Rhizosphere processes and associated phosphorus solubilisation during soil drying and rewetting

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August 2022

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Declaration

I hereby declare that, unless where otherwise credited, the contents of this thesis are my own original work and have not been previously submitted, in part or in full, for the award of a higher degree elsewhere. Some chapters of this thesis are presented in the international conferences through the alternative format. The presentation details are stated in each experimental chapter. The chapters are not published, they are written in the appropriate style and are intended for submission.

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I confirm that the above statement is correct.

P. M. Haygarth, Supervisor

August 2022

Acknowledgements

This work was funded by the Research EU and China Partnerships under SEW-REAP Europe Aid Programme; projects ECRIP ICI+/2014/348-010, and supported by Lancaster Environmental Centre, Lancaster University and in collaboration with the Rhizosphere Laboratory of Plant and Soil Interactions, China Agriculture University, Beijing, PR China.

First and foremost, I wish to express my tremendous thanks for the positivity, encouragement, enthusiasm, motivation and scientific guidance throughout all stages of my research from my supervisor, Prof. Phil Haygarth (Lancaster University, UK) who gave me this incredible opportunity to develop practical and theoretical research expertise in the "World of Phosphorus" under his supervision and leadership. I am also deeply grateful to my supervisor Prof. Jianbo Shen (China Agriculture University, CAU, China) and my fellow colleagues on the rhizosphere team in China Agriculture University, who have been exceptionally supportive for providing knowledge and resources, and for teaching me laboratory and experimental techniques with their time, expertise, patience, kindness and friendliness.

A very special warm thank-you to Dr. David Tyfield and people in SEW-REAP Europe Aid Programme for the funding of this studentships and supports that facilitated fulfillment of my laboratory studies in China. I am so grateful to have been a part of this programme that I wish to acknowledge.

My sincere thanks also go to Prof Haigang Li, Xiaoqiang Jiao and Zhihui Wen for all the stimulating discussions and many helpful suggestions for the production of Chapter3.

I greatly appreciated Dr. Malika Mezeli for the precious support and inspiring discussions, which led to improvement of Chapter 6. I would like to thank for your patience and time with listening to me and commenting back on my works in the discussion of this thesis.

Finally, I am always grateful to my wonderful family for love, care and a great deal of support along the PhD Road at each and every moment.

Abstract

Phosphorus (P) is at the heart of modern agriculture, which helps to sustain crop production. Many of the key processes centered around P solubilisation in the rhizosphere govern both plant uptake or potential leaching. However, the processes of controlling P solubilisation in the rhizosphere are of varying efficiency and now subject to constant changes under the varying soil conditions due to the climate change. In turn it is thought to have a potential onward effect of drying and rewetting (DRW) on the rhizosphere, which is one of the central components of this thesis. Thus, the focus of this PhD is to assess the P solubilisation processes in the rhizosphere with respect to the immediate (*i*-DRW) and delayed (*d*-DRW) DRW effect.

Alongside this, the thesis is also focussed in assessing the behaviour of different types of phosphate fertiliser and their potential efficiency in the rhizosphere, particularly widely available new low-grade phosphate rock (PR) for potential crop benefit. The low-grade PR material is newly emerging into the market and can be used as a potentially more sustainable type of P fertiliser over time, as a substitute higher-grade PR.

The overarching hypothesis tested was “P solubilisation in the rhizosphere can be affected by climate driven changes of soil associated with immediate and delayed effects of DRW events and root-zone acidification, under variable P amendments, with a particular consideration of the effects of low-grade phosphate rock”. To approach this, eight sub-hypotheses were tested, each being part of four different controlled experiments in the greenhouse using soils from four distinct agricultural regions of China. All laboratory experiments of P solubilisation (and potential leaching) in the rhizosphere were undertaken in three steps, which were dealt with chapters 3, 4 and 5.

Overall, the key findings indicate that the P solubilisation in the rhizosphere (with corresponding plant productivity) is highly dependent on soil type, in particular the biological and hydrological characteristics. The results show substantially different levels of soil P solubilisation, related to exudative processes of the rhizosphere. Rhizosphere manipulation by inducing soil acidity via chemicals and legumes, had significant effects on soil P solubilsation and plant growth response (chapter 3). The P solubilisation processes also performed better on the soil with recent history of a series of DRW events, than soil with constant moisture treatment (chapter 5). This data supports the practical implications in the crop productivity over arid agricultural regions, especially with soils having low P availability used in these experiments. However, despite the assumptions of an increase in P solubilisation associated with *i*-DRW, the results of parallel study (chapter 4) did not prove to have a statistically significant difference across all treatments when soils were treated with frequent DRW events immediately.

With regards DRW induced P leaching, the concentration of all P forms in leachate water varied in each cycle against soil type and the amount of P leaching following DRW was also proportionate with the rate of new P application. Concentration of P in the leachates of soils receiving different P sources varied from one *i*-DRW cycle to another, in respect of frequency and rate of DRW. Taken together, the evidence from the thesis led to the acceptance of the overarching hypothesis of this study.

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Abbreviations

AMF	Arbuscular mycorrhizal fungi
CPR	Commercial phosphate rock
***cit*-P0**	Citric acid treatment, without adding any phosphorus supply
***cit*-PR**	Citric acid treatment, with addition of phosphate rock
CAU	China agricultural university
SP	Calcium perphosphate monohydrate
DAP	Days after planting
DAPR	Direct application of phosphate rock
DPS	Degree of phosphorus saturation
DP	Dissolved phosphorus
DWPA	Diffuse water pollution from agriculture
DRW	Drying and rewetting
***i*-DRW**	Immediate drying and rewetting
***d*-DRW**	Delayed drying and rewetting
EPC	Equilibrium phosphorus concentration
MBP	Microbial biomass phosphorus
M&M	Monocropped maize
M&F	Intercropped maize with faba bean

M&A	Intercropped maize with alfalfa
P	Phosphorus
P0	Treatment without phosphorus addition
PUE	Phosphorus use efficiency
TP	Total phosphorus
PR	Phosphate rock
PR-SA	Phosphate rock with addition of sulphuric acids
PR-AS	Phosphate rock with addition of ammonium sulphate
R/S ratio	Root to shoot ratio
TOC	Total organic carbon
TN	Total nitrogen
SRL	Specific root length
UP	Urea phosphate

CHAPTER 1

General introduction

1.1 Introduction

Phosphorus (P) is the one of the essential elements, helping to meet the demands on agricultural production as well as addressing food needs for the growing world population. The current global population is 7.6 billion and is projected to reach 9.8 billion by 2050, and further increase to11.2 billion by 2100 (Costello et al., 2020). Inevitably, this means that intensive agricultural practices that utilise more land, water, and fertiliser may need to be increasingly adopted (Tilman et al., 2002). Our contemporary agriculture has already occupied nearly 40% of the global land surface area (Foley et al., 2005). For example, in 2008, approximately 70% of the total land area in the UK was utilised by agriculture at around 17.2 million hectares (Defra, 2008), including croplands, uncropped arable land, rough grazing, temporary and permanent grassland, and land for outdoor pigs (Angus et al., 2009).

1.2 Phosphorus uses in agriculture

One of the concerns of modern and future commercial crop farming is how to meet crop production targets given potential concerns of limitations and inefficiencies of P supply. On the face of it, the P fertiliser production obtained from mined and purified phosphate rock (PR) appears to be a potential solution. The consumption of inorganic phosphate fertilisers has already quadrupled since the mid-1900s consequently (Smil, 2000). However, crops are inefficient, and only take up around 10% of the applied P fertilisers (Haygarth and Jarvis, 1999), and a substantial amount of surplus of P gradually accumulates in the topsoil over time (Sattari et al., 2012; Syers et al., 2008; Wolf et al., 1987), because of the inherent low mobility of applied P (Johnston and Poulton, 1992). This results in a steady increase in level of accumulated P in the soil what is called soil “legacy” P. For example, over the past 65 years, the agricultural area has, on average, built up a P surplus approximately 1000 kg ha^{-1}, with 13% of them accumulated as Olsen-extractable form (Withers et al., 2010). The large amount of residual P built-up in agricultural topsoil has a potential for unintended consequences of P transfer via surface runoff and leaching ways to tile drains (Svanback et al., 2014) in the form of sediment bound and dissolved solids (Sharpley et al., 1992), as it is interacted with soil hydrology (Haygarth et al., 2000; 1998; Sims et al., 2000).

By 1999, the estimated P export to waterways in the UK had reached at around 1 kg ha^{-1} $year^{-1}$ in total (Haygarth et al., 2009; Haygarth and Jarvis, 1999), when concurrent range of annual fertilisers and manure inputs was ranged between 20 and 50 kg P ha^{-1} $year^{-1}$ (Cameron et al., 2002; Haygarth et al., 2005; Loganathan et al., 2001). The level of total P export has direct consequences for water quality, for example, diffuse water pollution agriculture (DWPA). Even though net P inputs have been reduced worldwide by 2%, due to increased levels of environmental awareness of freshwater quality (Mueller et al., 2013), the P transfer into watercourses is still attributed as becoming one of the main causes of eutrophication and it poses a long-term threat through the remobilisation and accumulation of legacy P in soils (Powers et al., 2016). With increasing evidence that the cumulative surplus P can be further amplified by manure recycling and P import that originated from animal feed stuffs, recycled bio-resources to soil and crop residues (Haygarth et al., 2005). The accumulation of surplus P in the soil can be further increased through various practices, including manure recycling and P importing from animal feed stuffs, recycled bio-resources to the soil and crop residues (Haygarth et al., 2005). Once eutrophic symptoms are established, it is extremely challenging to mitigate as demonstrated by an historical lack of success in stemming toxic algal growth (Jarvie et al., 2013). It perpetuates further symptoms under the condition of changing climate (Bennion et al., 1996; Johannesse and Dahl, 1996). Under the condition of a changing climate, these practices continue to give rise to further symptoms and additional consequences.

Environmental stresses (e.g., intense drying and high discharge) often manipulate nutrient cycling leading to widespread elevated annual total P export to waterbodies (Ockenden et al., 2016). The reason for this is because the natural occurrence of frequent soil drying and rewetting (DRW) caused by extreme weather events such as drought and flooding has a significant impact on soil abiotic and biotic properties, including soil hydrology, thereby altering the P forms, timings of fluxes and their ability to retain or move. The movement of P in response to sequential DRW events may also be exacerbated by rhizosphere processes, as frequent soil moisture fluctuations have the potential to induce activities in the rhizosphere that solubilise P. Two levels of activities are predicted to occur under the effect of DRW: (a) the immediate response of soils with onward leaching of P through the soil and (b) a longer-term response of soil where the soil fertility can be upgraded via increased P solubilisation, without any rhizosphere manipulation. However, in practice of agroecosystem, there has been little attention paid to the role of rhizosphere performance under extreme weather conditions, regardless of the approaches to improve crop productivity through the rhizosphere engineering approach (rhizosphere manipulation). However, there has been little attention paid to the role of the rhizosphere performance under extreme weather conditions.

The rhizosphere is a critical zone that is capable of buffering against waterway pollution and improving efficiency of P in the cropping system. However, the rhizosphere is highly vulnerable to extreme weather events that may change the level of processes in the rhizosphere, such as exudation, and accelerate P solubilisation (and in turn, potential P leaching). Conceptually, this could be considered an ‘enabling environment’ for improving plant performance, soil function and species biodiversity via recycling P in the rhizosphere over time with no adding fertiliser inputs (Keesstra, 2016; Lambers et al., 2013; Withers et al., 2015). The rhizosphere, with its accompanying exudation processes, is therefore the central component of this thesis. The work focusses on achieving improved phosphorus use efficiency (PUE) in the rhizosphere, optimising plant acquisition whilst reducing P losses into waterways, with particular attention to the climate change.

1.3 Phosphorus transfer continuum

The P transfer continuum was initially conceptualised by Haygarth et al. (2005) and modified under climate change by Forber et al. (2018). Tiers 2, 3 and 4 of the continuum models emphasise the P transfer across landscape to water (Fig. 1.3.1). The mobilisation (tier 2) initiates P movements from land via mechanisms of physical detachment and solubilisation based on the P compound size and biochemical properties of the soil. This thesis focuses on P mobilisation via solubilisation, with a particular consideration on the zone of the rhizosphere where plant and soil actively interact but seen in a climate change context as defined by Forber (2018). This requires a wide range of comprehensive research approaches, from cell (expression of candidate genes to tolerate moisture and P deficiency) to soil ped and profile (inherent buffering properties and long-term field evaluation of soil), including rhizosphere (plant root architecture, P solubilising exudates in root medium, soil pH and symbiotic associations of microorganisms). In this work, I will focus my thesis on climate-driven bio-physiochemical changes of soil, with respect to the potential efficiency of the P solubilisation process under drying and rewetting stimulation, associated with both the immediate (*i*-DRW) and delayed (*d*-DRW) effects.

