13$^{th}$ of July, when 6 hour $P_{gel}$ is still unmeasurable.

Therefore, it is well conceivable that 24 hour $P_{gel}$ depletes to buffer the pools measured by 6 hour $P_{gel}$ when plant uptake is high, and gets refilled by the 72 hour $P_{gel}$ pool, relatively more quickly than the 6 hour $P_{gel}$ gets refilled by 24 and 72 hour $P_{gel}$.

## 4.5 Buffering and development of Olsen P

The buffering of available fractions enables plants to take out large amounts of P without large changes in 24 and 72 hour $P_{gel}$ or Olsen P: The wheat yields on the "Exhaustion Land" 2015 took out P equalling 25 – 50 % of Olsen P without Olsen P changing.

According to Johnston *et al.* (2014), Olsen P will decrease by half over 6.6 years on the "Exhaustion Land" with the agricultural practises of 1993 – 1999, and no further applications of P. If the agricultural practises of 2015 mine the soil P reserves in the same way, plot 1 could go one half life for Olsen P without either P fertilisation nor a drop in yield. (Poulton *et al.* 2013). During those 6.6 years, Olsen P would drop from 30 to 15 mg/l, and the yields would have removed 165 % of present Olsen P. This shows the Olsen P fraction to be quite well buffered.

This study shows it to be even more well buffered as Olsen P declines, and Johnston *et al.* (2016) has demonstrated the same at a long-term experiment, where a plot with initial Olsen P of 5 mg/kg would need to take out over 500 % it's initial Olsen P to reduce it to 2.5 mg/kg.

The buffering of Olsen P seems to be both on a long-term, multi-annual and a short-term seasonal scale. (Johnston *et al.* 2016) explains the long-term buffering with Johnston *et al.* (2014) four compartment model (figure 3), where **pool 3** and **4** buffers **pool 1** and **2**, and increasingly so as Olsen P decreases.

This buffering is likely to also happen on the seasonal scale, as neither Olsen P nor 72 hour $P_{gel}$ (except for the harvest 3$^{rd}$ of March) changes significantly during the growing season. But this may also partly be because the Olsen method disrupt the soil equilibria of measured soils, which can quicken the P buffer and drown out (parts of) seasonal variations:

Soil solution ionic strength is typically 0.005 M (Edmeades *et al.* 1985), while the Olsen method utilises 0.5 M $NaHCO_3$. Sieving will by it's nature split up soil particles, and shaking may divide them further (Sposito 1984). The soil:solution ratio is higher than ambient, which may also cause a shift in desorbable P (Limousin *et al.* 2007), and the very

rewetting of air-dried soil may mobilise P (Blackwell *et al.* 2013). Different temperatures in lab and field can also affect sorption (Limousin *et al.* 2007), as well as storage and pretreatment of soil (Condron & Newman 2011).

In sum, this means Olsen P measures a pool not present *in situ*, but a pool empirically correlated with fractions *in situ*.

As Olsen P and other agricultural soil tests, measures empiric pools, it may be they are less suitable for fine scale scientific work, though the very extensive literature utilising agricultural soil tests would suggest they are not entirely unsuitable. Scientific suitability wouldn't be their goal either, as as they are mainly agricultural tests. The tests would optimally predict P status and uptake independent of soil and seasonal factors. Although there was a certain increase in Olsen P during the latter half of the season coinciding with an increase in 72 hour $P_{gel}$, there were no significant differences during the growing season, which, together with the fact that it has predicted 77 – 99 % of variance in yield (Poulton *et al.* 2013), is a testament to the usefulness of the Olsen P method on the "Exhaustion Land" and equivalent soils.

Olsen P is thus an empiric test to test whether there is enough P in the soil for agricultural purposes, and also for annual or longer term scientific work. Sequential extractions such as Singh *et al.* (2005) or Blake *et al.* (2003) are likely to discern which soil constituents bind the P, while DGT techniques will better describe the diffusive behaviour and mobility of P in soils. These properties are of course related, as the covariances of 72 hour $P_{gel}$ and Olsen P ($r = 0.61$) shows, but will answer different questions.

There being significant seasonal differences for DGTs exposed for 6 and 72 hours (although I do not try and explain the significantly lower 72 hour $P_{gel}$), suggests it could be a useful tool in future studies looking at the intra-annual variability of soil P.