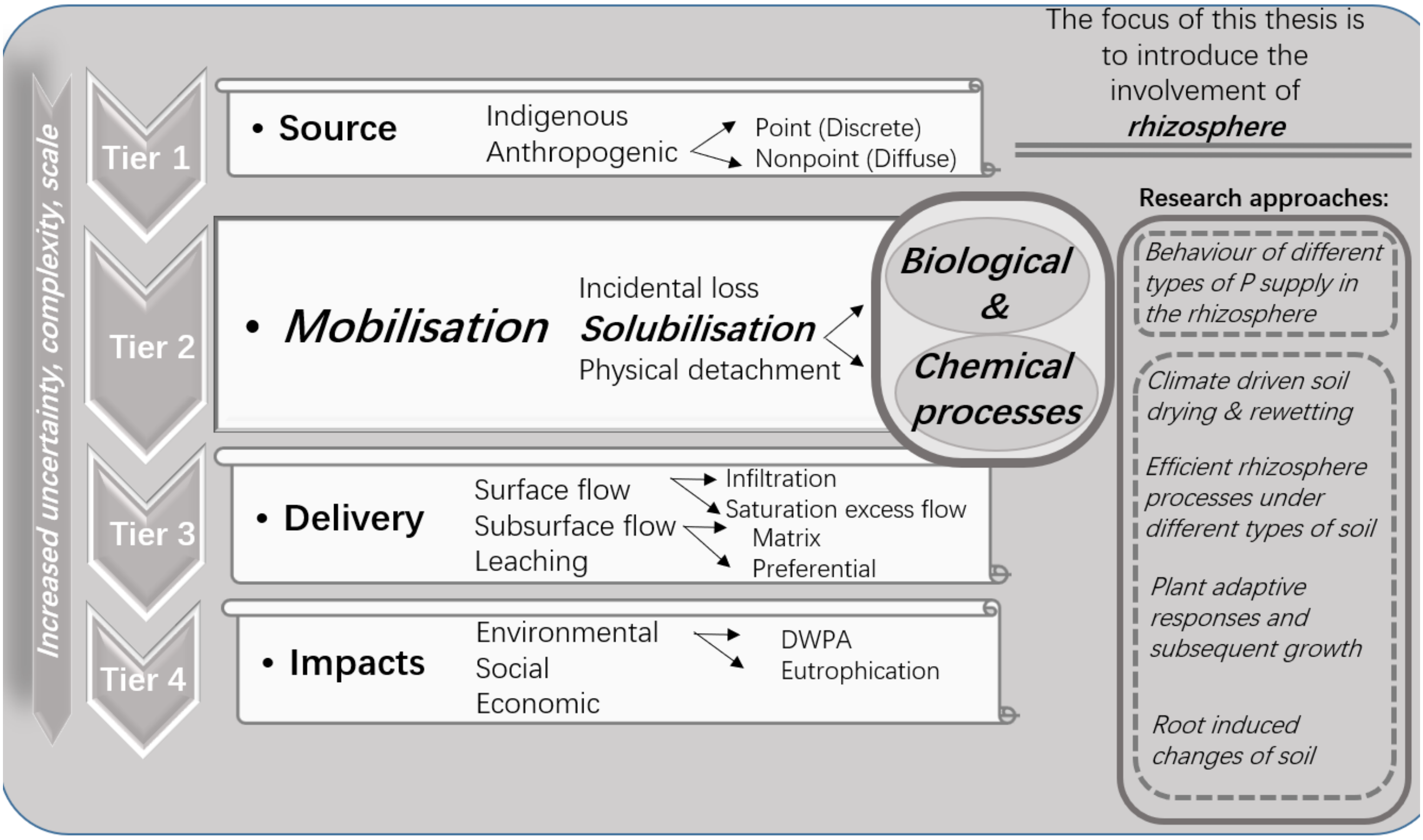


Figure 1.3.1 The 'Phosphorus Transfer Continuum' (adapted from Haygarth et al. 2005). This thesis will focus on mobilisation, and in particular how this is affected by climate driven changes in and around the rhizosphere.

1.4 Objectives and hypotheses

The aim of this thesis is to determine the potential efficiency of P solubilisation processes in the rhizosphere, in part. This also involves consideration of climate and root-induced changes generated from DRW and different root-zone acidification approaches. Additionally, the research was intended to assess, inform, and seek to improve the potential efficiency of P solubilisation processes in the rhizosphere, under the different types of P fertiliser application and rate, particularly in the direct application of the raw low-grade phosphate rock (PR).

In this thesis, I will be testing eight hypotheses, each framed under the original overarching hypothesis that is: P solubilisation in the rhizosphere can be affected by climate-driven changes of soil associated with immediate and delayed effects of DRW events and root-zone acidification, under variable P amendments, with a particular consideration of the effects of low-grade phosphate rock.

A brief overview schematic of the work was summarised in Figure 1.4.1.

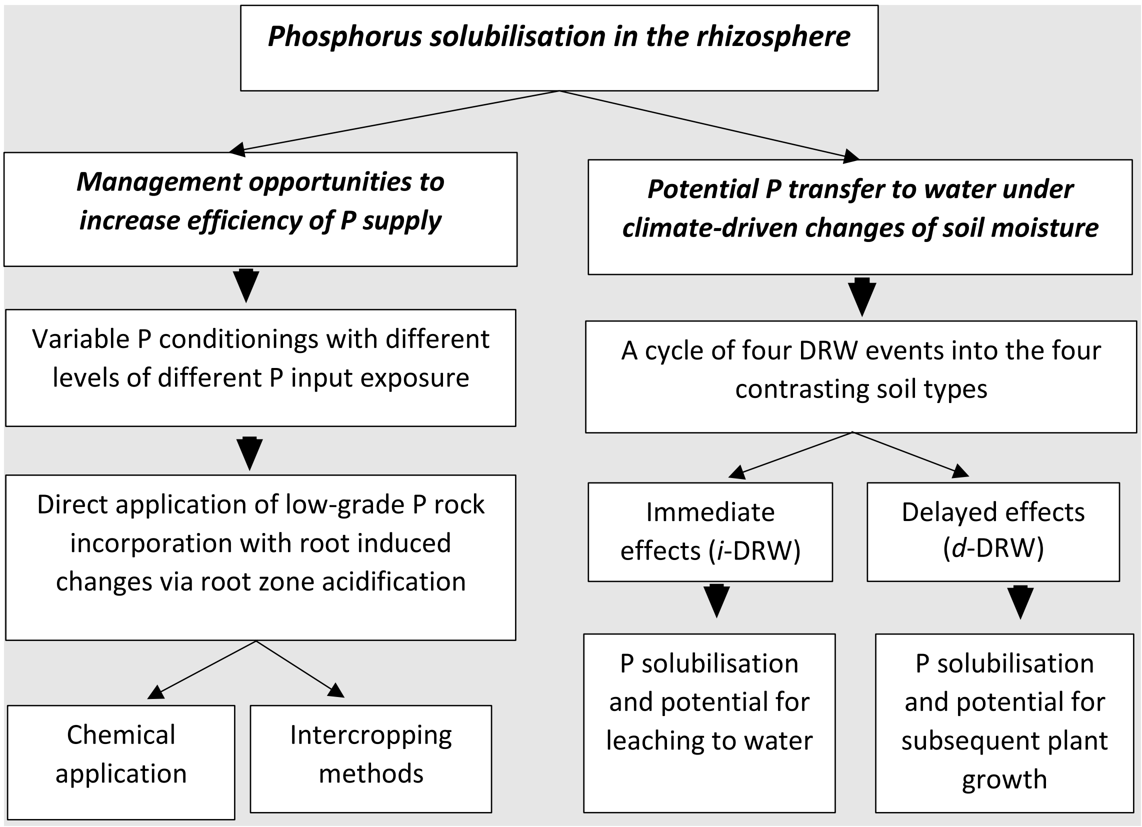


Figure 1.4.1 Schematic representative of overview of thesis structure

In doing this, the thesis will test the following eight sub-hypotheses in their associated chapters:

Chapter 2:

This chapter does not explicitly test any new hypothesises other than explore the literature related to the overarching thesis hypothesis.

Chapter 3

1. Aim: To assess the link between behaviour of P dynamics and P solubilisation processes in the rhizosphere, with corresponding plant performance under various levels of P exposure, using different types of PR, calcium perphosphate monohydrate and urea phosphate.

Hypothesis 1. Different types of P fertilisers have differing resulting effects on the P solubilisation in the rhizosphere.

2. Aim: To study the effects of rhizosphere P solubilisation processes in combination with different types of root zone acidification in soils treated with low-grade PR.

Hypothesis 2. The emission of organic acids in the rhizosphere at near neutral soil pH increases the P solubilisation of soils with different levels of P exposure.

Chapter 4

3. Aim: To study the immediate effects of DRW on the dissolved P forms in both soil solution and leachate water, using a further detailed assessment of P solubilisation processes within four contrasting soil types sampled from different agricultural regions of China, and at different P fertiliser application rates by testing the following five hypotheses:

Hypothesis 3. DRW increases the P solubilisation (and thus the potential for leaching).

Hypothesis 4. The amount of solubilised P has a potential for a leaching, being proportionate to the rate of P fertiliser applied.

Hypothesis 5. Dephosphorylating enzymes and organic acids affect the P solubilisation in the rhizosphere after DRW.

Hypothesis 6. The different soils respond differently to DRW.

Hypothesis 7. The forms of P in leachate water will vary depending on DRW event cycles and dynamics

Chapter 5

4. Aim: To observe a possible delayed effect of soil DRW stresses by studying P solubilisation in the rhizosphere, plant P acquisition and performance, and root growth on the soil with recent history of series of DRW events under different types of agricultural soils with different physio-chemical characteristics.

    Hypothesis 8. Agricultural soils with a recent history of drying and rewetting can trigger P solubilisation in the rhizosphere and a subsequent growth response of maize (Zea mays).

CHAPTER 2

Rhizosphere processes and associated phosphorus solubilisation during soil drying and rewetting – A review

2.1.1 Abstract

The rhizosphere is a biochemically active small volume of soil entwined with the root architecture. It is the critical interface and the nexus for improving phosphorus (P) use efficiency and mitigating water pollution, especially from soils of agricultural land. Within this narrow zone, P solubilisation processes can be performed to maintain the optimal P concentration for feeding the plant and organisms that live on through the support from exudative substances released into the root medium. The amount and composition of these exudates in the rhizosphere are strongly affected by the types of plant, insect, nutrient deficiency, physical and biochemical properties of surrounding soils and dynamic changes of soil moisture that are predicted during climate change. Climate change predictions indicate the changes in hydro-meteorological events and patterns of river flow and runoff with more frequent and extreme characteristics. It may have the potential onward effect on the rhizosphere. Likewise, the P loadings and their fate in the environment are estimated to increase the total P export from agricultural land to water under the changing climate, via mobilisation and delivery. Such an increase in P export into water is likely to have a knock-on effect on water quality. Consequently, the degradation of aquatic ecosystems has considerable social, economic, and ecological impacts. Here, I used a detailed literature review framed around rhizosphere research linking to the four tiers of the P transfer continuum (Haygarth et al., 2005), and particularly focussed on the potential value of P solubilisation processes in the rhizosphere, as a subcomponent of mobilisation in the wider transfer continuum, to help inform potential ways that improve P use efficiency and reduce P leakage. My appraisal suggests, the frequent drying and rewetting (DRW) events associated with the changing

patterns of precipitation in terms of degree, rate, and frequency, may impose stress on P cycling in the soil- rhizosphere- plant- water system. The purpose of this literature review is, therefore, to focus on every component of the P solubilisation process in the rhizosphere with their role in the mobilisation tier of the phosphorus transfer continuum and the efficiency of these processes under the climate change scenarios, as a basis for framing understanding of the later experimental chapters. Within the broad perspective of the review, I will also consider the various aspects of the P cycle in the soils including the nature of soil P chemistry in the relative order of importance from absorption to transfer ranging from biogeochemical eras to minutes and ecological impacts. The schematic representation of the overview of the whole systems reviewed in this chapter is visualised in Figure 2.2.1.

2.2 Introduction

The rhizosphere is a biochemically active small portion of soil around the root that adheres to the root segments, known as rhizosphere and rhizoplane, respectively (Marschner, 2011). While feeding the organisms that live on the soil surface (Morrissey et al., 2004), the rhizosphere contributes to plant P acquisition as well as minimizes P losses from soil to water by maintaining optimal P concentration in soil solution. The soil-based P cycle involving the rhizosphere involves complex small-scale process, that can respond to localized changes (Frossard et al., 2011) within a relatively short period of time. However, the rhizosphere can be viewed as fundamentally structural and functional units to play an essential role in nutrient cycling throughout the water-soil-atmosphere continuum through a wide range of activities. These activities also allow the rhizosphere soils to be viewed as a target area to meet the global challenges on food demand, providing security and environmental wellbeing. A detailed analysis of P solubilisation processes of the rhizosphere is critical in my research, as the consequential changes of soil moisture associated with climate change have a potential to influence P transfer to water bodies.

From an agronomic point of view, the rhizosphere ranges from nm to km scales due to spatial and temporal heterogeneities (Young and Ritz, 2000). The processes in the rhizosphere and the outputs are directly linked to the volume of soils where their abiotic and biotic factors are presented, including soil texture, water content, organic matter content, binding agents, and root and microbial rhizosphere exudates. The rhizosphere exudates strongly influence chemical properties of the soil, suggesting the critical factors of ecosystem functioning. It consists of high and low molecular weight compounds. The high molecular weight compounds (HMW) include: glycoproteinaceous mucilage, polysaccharide, proton, various enzymes, and phosphatases; and the low molecular weight (LMW) organic exudates and acids consisting of tribasic citrate, dibasic oxalic acids, organic mixture of acid anions, carboxylate, phytosiderophores, phenolic sugar, amino acids (Jones et al., 2009). The concentration gradients of organic exudates in the rhizosphere attract beneficial organisms through chemical signalling, molecular communication, quorum sensing and endophytes (Badri et al., 2009). It includes glycoproteinaceous mucilage, sugars, organic acids, amino compounds and polysaccharide, and some signalling substances (Jones et al., 2009). The increased efflux of exudates into the rhizosphere (Hedley et al., 2010; Hoffland, 1992) have been observed during conditions of both P and water deficiencies (Lyons et al., 2008) associated with the upregulation of candidate gene expression of plants and microbes that can synthesize the enzyme to stimulate P solubilising exudation.

Within this narrow space of the rhizosphere, the plant and microbial associations interact (Toal et al., 2000). The rhizosphere microbial communities include microflora (plant-growth promoting rhizobacteria, endophytes, and fungi, even pathogenic microbes), microfauna (parasites, nematodes, and protozoa) and microarthrops (worms and ants), other plants and invertebrate herbivores (Raaijmakers and Weller, 2001). The underground chemical, biological, and physiological processes of the resident microorganisms and plant roots (York et al., 2016) play a key role in the P use efficiency (Yang and Finnegan, 2010). The rhizosphere community enhances the nutrient solubilisation and acquisition through (a) turnover of microbial biomass; (b) acceleration of root, root hair and plant growth through indirect hormone-induced stimulation of rhizobacteria; (c) increase in root surface area by mycorrhizal association; and (d) displacement of sorption equilibrium between different pool by altering the distribution of organic and inorganic P forms. Upon dissolution of P in the root medium, the rhizosphere community can also remobilize stored P, particularly in leaf senescence during the autumn (Roberts et al., 2012) to improve the nutrient acquisition of living organisms (Shen et al., 2011).

Despite this generally accepted view in respect of the mutually beneficial relationship between rhizosphere communities and plants, in my view, there is an arguable case to suggest that there might have a constant competition for nutrient uptake in life beyond the rhizosphere at the same time, whereby soil microbes compete better through an efficient biological mechanism to enhance level of P solubility. However, the soil microbes can act as a P source (Achat et al., 2010; Zhang and Marschner, 2018), through their biomass specifically under a pulse of soil drying and rewetting exposure (Pachauri et al., 2014), resulting from drought and flood; and excessive irrigation, where the sudden change of accessible water for plant and microbial activity will be likely to alter the biotic and abiotic properties of the soil. A soil water potential (SWP)of around -0.7MPa is considered optimal for plants and soil microbial communities (Whalley et al., 2013) by. When SWP is reduced to -2 MPa, the mortality of microbial biomass has been found to increase (Turner and Haygarth, 2001) by 35-50%, with water-soluble organic P originated from soil microbes via bacterial cell wall rupture and lysis of microbial cells (Sparling et al., 1985), and decomposition of dead cells (Van Gestel et al., 1991). The remaining survivor microbes reduce their physiological activities, for example, a turndown of respiration to 5-8% at -1.5 MPa (Chowdhury et al., 2011).

Given that the rhizosphere holds processes of soil P solubilisation at varying rates of speed (Haygarth et al., 2005; Weiler and Naef, 2003) associated with the level of soil moisture fluctuation, it can also directly influence the P movement into the surrounding soil and drainage water through the onward leaching of solutes via eluviation (Wagenet et al., 1990) and infiltration processes across the soil matrix and root-induced subsurface channelling. The subsurface channelling, which was established under the natural root growth, such as elongation, anchorage and branching supported by exudation processes, border cells and lubricating mucilage (Shen et al., 2011), and bio pores derived from rhizosphere invertebrates can drastically alter the porosity (Feeney et al., 2006) and hydrological behaviour of the soil (Aravena et al., 2011; 2014). Specifically in arid lands, an occurrence of persistent soil drying enlarges the size of pore spaces around the root (Allen, 2009) that can create “enabling conditions” of the preferential flow to transfer the nutrients physically (Sims et al., 1998).

The percolation pathway facilitates the movement of dissolved and particulate P forms alongside the root channel and throughout dry soil cracking under rainfall events (Blackwell et al., 2009; Gordon et al., 2008; Turner and Haygarth, 2000). However, the interconnection of soil channels and pore networking that can carry out a flow is a complex natural phenomenon. In this review, I will particularly focus on a deeper insight into P solubilisation processes in the rhizosphere (a key component of P mobilisation discussed in the previous chapter) under the immediate (*i*-) and delayed (*d*-) drying and rewetting effects, in order to highlight an importance of the rapid and subsequent responses of the soil for the potential leaching and plant benefits respectively with the further detailed exploratory laboratory studies of rhizosphere process.

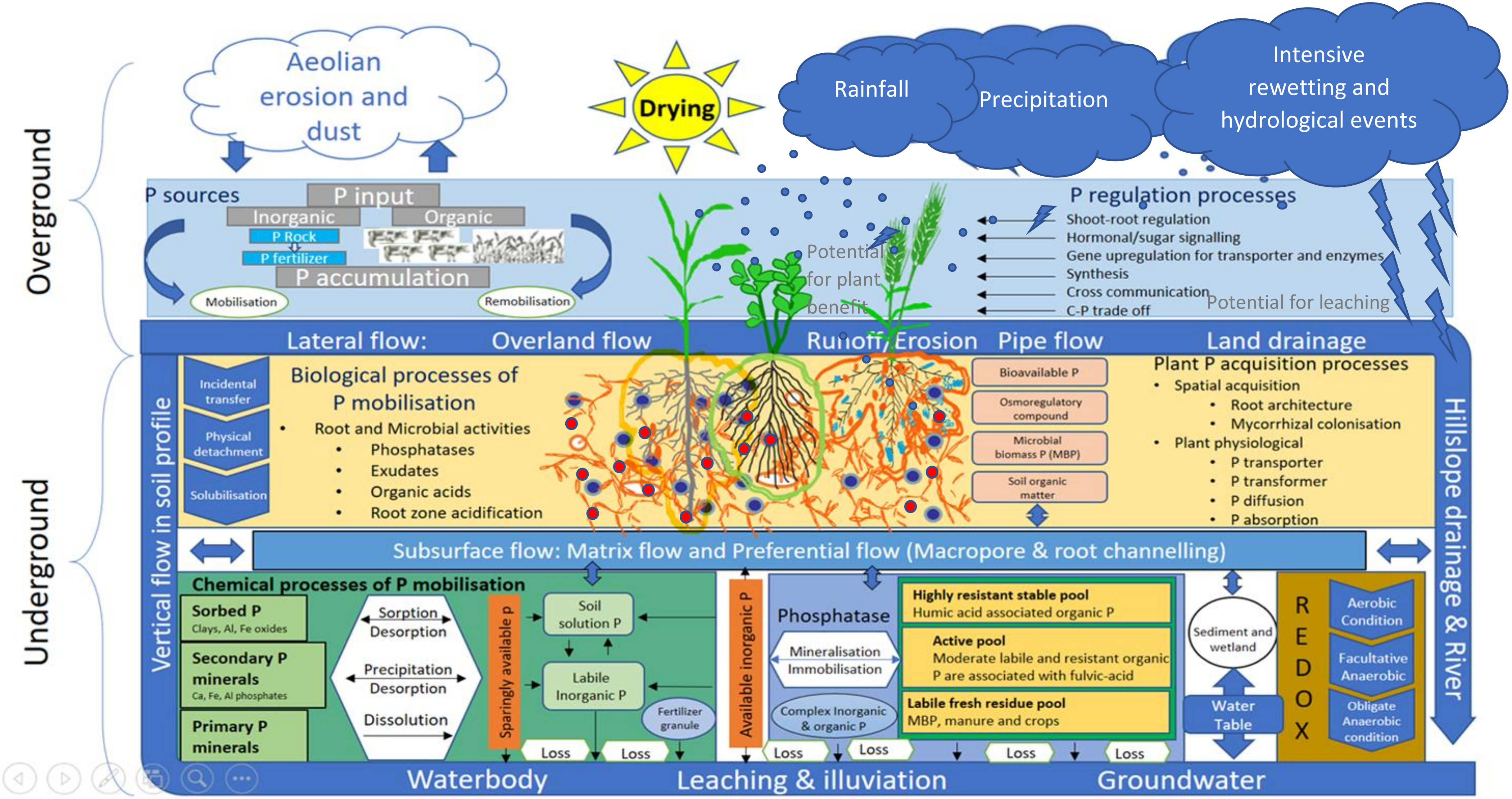


Figure 2.2.1 Rhizosphere processes and associated phosphorus solubilisation in water-soil-rhizosphere-atmosphere continuum including interaction between plants, soils and microorganisms

2.3 Immediate and delayed effects on soil drying and rewetting

Climate change has a potential to influence P solubilisation in the rhizosphere. Global warming leads to change the sequence of weather and hydrological events with the increased heatwave frequency by 2-4-fold (Christensen et al., 2013). Indeed, the surface temperature of the planet has increased by 0.7-0.9^{o}C per century since 1901 and doubled since 1975. Further, this figure is predicted to rise continuously, for instance, more than 0.5 ^{o}C on the average of 1985-2005 (www.climate.gov). Accordingly, the increased global temperature would certainly affect the pattern of total annual precipitation over the land area with an increasing risk of intense rewetting over time (O'Gorman and Tapio, 2009). However, despite being wetter than an average of 0.002 m per decade (between 2000 and 2015), some portions of the land area had been also affected by drought at the same time. Consequently, 20-70% of the U.S. land area has been abnormally dry at any given time (www.epa.gov).

This precipitation and temperature fluctuation can cause onward effects on drying and rewetting (DRW) in the soil. Many agricultural regions of the world are indeed vulnerable to climate change effects such as intense rewetting and drying during the cropping period (Pearce et al., 2007), due to the changing patterns of seasonal rainfall. One example is around the Yangtze River basin and other semi-arid areas of China. Such natural occurrence of soil DRW can also raise environmental concerns of accelerated of P fluxes from land to water (Ockenden et al., 2016). It can also affect the P cycling across all levels from global and geographically local scales on a seasonal and annual basis. The underlying mechanisms of the P mobilisation tier including physical and biochemical processes operating during DRW events are illustrated in Figure 2.3.1.