## 4.6 Exposure times for DGTs

In this experiment, more than 90 % of samples exposed for 6 hours were below the limit of detection. It may therefore raise questions if such a exposure time is a bit too short. Still, it is here the most interesting results occur. Thus, future studies would be advised to increase exposure time to ensure results above the limit of detection, but also keep it as short to ensure informative results. The best would be to achieve a lower limit of detection than the present study.

All the exposure times in this study are arbitrary, which DGT exposure times usually are (Degryse *et al.* 2009). Because root hairs are important in P uptake and only are active for a few days (McElgunn & Harrison 1969), their average lifetime might be a prudent exposure time if simulating plant uptake. Root hairs generally follow the growth of the root apex, and will thus only explore a set volume of soil for a short time before the soil is allowed to recover (Jungk 2001). DGTs will also not be able to exhaust the soil for more than a few days, as the risk of their iron oxide gels to become saturated increases, and thus the linear relationship between gel P content and soil P content breaks down (Christel *et al.* 2016).

Therefore, the life time of the root would be an alternative to arbitrary exposure times, as the root hairs may be responsible for the largest part of the P uptake, up to 90 % under low P conditions (Föhse *et al.* 1991) and the life time is sufficiently short to allow the DGT to simulate it's full operating time.

On the other hand, experiments would increase in size, as the lifetime of root hairs have to be determined in addition to the original point of the investigation.

# 5 Conclusions

It is prudent to avoid a wasteful use of P as it can cause both eutrophication and resource depletion or monopolisation, in addition to wastefulness being costly. On the contrary, it is necessary to increase P use efficiency in food production to sate the needs of an increasing world population at minimal environmental costs.

As P binds strongly to soil components, it is necessary to investigate these bindings, and how they are affected by seasonal and biotic factors.

Testing soil P by Olsen P and DGTs troughout the growing season, this study indicates that soil biota transforms readily available soil P (**pool 2**) to immediately available soil P (**pool 1**) prior to uptake, and prior to the major part of their P uptake.

Plants do not seem to increase total pools of plant available P as measured by Olsen P and 72 hour $P_{gel}$ in the early parts of the growing season, but later on, these fractions are on the rise. The increasing trend in the latter half of the growing season are likely geochemical rather than biogeochemical in nature, as plant P uptake mostly is done. Thus, there is no reason for the plant to solubise P.

The plants no not seem to deplete total pools of plant available P over the course of a single growing season, but there is a visible depletion of immediately available soil P as measured by 6 hour $P_{gel}$. It stands to reason that this depletion is caused by plant P uptake, as it is concurrent with the highest daily P uptake. The lack of a depletion of total plant available P is likely caused by the available pools (**1** and **2**) being buffered by the inavailable pools (**3** and **4**).

Extraction methods such as Olsen P or P-AL seems to be better suited for agricultural P tests on the “Exhaustion land” and similar soils, not necessarily because they give better results, but rather because there are fewer material requirements and and concentrations in the mg/l range allows for more operator error than concentrations in the µg/l range.

As there was no significant depletion of P during the growing season, there was no significant build up towards a winter maximum either.

Knowledge of the development in plant P uptake and plant available P in soil may allow the farmer to better time his fertilisation, plant breeders to select for plant uptake when the conditions are suitable, and environmental advisor to asses the risks of P run-off.

Although this study helps develop this understanding for wheat, there are still contradictions regarding the seasonal variation of soil P, and it's causes are still not properly understood.

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# National Institute of Standards & Technology

# Certificate of Analysis

## Appendix 1: Standard Reference Material 1547

### Peach Leaves

This Standard Reference Material (SRM) is intended primarily for use in evaluating the reliability of analytical methods for the determination of major, minor, and trace elements in botanical materials, agricultural food products, and materials of similar matrix. A unit of SRM 1547 consists of 50 grams of dried peach leaves of the Coronet variety.

Certified and Noncertified Concentrations of Constituent Elements: The certified concentrations of the constituent elements are given in Table 1. These concentrations are based on the agreement of results from at least two independent analytical methods or the mean of results from a method of known accuracy. Noncertified concentrations of constituent elements are provided for information only in Table 2.

**Notice and Warnings to Users**

Expiration of Certification: This certification is valid for five years from the date of shipment. Should any of the certified values change before the expiration of the certification, purchasers will be notified by NIST. Please return the attached registration card to facilitate notification.