A significant fluctuation of soil moisture as a result of lengthy intensive warm spells and heavy rainfall events leads to the pulse emission of carbon dioxide (CO_2) into the atmosphere. The soil organic material associated with carbon Fe and Al becomes oxidised as a result of the flush of soil respiration (Bartlett and James, 1980; Haynes and Swift, 1985; Schlichting and Leinweber, 2002), as carbon (C) fundamental building block of all organic molecules. During the immediate rewetting of drying soil (*i*-DRW), the soil respiration rate reaches a level five times higher than stable moistened soil within an hour (Iovieno and Bååth, 2008; Rey et al., 2005; Rudaz et al., 1991). This state can persist for ten days (Fierer and Schimel, 2003; Franzluebbers et al., 2000; Pesaro et al., 2004) under the delayed DRW condition (*d*-DRW). However, P and C cycles seem to be linked closely. This period with stronger and more significant P pulse events (Bünemann et al., 2013) lasts longer than C dynamics in the soil (Chen et al., 2016a). The performance of P transformation between the pools under the *d*-DRW is associated with subsequent stimulation of P mineralization (Styles and Coxon, 2006), by intracellular phosphatase enzymes in soil solution released from microbial cells, outlined in Figure 2.3.1.

The accessibility of water level for plants is measured based on soil water potential (SWP) associated with soil particles (Whalley et al., 2013), which consists of three components; (i) gravitational; (ii) osmotic; and (iii) matric (pressure) potential. Severe drying (SWP < -0.02 MPa) reduces yields by 22.6 % imposed throughout the crop life cycle (Carrijo et al., 2017). Decreasing the level of water contents and SWP may contribute to a similar process for nitrogen (N) (Fierer and Schimel, 2003) that is known as the "Birch effect" (Birch, 1958). However, the strength of 'flush' activity heavily relies on many factors, such as frequency and intensity of the DRW cycles (Butterly et al., 2009), soil characteristics including high organic carbon and microbial biomass in their contents (Nguyen and Marschner, 2005; Styles and Coxon, 2006), and changes of microbial community composition (Blackwell et al., 2010a; Gordon et al., 2008a; Turner and Haygarth, 2001b). It has been assumed that plant growth after the rewetting of soils depends on the recovery of photosynthesis and plant function (Yang and Zhang, 2006). Yet, the mechanisms underpinning subsequent plant responses to soil DRW are uncertain. Therefore, an in-depth understanding of the soil response to immediate and delayed DRW events can be understood to help optimise the management techniques for improving P use efficiency and reducing P transfer in the rhizosphere under climate change.

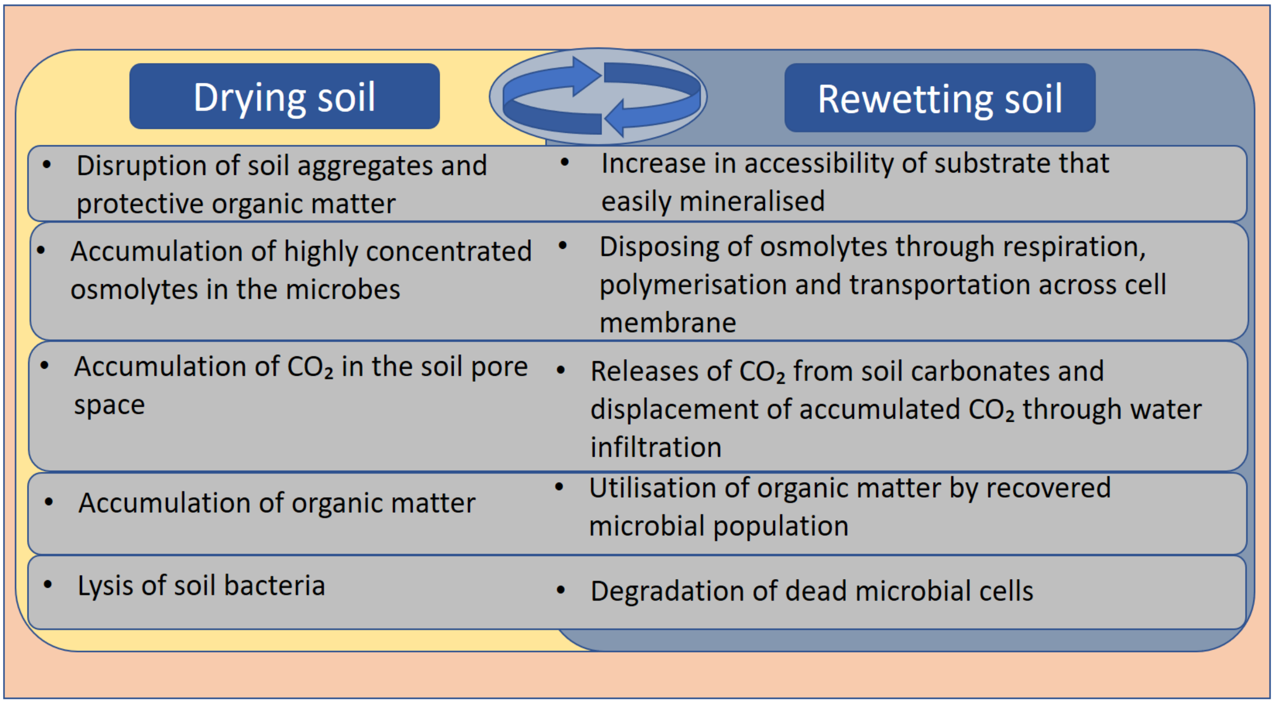


Figure 2.3.1 Schematic representative of immediate drying rewetting effects on processes in the soil, adapted from Kim et al. (2010).

2.4 Phosphorus biogeochemical cycle

The P biogeochemical cycle is the movement of P through the lithosphere, hydrosphere, atmosphere, and biosphere (Fig. 2.4.1), which leads to the natural distribution of P in the soil and water. In ecosystems, P is efficiently cycled throughout the soil-plant-water-atmospheric systems under a restricted way with tight control of soluble P, as it naturally keeps the proportion of the soluble P forms into the soil solution to a minimum through the natural releases of mineral breakdown processes. At the global scale, the P cycle usually takes many thousands of years to complete, involving several major components, including weathering of rocks, riverine transport, and sedimentation. However, the P biogeochemical cycle has already been disrupted at global, national, regional, and farm scales (Haygarth et al., 2014; Sharpley and Jarvie, 2012; Ulrich et al., 2013) in ways of altering reservoirs and accelerating fluxes. As a matter of fact, the fluxes of P from land to water far exceeds the natural pre-human era levels, due to the anthropogenic impacts, originating from a wide range of agronomy orientated action and other human activities (Haygarth et al., 2005; Withers and Jarvie, 2008). An increased P input into agricultural land associated with human activities coupled with organic matter and sediment resuspension would influence in P flux resulting in more reactive P in the waterbodies in nowadays than the prehumen era, when total P fertiliser application into the croplands was estimated to increase globally from around 14.2 Tg of P yr^{-1} (2005) to 22-27 Tg of P yr^{-1} (2050) (Mogollón et al., 2018). A flux of potential reactive P was estimated at around $4.8x10^{12}$g per year (prehumen era) and $3.4\text{-}10x10^{12}$g per year in the present day (Compton et al., 2000).

The primary P-containing parent materials are the indigenous P sources of land. The natural occurrence of weathering processes of the P sources is initiated by the tectonic uplift activities, including landslides, earthquakes, and volcanic eruptions (Reynolds and Davies, 2001),. The P mobilised from weathered rock is estimated to have been distributed in soil and water at the rate of 15 tg y^{-1} over a long period of time by rainfall action (Bennett et al., 2001). The rainfall is the driving force behind the P distribution in the whole system by providing the energy to support the P transport. Chemical weathering of apatite minerals can dissolve P via hydrolysis, oxidation, and carbonation reactions and then facilitate the bioavailability of soil P stocks in the terrestrial system (Filippelli, 2008). As a result of the weathering reaction, dissolved P (DP) and carbon dioxide form the carbonic and phosphoric acids. The reaction is as follows:

$Ca_5(PO_4)_3OH + 4H_2CO_3 \leftrightarrow 5Ca^{2+} + 3HPO_4^{2-} + 4HCO_3^{-} + H_2O$

A large proportion of DP fractions and weathered P containing materials constitute the base of the food of terrestrial and aquatic systems by assimilating into the biosphere through plant uptake processes (Schulze et al., 2002). In each growing season, only 1% of the total soil P around 400–4,000 kg P ha^{-1} in the top 30 cm is assimilated into living plant biomass at 10–30 kg P ha^{-1} (Blake et al., 2010; 2000). Once animal production and plant decay are returned to the soils, the transformation of insoluble organic compounds takes place concurrently. However, some organic P deposits in the biomaterials are very difficult to mineralise through the P solubilising microbial and faunal communities. The phosphorus journey across the terrestrial system ends the sediment deposition, where energy eventually runs out at this final stage.

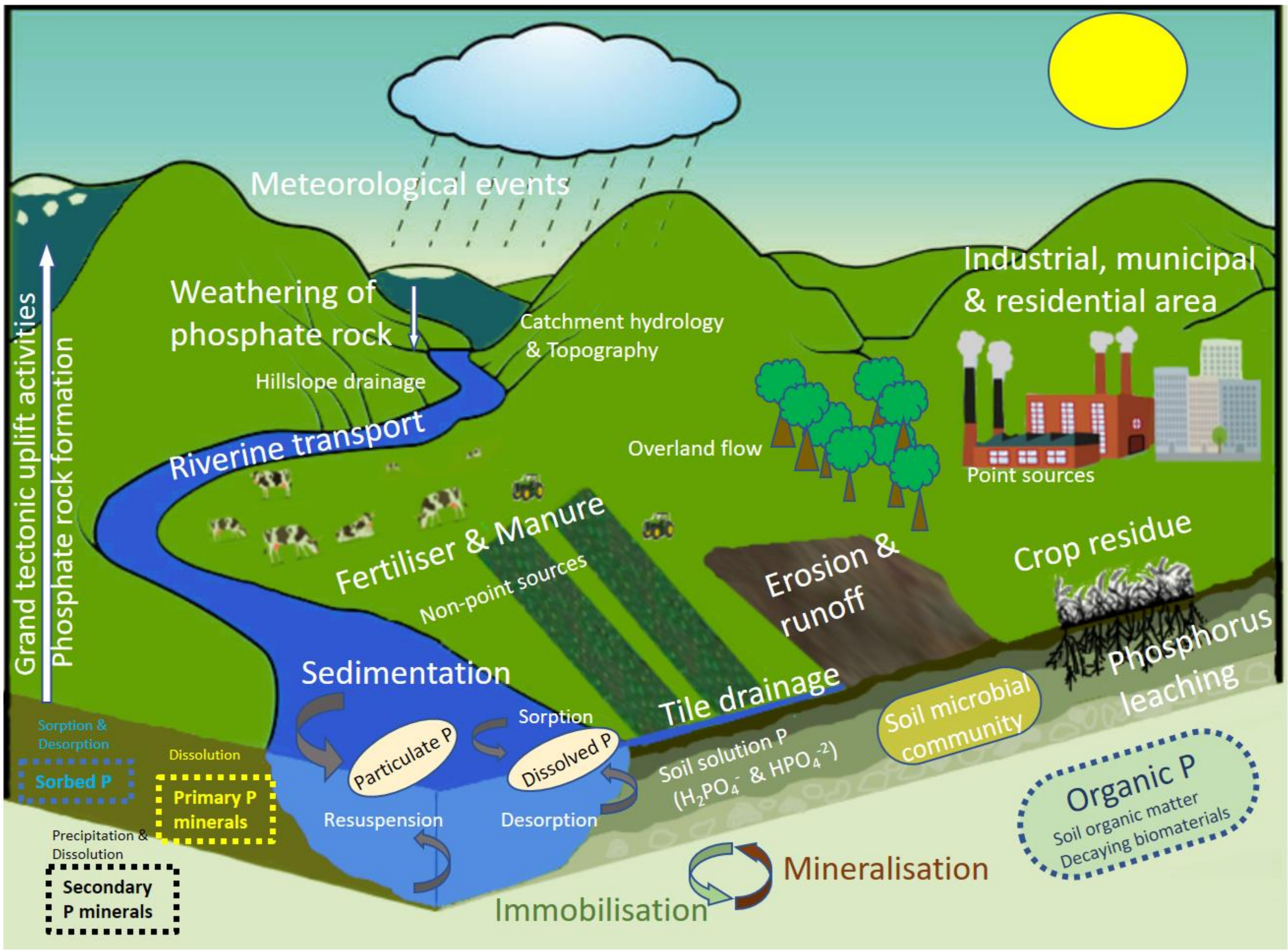


Figure 2.4.1 Phosphorus biogeochemical cycle

The level of available P stocks is found the greatest in young soils and then continuously declines during the pedogenesis (Walker and Syers, 1976), because of organic P occlusion (Turner et al., 2013). Whereas, the low total P (TP) concentration in the soil commonly occurs in many parts of the world that are associated with the old landmasses known as global hotspots, for example, Australia, South Africa, and South America (Myers et al., 2000). According to a study in a chronosequence from Cooloola, Australia spanning 500-460,000 years (Jones et al., 2015; Thompson, 1981), the soil P stocks, which are derived mostly from parent materials of P mineral sources and apatite, are linearly reduced within the dune age.. A gradual depletion of soil bioavailable P in terrestrial ecosystems can be explained by (a) organic P occlusion over some time via biological and geochemical processes (Turner et al., 2013) and (2) direct loss of bioavailable and other P fractions via crop uptake, soil erosion and runoff transport to the aqueous environment.