Stability: This material was radiation sterilized ($^{60}Co$) at an estimated minimum dose of 27.8 kGy for microbiological control. However, its stability has not be rigorously assessed. NIST will monitor this material and will report any substantive changes in certification to the purchasers.

Storage: The material should be kept tightly closed in its original bottle and stored in the dark at a temperature between 10 and 30 °C. It should not be exposed to intense sources of radiation. Ideally, the bottle should be kept in a desiccator under the conditions indicated above.

Use: The bottle should be thoroughly mixed by rotating and/or rolling the bottle before each use. Allow the contents to settle for one minute prior to opening. A minimum sample of 150 mg of the dried material, dried as described in the section on "Instructions for Drying", should be used to relate analytical determinations to the certified values in this certificate. In some cases, especially for volatile elements such as mercury, it is preferable to analyze samples from the bottle without drying, determine the moisture content on a separate sample from the same bottle, and correct the analytical results to a dry weight basis.

Dissolution of SRM 1547: Digestion procedures should be designed to avoid loss of volatile elements, such as arsenic, mercury, etc. Digestion of the SRM in nitric and perchloric acids was found to be incomplete with a small residue of siliceous material remaining. This residue must be considered an integral part of the SRM and should be dissolved with a small amount of hydrofluoric acid to obtain total dissolution.

Coordination of all analytical measurements used in the characterization of this SRM was performed by D.A. Becker of the NIST Inorganic Analytical Research Division.

Statistical analysis of the experimental data was performed by W. Guthrie and S.B. Schiller of the NIST Statistical Engineering Division.

The technical and support aspects involved in the certification and issuance of this SRM were coordinated through the Standard Reference Materials Program by R. Alvarez and T.E. Gills.

Gaithersburg, MD 20899
January 22, 1993
(Revision of certificate dated 7-2-91)

William P. Reed, Chief
Standard Reference Materials Program

Instructions for Drying: Samples of this SRM must be dried only by one of the following two procedures.

1. Drying in a desiccator at room temperature (approximately 22 °C) for 120 hours over fresh anhydrous magnesium perchlorate. The sample depth should not exceed one cm.

2. Freeze drying for 24 hours at a pressure of 13.3 Pa or lower and a shelf temperature of -5 °C or lower after having frozen the sample (not to exceed one cm in depth) at -40 °C or lower for at least one hour. At the end of the 24-hour period, samples are placed immediately in a desiccator with fresh anhydrous magnesium perchlorate. Samples are weighed after allowing a minimum of four hours to establish temperature equilibrium.

**NOTE:** Vacuum drying at room temperature and oven drying at elevated temperatures have resulted in excessive weight losses and therefore are not recommended.

Homogeneity Assessment: Samples from randomly selected bottles of SRM 1547 were tested for homogeneity by instrumental neutron activation analysis. No evidence of chemically significant inhomogeneity was observed (Ref. 1).

Table 1. Certified Concentrations of Constituent Elements

| Element | Concentration, wt. percent |
|---|---|
| Calcium | 1.56 ± 0.02 |
| Magnesium | 0.432 ± 0.008 |
| Nitrogen (Total) | 2.94 ± 0.12 |
| Phosphorus | 0.137 ± 0.007 |
| Potassium | 2.43 ± 0.03 |

| Element | Concentration, µg/g | Element | Concentration, µg/g |
|---|---|---|---|
| Aluminum | 249 ± 8 | Mercury | 0.031 ± 0.007 |
| Arsenic | 0.060 ± 0.018 | Molybdenum | 0.060 ± 0.008 |
| Barium | 124 ± 4 | Nickel | 0.69 ± 0.09 |
| Boron | 29 ± 2 | Rubidium | 19.7 ± 1.2 |
| Cadmium | 0.026 ± 0.003 | Selenium | 0.120 ± 0.009 |
| Chlorine | 360 ± 19 | Sodium | 24 ± 2 |
| Copper | 3.7 ± 0.4 | Strontium | 53 ± 4 |
| Iron | 218 ± 14 | Vanadium | 0.37 ± 0.03 |
| Lead | 0.87 ± 0.03 | Zinc | 17.9 ± 0.4 |
| Manganese | 98 ± 3 | | |

Certified Concentrations and Uncertainties: The certified concentrations are equally weighted means of results from two or more different analytical methods or the mean of results from a method of known accuracy. In the case of two or more methods, each uncertainty is the sum of a 95% confidence limit and an allowance for systematic error between the methods used. In the case of a method of known accuracy, each uncertainty is the sum of a 95% confidence limit and the known systematic error of the method.

# Appendix 2: Reference material 2, internal grass standard

| **In house STANDARD GRASS** (JULY 1996 PL 1), laboratory determined september 2007<br>Values in mg $kg^{-1}$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **P** | **K** | **Ca** | **Mg** | **Na** | **S** | **Fe** | **Al** | **Ti** |
| **-2 SD** | 3084 | 23786 | 7578 | 1356 | 1935 | 1958 | 513 | 299 | 16,35 |
| **mean** | 3160 | 24502 | 7758 | 1404 | 2008 | 2007 | 589 | 319 | 18,37 |
| **+2 SD** | 3235 | 25218 | 7938 | 1445 | 2080 | 2055 | 665 | 339 | 20,37 |
| | | | | | | | | | |
| | **Mn** | **Zn** | **Cu** | **Cr** | **Ni** | **Pb** | **Mo** | **Cd** | **Co** |
| **-2 SD** | 58,17 | 21,85 | 5,247 | 0,828 | 1,563 | 0 | 0,499 | 0,183 | 0,376 |
| **mean** | 59,61 | 25,01 | 5,52 | 1,836 | 2,425 | 1,729 | 0,943 | 0,426 | 0,884 |
| **+2 SD** | 61,05 | 28,17 | 5,793 | 2,844 | 3,287 | 3,287 | 1,387 | 0,669 | 1,464 |

# Appendix 3: Tables of plant dry matter and P and soil P from March to August

Table A3.1: Soil P content by treatment (treat) and harvest.
Values are commonly average of 3 ± SD. When there are fewer data points behind the value, SD is given as na instead.