2.5 Phosphorus forms in soil

The environmental P distribution and speciation are highly variable in space and time. The common concentrations of TP in soil range from 10^1 to 10^3g P kg^{-1} reviewed by Kruse et al. (2015), which is a combination of all forms of P such as primary minerals, soluble, labile, secondary minerals, organic and occluded (Fenton, 1999; Turner et al., 2013). Thirty to 65% of them are derived from biomaterials (Harrison, 1987). As soil horizon formation increases, the degree of solubility and stock abilities also differ a great deal from the topsoil, to deeper layers within the soil profile (Mcdowell and Sharpley, 2001). It is being incorporated with the differentiation of native vegetation species and biodiversity (Fenton, 1999). For example, total P contents in soils (up to 25 cm deep) are estimated to range from 150 to 700 μg P g^{-1} (Wild, 1988). However, it depends on the soil's inherent and anthropogenic factors, including the history of P fertiliser application, the time span since the last fertiliser applications (Mcgechan and Lewis, 2002), amount of net P losses, reductions in soil quality, total organic carbon (TOC), organic matter and crop yields.

2.5.1 Inorganic phosphorus pool

The inorganic P forms can be practically divided into several different pools that are applicable for uses of a broad scope of purposes in relation to plant, microbial and soil P (Turner et al., 2013). This contains soil solution and active and fixed pools based on soluble, loosely labile and stable mineral phosphate fractions respectively. These P fractions are in a constant state of shift within the pools, in accordance with the concentration gradient under the various time spans. The organic and mineral P fractions can also be transformed within the pools, visualised in Figure 2.5.1. In the soil solution pool, the solubilisation processes lead to maintaining the soluble P at the minimal level sufficient for the plant uptake which is approximately 1% of the total proportion of P through the rapid adjustment of equilibrium for replacement of the amount removed from the solution pool.

The soluble P in the soil solution is typically about 1 kg ha^{-1} at a given time (Pierzynski, 1991), which is equivalent to 1-5 µM (Bieleski, 1973). Once P in soil solution reaches lower than 0.5 µm (McDaniel and Wild, 1988) shortly after the removal by plants and microbes, it is continually replenished from the solid phase. The orthophosphate ions in soil solution could possibly be replaced 20 to 50 times per day at a different rate (Marschner, 1995), according to the P demand of living organisms (Menezes-Blackburn et al., 2016b). The fertiliser application that can directly contribute to the soluble pool (www.nrcs.usda.gov) for example, ranges from below 2 µM in the non-P added soil (Menzies et al., 1994) to more than 20 µM in the highly fertilised soil (www.grdc.com.au). The soluble P can also enter a labile pool or transform into secondary minerals.

The active pool contains the labile P forms that are attached to the small soil particles. The P in the soil solution is in equilibrium with chemisorbed P on the surfaces of Fe and Al oxides and carbonates. The active pool produces soluble solids as it reacts with elements such as Fe, Al, Mg, and Ca. The chemisorbed P is isotopically exchangeable or anion resin extractable. The amount of phosphate within this active pool is linked closely with the dissolution kinetics of secondary minerals. Many secondary minerals, including dicalcium phosphate dihydrate or octocalcium phosphate, equilibrate rapidly with labile and soluble P. Equilibrium of some of them is seldom attained for example hydroxyapatite. Phosphorus compounds absorbed on the inner surfaces of soil components are less accessible for plants. Again here, non-labile P can also be transformed into labile and soluble forms to substitute the plant available P throughout desorption and dissolution of inorganic P as a concentration of P in the soil solution changes.

The fixed pool contains the non-labile P forms, including insoluble inorganic P compounds and strongly bonded P sources, which take many years to solubilise (Syers et al., 2008). For example, occluded P is comprised of physically encapsulated minerals, some of them have no P in their structure (Fenton, 1999). The primary mineral is represented in this P pool as parent material and apatite, such as octocalcium phosphate strengite, vivianite, variscite, and wavellite. Approximately 95% of apatite occurs as fluorapatite (Holtan et al., 1988). Apatite can be hydroxylapatite, fluorapatite, and chlorapatite, based on the dominant ion contents in their crystalline, such as OH^-, F^-, and Cl^-, respectively.

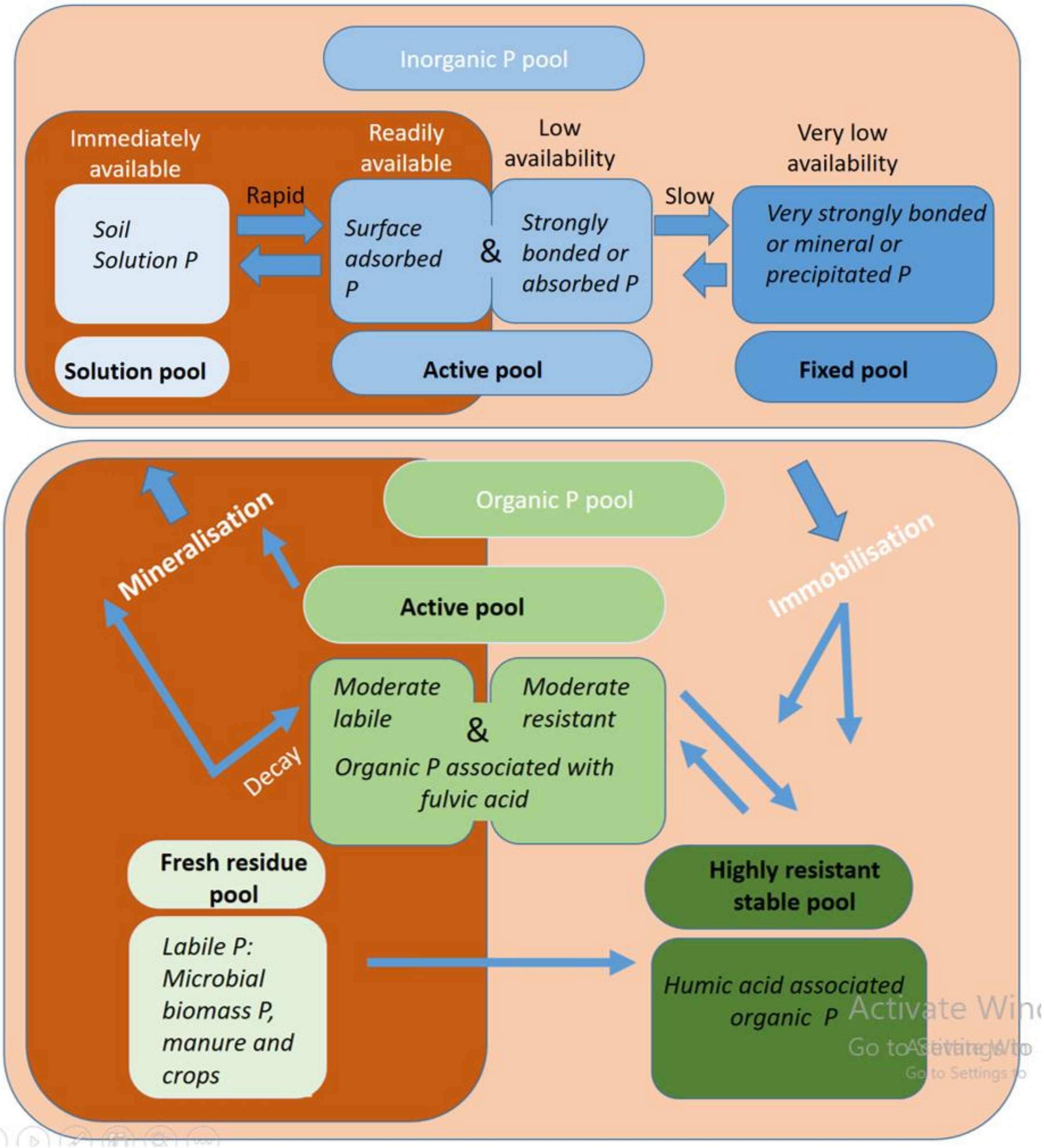


Figure 2.5.1 Schematic representation of the phosphorus pool in soil, redrawn from Johnston et al. (2014).

2.5.2 Organic phosphorus pool

The quantitative relation of soil solid part is at a ratio of 1:9 organic materials to minerals including the fraction of soil organic matter (SOM) ranging around 1-6% (Brady and Weil, 1999). This is a blend of biomaterial decay and newly formed stable organic compounds. Despite a relatively small proportion, the accumulation of soil organic P, however, has a great potential to provide a P source (Cole et al., 1977) of the P transfer. For example, the presence of a large amount of the total P in soil solution and leachates exist as a form of organic P (Turner and Haygarth, 2000; 2001a; 2001b), suggesting the role of organic P across the global P cycle (Hayes. 2000).

Organic P can be classified into labile, moderately labile; moderately resistant; and highly resistant stable forms. It is divided into three pools, including fresh residue, and active and highly resistant stable pools (Fig.2.5.1). The proportion of P forms between pools is controlled by the various bio-physiochemical and environmental factors balancing between the mineralisation and immobilisation process. A labile form in a fresh residue pool consists of P in microbial biomass, manures, and crop residues. Both moderately labile and resistant organic P are associated with fulvic-acid, presenting in an active pool where mineralisation of organic P occurs. Highly resistant stable organic P belongs to humus pools. The humus pool contains humic acid associated organic P fractions in soil and water (Borie et al., 1989), which is very resistant to mineralisation (Bowman and Cole, 1978; Randriamanantsoa et al., 2015). The humus can improve the soil structure, specifically porosity, through its colloidal nature. The humus acts as a bridge between large particles and the surface charge of mineral aggregates through granular structure, which has a huge impact on holding the water within the soil (Brady and Weil, 1999). Although it appears that humus can minimise leaching by storing nutrients, in the long term the accumulation of such organic P in the soil has the potential to become a main source of the P transfer (Cole et al., 1977), even in the future circumstances of net P input decline in land (Powers et al., 2016).

The organic P compounds consists of 3 types in the natural environment: (1) phosphomonoester (H_2O_4PR): *myo*-inositol phosphate (IP) known as phytate (2) phosphodiesters: RNA, DNA and phospholipid and (3) organic polyphosphates: ATP. Some organic P compounds are very insoluble, as they fix firmly with minerals and soil constituents. However, the presence of the most dominant organic P fraction in the soil (around 60-90%) is characterized as weakly sorbed orthophosphate monoesters and diesters (Chang and Jackson, 1957). The free phosphate ions can be easily released from most monoesters and diesters via the cleavage of phosphomonoesterase. Therefore, there has been a debate over the plant availability of organic P compounds, specifically weakly sorbed simple phosphate monoesters e.g. D-Glucose 6-phosphate (Turner, 2010), as it looks increasingly likely to act as bioavailable P for sustainable crop production through the dissolution process.