| Harvest n | Date | Treat | Olsen mg/l | ± | $P_{DGT}$ mg/l | ± | 6 h $P_{gel}$ µg/gel | ± | 24 h $P_{gel}$ µg/gel | ± | 72 h $P_{gel}$ µg/gel | ± |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 04.03.15 | 1 | 33 | 3,7 | 0,09 | 0,041 | 90 | 40 | 140 | 60 | <LOD | |
| 1 | 04.03.15 | 2 | 31 | 1,4 | < LOD | | < LOD | | < LOD | | <LOD | |
| 1 | 04.03.15 | 3 | 21 | 2,4 | 0,07 | 0,036 | < LOD | | 100 | 52 | 150 | 79 |
| 1 | 04.03.15 | 4 | 8 | 2,0 | 0,07 | 0,038 | < LOD | | 100 | 55 | 150 | 68 |
| 2 | 27.03.15 | 1 | 32 | 2,7 | 0,11 | na | 110 | 54 | 150 | na | 262 | na |
| 2 | 27.03.15 | 2 | 31 | 2,4 | < LOD | | < LOD | | < LOD | | 216 | na |
| 2 | 27.03.15 | 3 | 20,4 | 0,24 | < LOD | | < LOD | | < LOD | | 233 | na |
| 2 | 27.03.15 | 4 | 7,0 | 0,49 | < LOD | | < LOD | | < LOD | | <LOD | |
| 3 | 23.04.15 | 1 | 31 | 1,7 | < LOD | | < LOD | | < LOD | | 220 | 33 |
| 3 | 23.04.15 | 2 | 28 | 1,4 | 0,06 | 0,029 | 100 | 50 | 90 | 42 | 200 | 10 |
| 3 | 23.04.15 | 3 | 17,9 | 0,96 | < LOD | | < LOD | | < LOD | | 100 | 59 |
| 3 | 23.04.15 | 4 | 6,7 | 0,25 | < LOD | | < LOD | | < LOD | | <LOD | |
| 4 | 06.05.15 | 1 | 30 | 5,2 | 0,10 | 0,056 | 130 | 61 | 150 | 81 | 200 | 110 |
| 4 | 06.05.15 | 2 | 29 | 3,4 | 0,11 | 0,062 | 100 | 62 | 160 | 88 | 200 | 10 |
| 4 | 06.05.15 | 3 | 23,0 | 0,2 | 0,06 | 0,029 | 120 | 45 | 90 | 42 | 100 | 57 |
| 4 | 06.05.15 | 4 | 7 | 1,2 | < LOD | | < LOD | | < LOD | | <LOD | |
| 5 | 21.05.15 | 1 | 30 | 3,9 | 0,09 | 0,042 | 110 | 66 | 140 | 60 | 200 | 140 |
| 5 | 21.05.15 | 2 | 28 | 3,9 | 0,07 | 0,034 | < LOD | | 100 | 49 | 190 | 17 |
| 5 | 21.05.15 | 3 | 20,71 | 0,083 | 0,07 | 0,034 | < LOD | | 100 | 49 | 200 | 110 |
| 5 | 21.05.15 | 4 | 7 | 1,2 | < LOD | | < LOD | | < LOD | | <LOD | |
| 6 | 04.06.15 | 1 | 28 | 0,9 | < LOD | | < LOD | | < LOD | | 200 | 35 |
| 6 | 04.06.15 | 2 | 29 | 4,6 | 0,11 | 0,011 | < LOD | | 160 | 16 | 270 | 37 |
| 6 | 04.06.15 | 3 | 22 | 1,2 | 0,07 | 0,031 | < LOD | | 90 | 45 | 185 | 8,8 |
| 6 | 04.06.15 | 4 | 7 | 1,2 | 0,07 | 0,031 | < LOD | | 90 | 45 | <LOD | |
| 7 | 16.06.15 | 1 | 30 | 4,6 | 0,08 | 0,029 | < LOD | | 120 | 42 | 230 | 16 |
| 7 | 16.06.15 | 2 | 29 | 1,7 | 0,07 | 0,034 | < LOD | | 100 | 50 | 200 | 9 |
| 7 | 16.06.15 | 3 | 19,7 | 0,61 | < LOD | | < LOD | | < LOD | | 100 | 52 |
| 7 | 16.06.15 | 4 | 9 | 3,0 | < LOD | | < LOD | | < LOD | | <LOD | |
| 8 | 01.07.15 | 1 | 33 | 2,7 | 0,106 | 0,0070 | < LOD | | 150 | 10 | 270 | 27 |
| 8 | 01.07.15 | 2 | 30 | 2,4 | 0,09 | 0,036 | < LOD | | 130 | 52 | 250 | 20 |
| 8 | 01.07.15 | 3 | 20 | 2,2 | < LOD | | < LOD | | < LOD | | 130 | 54 |
| 8 | 01.07.15 | 4 | 9 | 2,7 | < LOD | | < LOD | | < LOD | | <LOD | |
| 9 | 13.07.15 | 1 | 34 | 4,6 | 0,09 | 0,040 | < LOD | | 130 | 58 | 290 | 54 |
| 9 | 13.07.15 | 2 | 32 | 5,8 | 0,10 | 0,049 | < LOD | | 150 | 71 | 300 | 110 |
| 9 | 13.07.15 | 3 | 20 | 2,7 | 0,08 | 0,030 | < LOD | | 120 | 43 | 250 | 220 |
| 9 | 13.07.15 | 4 | 7 | 1,5 | < LOD | | < LOD | | < LOD | | <LOD | |
| 10 | 05.08.15 | 1 | 30,4 | 0,10 | 0,09 | 0,037 | 100 | 58 | 130 | 53 | 170 | 85 |
| 10 | 05.08.15 | 2 | 28 | 2,2 | < LOD | | < LOD | | < LOD | | 300 | 160 |
| 10 | 05.08.15 | 3 | 19 | 1,2 | < LOD | | < LOD | | < LOD | | <LOD | |
| 10 | 05.08.15 | 4 | 6,1 | 0,61 | < LOD | | < LOD | | < LOD | | <LOD | |