The biggest representative of phosphomonoester is the phytic acid (the alternative name myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate). The structure of phytate is pH dependent (Shang et al., 1992), for example, the axial structure in a pH between 5 and 12, whilst the equatorial structure is out of this range. Due to the high anionic charge (Turner et al., 2002), soil phytate strongly correlates with P sorption capacity for binding sites of 12 ionisable protons, which is very similar to the orthophosphate (McKercher and Anderson, 1968). It behaves like a strong ligand that adsorbs with Al and Fe and amino acids to form the protein–phytate–mineral complexes (Kies et al., 2006). However, the retention capacity of phytic acid with Fe and Al oxides (Celi et al., 1999; Ognalaga et al., 1994; Shang et al., 1990) is weaker than the phyllosilicates (Celi et al., 2003). The enzymatic hydrolysis can break down phytate into inositol, phosphate, and other divalent elements that contain about 14 to 28% phosphorus, 12–20% calcium, 1 to 2% trace elements of iron and zinc. The release of P originates particularly from phytate (e.g., plant materials and monogastric manure) is likely to be easier and more sustainable over time, as a large part of organic P is made up of phytate.

2.5.3 The distribution of plant-available phosphorus in the soil

Maintaining bioavailable P in the soil is one of the considerable challenges, incorporating a broad spectrum of issues in P cycling ranging from macro- to micro-scales, particularly under climate change. At the macro geological level, the weathering of rock materials in the biosphere is not sufficient to fulfil the demand of large crop yields, due to the nature of the long-term breakdown processes of P bearing sources. In addition, the amount of P lost in runoff via rainstorms, soil erosion and dust storm to ocean deposits (Chadwick et al., 1999; Gilkes et al., 2002) are greater than the replenishment generated from bedrock weathering, as it interacts with extreme climatic events, including intense rainfalls and cyclonic winds. Further, excessive irrigation can also be taken into consideration. It has been found that the occasional interruption of soil rewetting through intense rainfall elevates the total P loading to water bodies (Ockenden et al., 2016). In arid land, P bounded to small soil particles is susceptible to a critical decrease through aeolian erosion by cyclonic winds (Katra et al., 2016).

At the cropland level, many types of modern agricultural activities appear to simultaneously affect P solubilisation and potential transfer, as it is coupled with environmental variable events, via heavy rainfall and long dry season during the cropping period at different temporal scales. The agricultural activities include the agricultural P loading, frequent removal of P from arable land with the harvesting of crop production, soil compaction, cropland irrigation, artificial tillage, drainage and heavy agricultural machinery. In particular, the conservation and conventional tillage practices can lead to an increase in tillage-driven P loss to waterways via runoff and leaching associated with the higher infiltration rates and total volume of runoff (Blevins et al., 1990; Wendt and Corey, 1980). Likewise, the use of heavy machinery in agricultural practices can often result in soil compaction, which in turn makes it difficult for plants to obtain essential nutrients at sufficient levels. For example, plants grown in compacted soil can only absorb approximately 60 percent of nutrient substances (Fugelsnes and Lie, 2011).

At the micro level, plants and soil microorganisms play an important role in a nutrient cycle through the processes in the rhizosphere. In the rhizosphere, the plants can distinctively reflect the underlying conditions of the P balance for intake. Generally, the proportion of P in the total dry weight of plant over-ground parts is surprisingly high, at approximately 25% (www.agric.gov.ab.ca). However, this is likely to be the case for young and actively growing ones. Plants tend to contain 1000 times higher P than their external root medium (Schachtman et al., 1998)as compared to the presence of P against the soil. Concurrently, the zone of P depletion around the root often occurs in the rhizosphere, after plant P uptake. Such depletion may be linked with the relative immobility and diffusion rate of P, ranging from 10^{-12} to 10^{-15} m^2 S^{-1} over a short distance (Rengel and Marschner, 2005a; Schachtman et al., 1998). As a result, plants employ several adaptive strategies to obtain P (Bucher, 2007)via an increase in active transport, root morphology, synthesis of dephosphorylating enzymes and organic acid excretion, and symbiotic interaction with soil microorganisms.

2.6 Phosphorus resources

The sources of P fertiliser input to the farmland are largely derived from mined and purified phosphate rock (PR). Currently, the world reserves of PR sources are estimated at 60 billion tons (Jasinski, 2011). The global P reserve is unevenly distributed worldwide (Fig 2.6.1) and more than 170 different P containing minerals have been identified (Holford, 1997). The phosphate rock is varied in terms of mineralogical, textural and chemical characteristics as an igneous and sedimentary rock, due to the deposit cycles, level of burial and heat. Ten percent of world P reserves exist as low-solubility igneous and metamorphosed sedimentary rock. Igneous PR deposits are associated with the apatite of magmatic and hydrothermal origins with 42% of P_2O_5 contents. It includes fluorapatite and chlorapatite. The igneous rocks are low-soluble materials. Some of them are found in hard crystalline forms, for example, the Kola deposit in Russia. Whereas, the sedimentary deposit is an unconsolidated and indurated rock, containing high carbon substitutes at the ratio 1:1 with 33% of P_2O_5 contents. As compared with igneous rock, sedimentary rock deposits are more soluble. Highly soluble sedimentary rock accounts for 1% of world reserves, according to the United States Geological Survey (USGS). However, the quality issues of some sedimentary PR, particularly from Morocco remain a major concern, due to the high level of cadmium (Cd) content at an average 32 mg kg^{-1}, which is toxic to humans.

Morocco, China, and the USA are the biggest phosphate mine producers, supplying 70% of global P production. Almost 90 % of them are utilised for food production (Cordell et al., 2009). According to commercial fertiliser producers, the process of soluble inorganic P fertiliser production is segmented into phosphoric acid, granular ammonium phosphate and superphosphate (Cheremisinoff and Rosenfeld, 2011). Briefly, the production of phosphoric acid is the initial step of a manufacturing procedure, using either a dry or wet method. The electric furnace is the dry method to produce pure white high-grade chemicals. The reaction with sulphuric acid is applied to employ, under the wet process, for the phosphoric acid production (P_2O_5) at the concentration rate of 72-76% (www.extension.umn.edu). The phosphoric acid reacted with anhydrous ammonia and PR and then produced AP and SP respectively. It is worth mentioning at this point about the mining operation. The raw material is beneficiated here by removing waste from mine ore and floating calcium to upgrade these products to meet the prevailing market grade. During this manufacturing performance, the large stockpiles of the by-products are generated that contain hazardous impurities such as heavy metals, sulphates and radioactive substances (Straaten, 2007; Tayibi et al., 2009). The attempts of direct application of PR (DAPR) are made to avoid such adverse impacts via detailed further in Chapter 3.

The PR is a finite non-renewable natural resource. This natural reserve would last from 300 to 400 years (Van Kauwenbergh, 2010) at the current P consumption rates of 160 to 170 million tonnes per year, according to the United Nations Environment Programme yearbook 2011 (Woertz, 2013). According to the United States Geological Survey (USGS) in 2017, the world mining production in the year 2016 reached 0.261 million tons to meet the global P demand, trending continuously upward. Likewise, the total global P inputs increased at rates of 35 Tg P yr^{-1} in 2005 and 41 Tg P yr^{-1} in 2010 with a projection of a large increase in the future, for example, 53 Tg P yr^{-1} in 2050 (Mogollón et al., 2018). As the pressure on the P budget would cause a severe crisis of high-grade PR shortage in the next few hundred years (International Fertiliser Development Centre report in 2010), the key priority of agroecosystems is to increase the efficient use of this scarce raw material and reduce P fertiliser inputs without lowering the size of yields. In turn, controlling the source of P transfer would be the most cost-effective mean of measure to minimize the adverse off-site substantial impacts on natural cycle of P and on water quality.

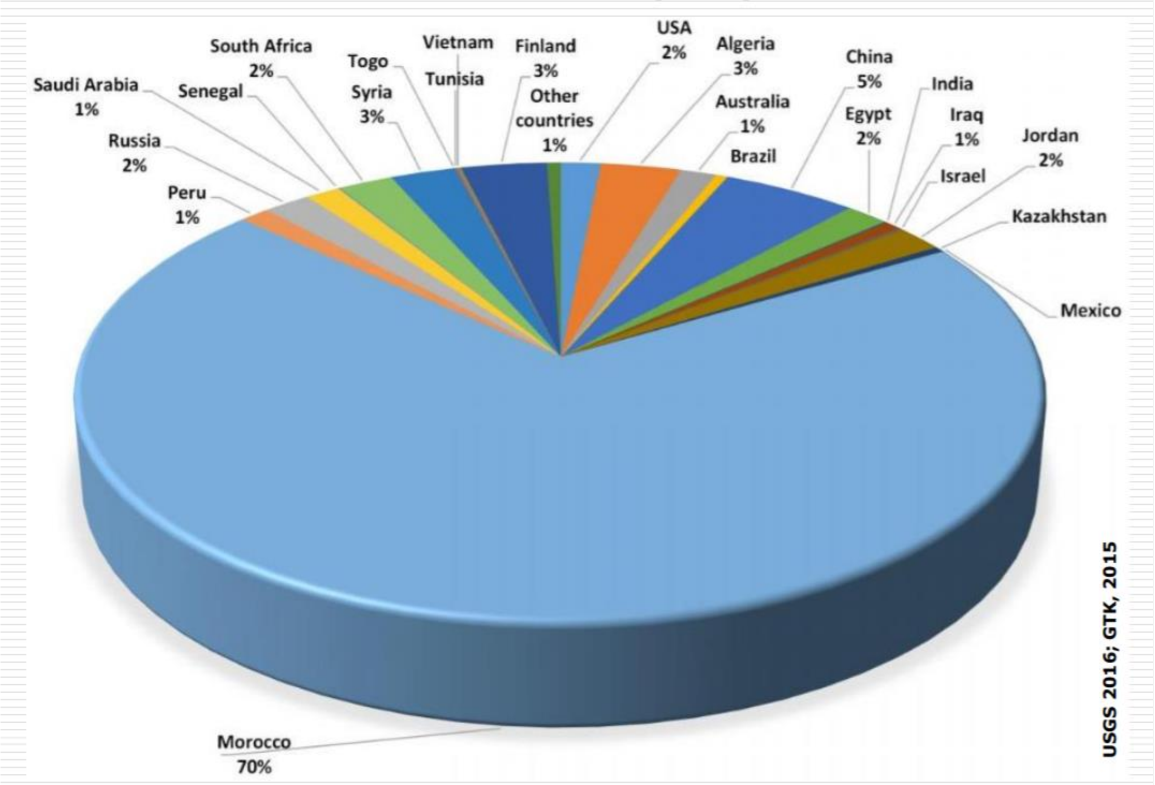


Figure 2.6.1 Global distribution of commercial PR reserves in year of 2016 (from www. minerals.usgs.gov)

2.7 Phosphorus mobilisation

The phosphorus journey across the land starts with the P mobilisation in the soil. Three different modes of P transfer via mobilisation have been discussed in the earlier chapter such as solubilisation, physical detachment and incidental transfer under the P transfer continuum of Haygarth et al. (2005). Solubilisation is the main type of mobilisation with relevance to this thesis focusing on the rhizosphere, but I will also for completeness, consider the alternative type of mobilisation. This involves the fundamental micro scale processes of P dissolution and operates over the relatively long-term involving biological (Gordon et al., 2008) and chemical components (Blake et al., 2010) of the rhizosphere. Phosphorus solubilisation mainly comprises mineralisation, via enzyme hydrolysis. It allows the sorption and desorption reactions, with subsequent displacement in the soil solution and root medium, particularly incorporated well with the anaerobic condition of wetted soil. After the release of P anions from an occluded or absorbed component, the solubilised P is ready for plant uptake (Shen et al., 2011), however, it can also be retained in the soil or be liable to leaching loss from the soil (Scalenghe et al., 2012; 2014). The physical detachment is a macro scale dispersion process of non-dissolved particulate and colloid P forms (Macleod et al., 2012). Particulate P loss in the water flow is often associated with cracking and root channelling of drying soil under the rewetting. The incidental transfer is a rapid and direct loss of dissolved and particulate P forms recently applied to soil, in runoff. It can occur when freshly applied fertiliser and manure interact with a subsequent rainfall event (Preedy et al., 2001).