Table A3.2: Plant dry weights by treatment (treat) and harvest.
Values are averages of 3 ± SD. Separate weighing of ears, leaves and stems started 4.6.15

| Harvest n | Date | Treat | Plant g/m² | ± | Ears g/m² | ± | Leaves g/m² | ± | Stems g/m² | ± |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 04.03.15 | 1 | 12 | 1,2 | | | | | | |
| 1 | 04.03.15 | 2 | 16 | 2,7 | | | | | | |
| 1 | 04.03.15 | 3 | 11 | 3,3 | | | | | | |
| 1 | 04.03.15 | 4 | 9,1 | 0,59 | | | | | | |
| 2 | 27.03.15 | 1 | 25 | 2,6 | | | | | | |
| 2 | 27.03.15 | 2 | 30 | 6,5 | | | | | | |
| 2 | 27.03.15 | 3 | 17,0 | 0,64 | | | | | | |
| 2 | 27.03.15 | 4 | 11 | 2,4 | | | | | | |
| 3 | 23.04.15 | 1 | 140 | 18 | | | | | | |
| 3 | 23.04.15 | 2 | 150 | 20 | | | | | | |
| 3 | 23.04.15 | 3 | 90 | 5,7 | | | | | | |
| 3 | 23.04.15 | 4 | 70,0 | 11 | | | | | | |
| 4 | 06.05.15 | 1 | 239 | 2,0 | | | | | | |
| 4 | 06.05.15 | 2 | 230 | 18 | | | | | | |
| 4 | 06.05.15 | 3 | 190,0 | 42 | | | | | | |
| 4 | 06.05.15 | 4 | 110 | 19 | | | | | | |
| 5 | 21.05.15 | 1 | 570 | 29 | | | | | | |
| 5 | 21.05.15 | 2 | 530 | 35 | | | | | | |
| 5 | 21.05.15 | 3 | 500 | 37 | | | | | | |
| 5 | 21.05.15 | 4 | 300 | 30,0 | | | | | | |
| 6 | 04.06.15 | 1 | 1000 | 75 | 30 | 18 | 360 | 26 | 620 | 36 |
| 6 | 04.06.15 | 2 | 1000 | 100 | 20 | 17 | 380 | 40 | 640 | 91 |
| 6 | 04.06.15 | 3 | 1000 | 100 | 17 | 6,8 | 360 | 37 | 700 | 130 |
| 6 | 04.06.15 | 4 | 650 | 66 | 9 | 8,2 | 200 | 36 | 450 | 39 |
| 7 | 16.06.15 | 1 | 1300 | 120 | 268 | 8,5 | 290 | 21 | 700 | 100 |
| 7 | 16.06.15 | 2 | 1300 | 110 | 270 | 29 | 301 | 6,2 | 720 | 78 |
| 7 | 16.06.15 | 3 | 1420 | 34 | 300 | 11 | 330 | 29 | 800 | 31 |
| 7 | 16.06.15 | 4 | 1000 | 210 | 200 | 47 | 190 | 36 | 600 | 120 |
| 8 | 01.07.15 | 1 | 1600 | 76 | 550 | 29 | 240 | 18 | 810 | 30 |
| 8 | 01.07.15 | 2 | 1520 | 61 | 490 | 10 | 230 | 16 | 800 | 48 |
| 8 | 01.07.15 | 3 | 1650 | 89 | 400 | 180 | 300 | 150 | 880 | 50 |
| 8 | 01.07.15 | 4 | 1200 | 220 | 410 | 80 | 150 | 39 | 600 | 100 |
| 9 | 13.07.15 | 1 | 2000 | 100 | 1080 | 75 | 230 | 12 | 670 | 29 |
| 9 | 13.07.15 | 2 | 1760 | 65 | 940 | 40 | 200 | 10 | 620 | 30 |
| 9 | 13.07.15 | 3 | 2100 | 280 | 800 | 510 | 500 | 460 | 800 | 200 |
| 9 | 13.07.15 | 4 | 1400 | 210 | 840 | 87 | 110 | 33 | 500 | 100 |
| 10 | 05.08.15 | 1 | 1800 | 120 | 1130 | 57 | 200 | 60 | 430 | 53 |
| 10 | 05.08.15 | 2 | 1700 | 230 | 1100 | 110 | 190 | 43 | 410 | 93 |
| 10 | 05.08.15 | 3 | 1900 | 100 | 1260 | 77 | 190 | 65 | 490 | 49 |
| 10 | 05.08.15 | 4 | 1100 | 300 | 700 | 220 | 80 | 16 | 320 | 69 |