2.7.1 Solubilisation via biological processes

Soil microbial communities within the whole soil profiles mediate the key processes of the soil e.g., degradation of soil organic matter, P cycling, and mineral weathering through their metabolic activities. Both the soil microbial biomass P (MBP) (Blackwell et al., 2010; Turner et al., 2003) and P released from microbial osmoregulatory compounds (Halverson et al., 2000) and soil organic matter (Wu and Brookes, 2005) via mineralisation may represent the significant P sources in soil (Cole et al., 1977). Most P solubilising microbes exist on the rhizosphere along with fungi. The P solubilisation potential of these microbes accounts for approximately 1-50% of soil (Chen et al., 2006). As compared with the rhizosphere, the bulk soil has relatively oligotrophic conditions with low rates of microbial activity and nutrient transformation (Ai et al., 2012). However, the bacterial biodiversity is greater in the bulk than in the rhizosphere soil (Marilley and Aragno, 1999). The microbial P value in soil ranges from 1.5 to 50 $\mu g\ g^{-1}$ soil (Chauhan et al., 1981) with increasing bioavailability over time after microbes die (cals.cornell.edu). The turnover rate of soil MBP may vary, for example, 2.5 years in England

(Brookes et al., 1984) and 0.4 years in China (Chen and He, 2002) depending on soil abiotic and biotic stresses including soil pH, temperature, moisture and vegetation type.

Phosphorus solubilising microorganisms in the soil have the potential to regulate the turnover of soil organic P (Oberson, 2005), particularly dissolved organic P (Chauhan et al., 1981) through enzyme catalytic hydrolysis (Marschner, 1996). The rate of organic P via microbial breakdown depends on substrate characteristics and availability of alternate electron acceptors such as aerobic (O_2), facultative anaerobic (NO, $Fe3^+$ and Mn^{4+}) and obligate anaerobic (SO_4^{2-} and CO_2) redox conditions on P mineralisation (Fenchel and Jørgensen, 1977; McLatchey and Reddy, 1998). The rates of soil organic P mineralisation and microbial biomass were approximately three-fold higher in soil incubated upon aerobic conditions than in soil with reducing circumstances of nitrate, sulphate, and bicarbonate (McLatchey and Reddy, 1998). For example, in sulphate-dominated soils, P can be released during the production of H_2S from the biological reduction of SO_4^{2-} and formation of Fe sulphides. The electron acceptors of Fe are generated through the metabolic processes of facultative organisms under anaerobic conditions.

Abiotic stresses of the soil lead to the lysis of microbial cells (Turner and Haygarth, 2001a; 2001b) at the rate between 17 and 58% (Kieft et al., 1987; Salema et al., 1982) associated with drying and rewetting and freezing and thawing cycles (Blackwell (Blackwell et al., 2010c). If the level of soil water potential (SWP) for optimal microbial activity (>-0.7MPa) is reduced to -2 MPa, the microbial biomass increases by 35-50%. Consequently, the concentration of soluble P in the soil significantly increases (Buenemann et al., 2013), through the nutrient transfer into the labile pool (Wu and Brookes, 2005). However, fungal biomass does not support the turnover biomass, due to the fungal hyphae that can remain in dry soil for more extended periods (Allen, 2009; 2007). The survivor microorganisms in the soils from osmotic pressure of DRW are: (a) reducing their physiological activities, particularly a turndown of respiration to 5-8% at -1.5 MPa (Chowdhury et al., 2011) and (b) forming the biofilms (Pailler et al., 2014) that protect against the environmental extreme conditions.

2.7.1.1 Releases of protons from organic acids

The protons released from organic acids synthesized from P solubilizing microbes (phenolic and siderphores) and roots (low molecular weight exudates) can modify the chemistry and pH of the rhizosphere. Organic acids are produced in periplasmic spaces of microbial cells through direct oxidation pathways (Zhao et al., 2014), varying from a few mM to 100 mM (Reyes et al., 1999). The half-life time of organic acids in the soil is 0.5 to 12 hours. Full and partial dissociation of carboxylic acid takes place in soil solution at pH 6.4 (Ryan and Jones, 2001). The cytosolic pH of root cells normally ranges between 7.1 and 7.5. In the root medium, the deficiency of nutrients, e.g. P, Fe and Mn can induce acidification (Hinsinger, 2001) by increasing carboxylate efflux from roots to the rhizosphere. Carboxylates are salts of organic acids. The flow of carboxylate from root cells to the rhizosphere is transported via the carboxylate ion channel, transporter carrier and H^+ pumping ATP-ase mechanisms (Lambert et al., 2008).

Organic acids can solubilise various forms of organic and inorganic P directly and indirectly. The direct way is, the organic anions in the surrounding soils decrease P sorption via alterations of surface characteristics of soil particles and clay, ligand exchange reaction, and chelation of cations Al and Fe in acidic and Ca in alkaline soils (Jones, 1998; 1994). As soil pH drops, P is released through the substitution of H^+ for Ca^+ (Goldstein, 1994). The binding strength of P adsorption can be affected by the electric surface potential of negative charge (Bowden et al., 1980). The negatively charged inorganic P complexed with Al and Fe mineral (Oburger et al., 2011) are solubilised via ligand exchange and ligand promoted dissolution of Fe and Al oxides (Johnson and Loeppert, 2006). The P solubilising effect of organic acids has been developed well in soils with significant amounts of carbonate or Fe and Al oxides (Oburger and Jones, 2009).

The reaction of P releases associated with organic acids is as follows:

Fe/Al oxide –P +L= Fe/Al oxide –L + P or Fe/Al oxide –P +L= Fe/Al oxide +Al^{3+}/Fe^{3+}-L + P

L represents the complex of organic ligand adsorption, increased with the number of carboxylic groups and concentration in soil solution (Jones and Brassington, 1998). It uses carrier proteins for transporting (Neumann et al., 1999; Ryan and Jones, 2001). In general, tri-carboxylic anions (citric acids) show higher P solubilisation potential than di-carboxylic acids (gluconate and oxalate). However, both citrate and oxalate have a greater potential for P solubilisation than other organic acids (Jones, 1998).

The indirect effect of organic anions is to solubilise the P bounded with the humic metal complex (Gerke, 1993) and then increase a subsequent dephosphorylating activity of phosphatase (Hayes et al., 2000) through the support from a process that releases organic P from soil (Pierzynski, 1991). The organic anions expand the efficiency of phytase catalysis reaction on complexed inositol phosphates (IP) forms (Menezes-Blackburn et al., 2016a; 2016b), as dephosphorylating inhibited by adsorption of phytase to soil particles and phytate complexion.

The appearance of root acidification is also inspired by the cation assimilation, particularly NH_4^+ (Alori et al., 2017; Asea et al., 1988; Illmer and Schinner, 1995). It has been generally accepted that the assumption of the cation assimilation theory is based on the denitrification processes. Nitrogen assimilation can increase the net cation released from ammonium (NH_4^+) and nitrate (NO_3^-) compounds. Nitrification is the conversion of ammonia to nitrite. Indeed, nitrification processes lead to greater N leaching to ground water, due to the higher water solubility of NO_3^- than NH_4^+. Ammonia oxidising nitrification processes in the rhizosphere are primarily facilitated by bacterial activities involving ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). Initially, AMO catalyzes NH_3 oxidation to NH_2OH, and then oxidation of NH_2OH is catalysed to HAO to NO_2^-. Although plants are not engaging in nitrification directly, plants can only impact on N transforming microbes and fungi nearby their rhizosphere through root exudates (Bardgett et al., 2014; 2003; Finzi et al., 2015) by inhibiting AMO and HAO. For example, root exudation of the invasive weed, *Fallopia* spp., was associated with the low denitrification potential of soil (Clément et al., 2015). Consequently, the root medium pH increases. In addition, plant uptake of nitrogen (N) ions is higher than P ion (Hinsinger, 2001). Upon ratios of cation and anion uptake, in agricultural practice, combinations of ammonium and superphosphate ($Ca(H_2PO_4)_2$) application have commonly been used in calcareous soil (Jing et al., 2010).

2.7.1.2 Releases of phosphatases

Mineralisation is one of the well-known reactions to release P slowly into the soil solution from organically bound material via extra and intracellular phosphatase enzyme activities. Phosphatases catalyse the hydrolysis of both esters and anhydrides of phosphoric acid P. The phosphatase activity is proportional to the rate of organic P mineralisation (George et al., 2002a). Mineralisation always accompanies microbial respiration and changes in redox potential. The balance between mineralization and immobilization depends on P deficiency (Vance, 2010), plant demand and growth stage (Dormaar, 1972), C and P ratio of organic matter and the type of electron acceptors that involves in the decomposition of microbial respiration in aerobic and anaerobic ways (Gould and Motsch, 1981). For example, the soils with high total organic carbon (TOC) consist in general of a significant proportion of organic P (Harrison, 1983). There are two types of phosphatase, acid and alkaline. Plant roots, mycorrhizal fungi, and microorganisms mediate acid phosphatases (Tarafdar et al., 1989). Acid phosphatase catalyses occurs in acidic condition pH below 7. Whereas soil microbes produce alkaline phosphatases. The reaction is as follows:

orthophosphoric monoester + H_2O → alcohol + H_3PO_4.

All forms of phosphatase have been classified based on hydrolysis, according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. It includes phosphomonoesterase (monoester bonds including mononucleotides and sugar phosphates and lower-order inositol phosphates); phosphodiesterases (one or two ester bonds in phosphodiester compounds and nucleases); phosphatesphosphoprotein phosphatases (phosphoester bonds of phosphoserines, phosphothreonines or phosphotyrosines); phytases (myo-inositol hexaphosphates and all six phosphate groups from inositol hexaphosphate); phospholipases and nucleotidases.

Phytase is the class of phosphatase enzyme that removes the phosphate group by initiating the phosphate hydrolysis at carbon 1, 3, or 6 in the inositol ring of phytate (Singh and Satyanarayana, 2011). Plants tend to have a limited capacity to obtain P from phytate unless six phosphate groups of phytic acid are released into the soil solution by phytases cleavage (Fig. 2.7.1). The phytases can effectively hydrolyse soluble forms of Ca- and Mg-IP, but ineffective in Al-, Fe-, Cu-, Zn- and sand sorbed IP (Lung and Lim, 2006; Tang et al., 2006). There are four classes of phytase enzymes, which are structurally distinctive crystalline in their catalytic properties to hydrolyse phytate, including histidine acid phosphatases (HAPs), β-propeller phytases (BPPs), purple acid phosphatases (PAPs) and protein tyrosine phosphatases (PTPs). The most well-known phytases are HAPs, which can cleave phosphate groups of phytic acids at different rates and in a separate order. According to the Enzyme Nomenclature Committee of the International Union of Biochemistry, phytases have been grouped into three discrete categories, 3-phytase, 4-phytase and 5-phytase on the bases of the position of phosphate dephosphorylation of the inositol ring.

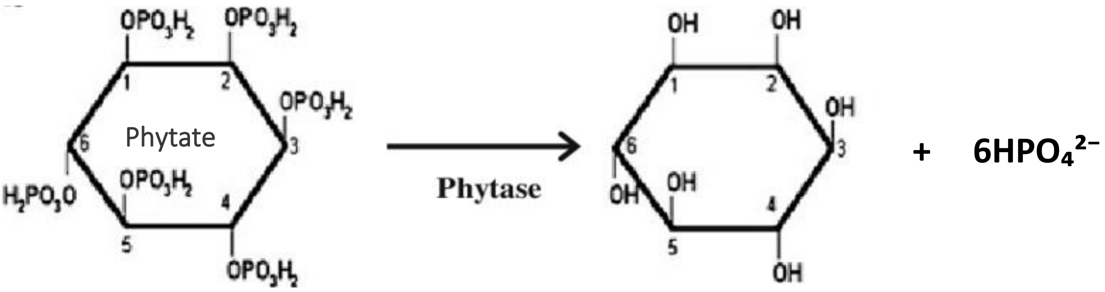


Figure 2.7.1 Schematic representation of the hydrolysis of phytate. The six phosphate groups are released from phytic acid by phytase (Singh and Satyanarayana, 2011).

The phytase can be produced by plants, animal tissues and microbes, for example, 3- phytases have been isolated from bacteria, fungi and *Aspergillus* species (Sajidan et al., 2004), 6-phytase from wheat grains and oilseeds and 5-phytases from alfalfa, beans, peas and *Selenomonas ruminantium* (Chu et al., 2004). The extracellular phytase activities derived from living and lysed microbial cells are more productive (Burns et al., 2013) than root-derived ones (Richardson et al., 2001). It can be an indicator of the soil biological capacity (Nannipieri et al., 1996) and long-term soil function (George et al., 2007a; Naidja et al., 2000). The phytase is also used in monogastric farming practices by adding the exogenous microbial phytase in the animal feed. It can mitigate P pollution originating from the manure and reduce the amount of inorganic P supplementation. For example, 500–1000 units per kg can replace 1 g inorganic phosphorus (Yi et al., 1996) and consequently, P in the manure lessens by 30-50% (Wang and Yang, 2007).