Table A3.3: Plant phosphorus content by treatment (treat) and harvest.
Values are averages o 3 ± SD. Separate weighing of ears, leaves and stems started 4.6.15

| Harvest n | Date | Treat | Plant mg/m² | ± | Ears mg/m² | ± | Leaves mg/m² | ± | Stems mg/m² | ± |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 04.03.15 | 1 | 47 | 6,2 | | | | | | |
| 1 | 04.03.15 | 2 | 60 | 12 | | | | | | |
| 1 | 04.03.15 | 3 | 32 | 8,8 | | | | | | |
| 1 | 04.03.15 | 4 | 14 | 1,9 | | | | | | |
| 2 | 27.03.15 | 1 | 90 | 13 | | | | | | |
| 2 | 27.03.15 | 2 | 100 | 22 | | | | | | |
| 2 | 27.03.15 | 3 | 50 | 3,2 | | | | | | |
| 2 | 27.03.15 | 4 | 23 | 4,8 | | | | | | |
| 3 | 23.04.15 | 1 | 320,0 | 43 | | | | | | |
| 3 | 23.04.15 | 2 | 340 | 36 | | | | | | |
| 3 | 23.04.15 | 3 | 180 | 18 | | | | | | |
| 3 | 23.04.15 | 4 | 110 | 29 | | | | | | |
| 4 | 06.05.15 | 1 | 630 | 51 | | | | | | |
| 4 | 06.05.15 | 2 | 590 | 51 | | | | | | |
| 4 | 06.05.15 | 3 | 400 | 120 | | | | | | |
| 4 | 06.05.15 | 4 | 204 | 52 | | | | | | |
| 5 | 21.05.15 | 1 | 1300 | 140 | | | | | | |
| 5 | 21.05.15 | 2 | 1300 | 140 | | | | | | |
| 5 | 21.05.15 | 3 | 1000 | 140 | | | | | | |
| 5 | 21.05.15 | 4 | 400 | 91 | | | | | | |
| 6 | 04.06.15 | 1 | 1700 | 280 | 90 | 49 | 900 | 200 | 700 | 47 |
| 6 | 04.06.15 | 2 | 1900 | 190 | 60 | 53 | 990 | 78 | 800 | 200 |
| 6 | 04.06.15 | 3 | 170 | 120 | 50 | 17 | 900 | 120 | 800 | 200 |
| 6 | 04.06.15 | 4 | 750 | 240 | 20 | 19 | 300 | 160 | 430 | 94 |
| 7 | 16.06.15 | 1 | 1900 | 210 | 710 | 20 | 570 | 51 | 700 | 180 |
| 7 | 16.06.15 | 2 | 1900 | 130 | 700 | 51 | 600 | 53 | 600 | 99 |
| 7 | 16.06.15 | 3 | 2100 | 190 | 740 | 93 | 700 | 140 | 620 | 76 |
| 7 | 16.06.15 | 4 | 110 | 380 | 500 | 150 | 300 | 100 | 300 | 120 |
| 8 | 01.07.15 | 1 | 2100 | 130 | 1370 | 99 | 290 | 27 | 470 | 28 |
| 8 | 01.07.15 | 2 | 2000 | 260 | 1200 | 280 | 290 | 33 | 590 | 65 |
| 8 | 01.07.15 | 3 | 2000 | 190 | 980 | 270 | 500 | 180 | 580 | 82 |
| 8 | 01.07.15 | 4 | 1200 | 430 | 800 | 200 | 140 | 90 | 300 | 100 |
| 9 | 13.07.15 | 1 | 2400 | 110 | 2000 | 100 | 120 | 39 | 280 | 2,8 |
| 9 | 13.07.15 | 2 | 2000 | 110 | 1700 | 65 | 120 | 19 | 230 | 29 |
| 9 | 13.07.15 | 3 | 2000 | 600 | 1400 | 800 | 300 | 300 | 360 | 60 |
| 9 | 13.07.15 | 4 | 1300 | 300 | 1200 | 230 | 30 | 20 | 120 | 55 |
| 10 | 05.08.15 | 1 | 2300 | 170 | 2100 | 170 | 80 | 15 | 90 | 19 |
| 10 | 05.08.15 | 2 | 2000 | 250 | 1900 | 220 | 60 | 15 | 80 | 15 |
| 10 | 05.08.15 | 3 | 2300 | 200 | 2200 | 200 | 40 | 15 | 100 | 12 |
| 10 | 05.08.15 | 4 | 900 | 450 | 900 | 440 | 11 | 3,4 | 30 | 13 |

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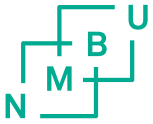


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