The phytases are also added to the soil, and the ability of enzyme activity is inhibited within 24 hours (Giaveno et al., 2010), via adsorption of the soil solid constituents and protein (Norde, 1986; Norde and Lyklema, 1991). The inhibition activities will vary depending on the soil characteristics (George et al., 2005; George et al., 2007b), for example, 40% of phytase remained active after 28 days in sandy soil, while only 5% remained active in soil with higher clay content (George et al., 2005). The absorption of phytases is associated with the intra- and intermolecular force (Giaveno et al., 2010; Quiquampoix et al., 2002), and it protects the enzymes against degradation for extending the period of persistence (Naidja et al., 2000). Clays and Al hydroxides reduce phytase activities much stronger than organic matter through interlaminar spaces more than 1:1 phyllosilicate (Nannipieri et al., 1996; Rao et al., 2000). However, this inhibition can be limited when these complexes interact with the soil proteinase, microbial degradation, metabolites (Nannipieri et al., 1996), the soil texture and minerals (Rao et al., 1994), the heterogeneous nature of pure clay and the presence of endogenous SOM (Giaveno et al., 2010).

2.7.2 Solubilisation via chemical processes

In the soil systems, changes in moisture content strongly affect soil chemistry and oxygen diffusion. Under prolonged periods of wet weather in the changing climate as well as long-term land drainage, the surface soil in the temperate zone has transient high-water tables and saturation that can create anaerobic conditions favourable for reduction (Edwards and Withers, 2010; Stoate et al., 2001). This condition can change the P equilibria and oxidation states even in the small zone of soil within a relatively limited period by generating a number of redox-active organic compounds to solubilise redox-sensitive minerals e.g., Mn- and Fe-oxides. Under reduced conditions, equilibrium P concentration in the soils is always higher than in oxidized states, which are regarded as having low P sorption capacity and high desorption potential (Patrick and Khalid, 1974). The extent of reduction can increase desorption potential and rates of P diffusion from soil to solution, which influences the proportion of orthophosphate between soil solution and solid phase by changing the P speciation.

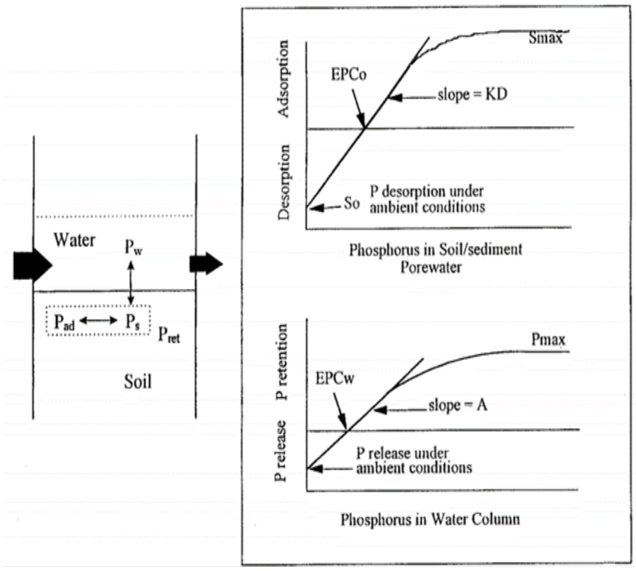
The equilibrium relation between P releases, and retention of soil is a very complex dynamic process, depending on P saturation, the physiochemical properties of soils (Barrow, 1983), concentration of P in solution (intensity), the ability of solid phase to replenish P into solution (capacity), the rate of P loading, management history of P loading, types of P and non-P components of the applied fertiliser and total organic carbon contents. The oxidation states of soil, however, are controlled by the temperature (Suplee and Cotner, 2002), moisture (Dijkstra et al., 2012) and pH. In the field conditions, the P solubilisation increases within the warm soils and remains longer in wetter conditions. Whereas the low moistened soils with high P sorption capacity can buffer against P loss, even if P has been applied to it (David et al., 2007). Under thermochemistry, the redox seems to be not equilibrium, due to the instability of water and carbon dioxide content.

Redox reactions can create new compounds through the alteration of the biochemistry of soils. With regards to soil DRW, the redox equilibria are highly likely to shift in P solubilisation via the reduction of organic fraction associated with carbonate dissolution under the decreased pH level (Scalenghe et al., 2012). Carbon reduction and subsequent oxidation are the processes known as photosynthesis and respiration respectively. Drying can induce organic P directly through the oxidation of soil organic carbon with Fe and Al associations (Bartlett and James, 1980; Haynes and Swift, 1985; Schlichting and

Leinweber, 2002). This is a soil type specific process (Gérard, 2016) linked with the P sorption sites in the surface area of Fe and Al oxides and clay minerals. The subsequent mineralization (David et al., 2006) and the reduction of soluble Fe oxyhydroxide of amorphous and poorly crystalline forms (Berkheiser et al., 1980; Gale et al., 1994; Richardson, 1985) can release a significant proportion of P from the non-microbial organic and inorganic sources (Blackwell et al., 2009; Buenemann et al., 2013; Butterly et al., 2009; 2011). Further, in the anaerobic condition of sulphate-dominated wetted soil, the production of H_2S and $FeSO_4$ can prevent P from fixing with iron through a largely created surface area for P sorption (Caraco et al., 1991).

2.8 The phosphorus transformations between soil solution and solid phase

The state of P equilibrium between the liquid and solid phases transformed through the dissolution-precipitation and adsorption-desorption processes. These reactions maintain P ion concentrations in the soil solution and the root zone (Fig. 2.8.2). To understand the behaviour of soluble P within environmental contexts, the adsorption and precipitation need to be discussed by certain extent as these are the reversed processes of desorption and dissolution. Prediction and sorption reaction in the equilibrium relations forms new solid compounds (Corey, 1981) through the fixation of P with soil constituents (Di et al., 1995), having an implication to control the soluble P in the soil solution.



K: partition coefficient related to P buffering capacity
EPCo: equilibrium P concentration, where adsorption equals desorption
So: native P present in sorbed phase under ambient conditions
A: phosphorus assimilation coefficient
Pmax: phosphorus retention maximum
P0: maximum P release potential of native soils or sediments
EPCw: equilibrium P concentration in the water column at which point P retention equals release.

Figure 2.8.1 Schematic representation of phosphorus (P) desorption and adsorption in soils and sediments, and following P release and retention in water column, noted the P adsorption on y-axis and P in solution on x-axis (Reddy et al., 1999).

When P is added to the soil, the sorption reactions occur until the reach of the new equilibrium (Hedley and McLaughlin, 2005). It is achieved through a two-step process consisting of rapid and slow phases, in which initial retention on a surface and diffusion into a solid phase known as adsorption and absorption respectively (Barrow, 2010; Portielje and Lijklema, 1993). The solubility equilibrium approach through dissolution-precipitation reaction can illustrate (Sposito, 1989) as follows:

$$MaLb\ (solid) = aM^{m+}(aq) + bL^{L-}(aq)$$

An initial process of retention components is P adsorption, which is irreversible. With high buffering and P sorption capacity, a rapid exothermic ligand exchange reaction can be performed in the soil solution between orthophosphates and reactive surface aquo and hydroxyl groups. In soil solution, orthophosphate tends to react with acids such as H^+, Ca^+, Mg^+ and Fe^+, which directly accept electrons through a vacant valence orbital to form soluble outer and inner sphere complexes. Inner surface sorption is the following slow process leading to higher strength of adsorption; thereby reducing the bioavailability of P compounds (Fig. 2.8.2).

Protonation and deprotonation of phosphate ions (H_2PO^{4-} and HPO_4^{2-}) act as ligands to produce ion pairs of mono-, bi- and multidentate two-dimensional complexes with Al and Fe minerals through the replacement of H_2O or OH^-. Sorption then continues to precipitate into a three-dimensional crystalline structure, for example formation of a fertiliser granule is the case in point. Generally, the adsorption and precipitation with higher denticity can store P in soils for long-term (Hedley et al., 1995), which are less reversible than monodentate. This can lower the P concentration in soil solution to preventing P ions to move toward the concentration gradient, particularly, in the soil with high sorption rate. In addition, the organic P can also form insoluble compounds by bridging ligands between negatively charged carboxyl and hydroxyl function groups of orthophosphoric acid esters with positively charged Fe and Al oxide in active and fixed pools (Gu et al., 1994; Hinsinger, 2001).

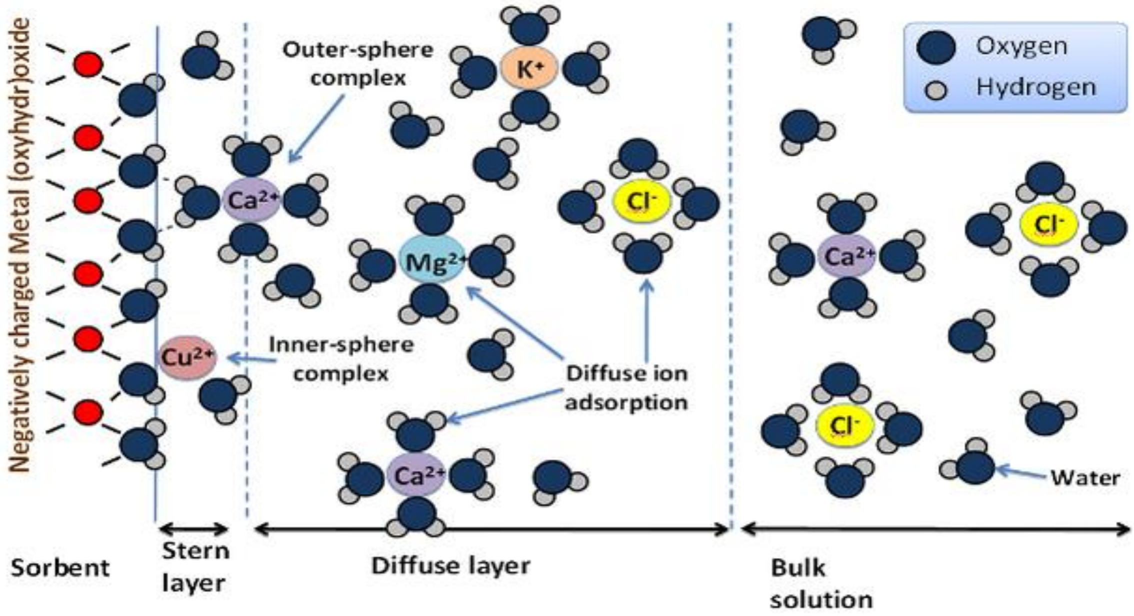


Figure 2.8.2 Schematic arrangement of soil sorption processes at a sorbent surfaces taken from Chorover and Brusseau (2008) and Maurice (2009).

2.8.1 The role of soil pH

The retention and release reactions are pH specific process of the soil that governs the solubilisation of Al-, Fe- and Ca-phosphates and the movement of soluble P through the soil pore. The bioavailability of P is the greatest in the soils with slightly acidic to neutral pH, dominated by the primary orthophosphate ions ($H_2PO_4^-$). In acid soils, the prevalent phosphate forms exist as Al- and Fe-phosphates. However, the organic P forms and concentrations have been weakly affected soil pH under aerobic conditions, particularly with pH above 4 (Turner and Blackwell, 2013). In alkaline soil conditions, precipitation reactions with Ca and Mg are predominantly employed (Lindsay et al., 1989), particularly calcium carbonate ($CaCO_3$) play an important role in the interaction with P (Fig. 2.8.3). The dominant form in alkaline soil is the secondary orthophosphate ions (HPO_4^{-2}). The calcareous soil gives rise to the creation of $CaHPO_4$ (Dicalcium phosphate DCP), which exhibits varying degrees of accessibility to plants. Calcium phosphate plays an essential role in assessing the outcomes of applied fertiliser reactions. In the long term, DCP can transform into more resilient forms such as dihydrate octocalcium phosphate and hydroxyapatite. The P sorbed on the solid phase typically remains bound as the neutral salts such as NaCl and KCl do not conform to the regular adsorption behaviour. This phenomenon is referred to as the hysteresis effect.

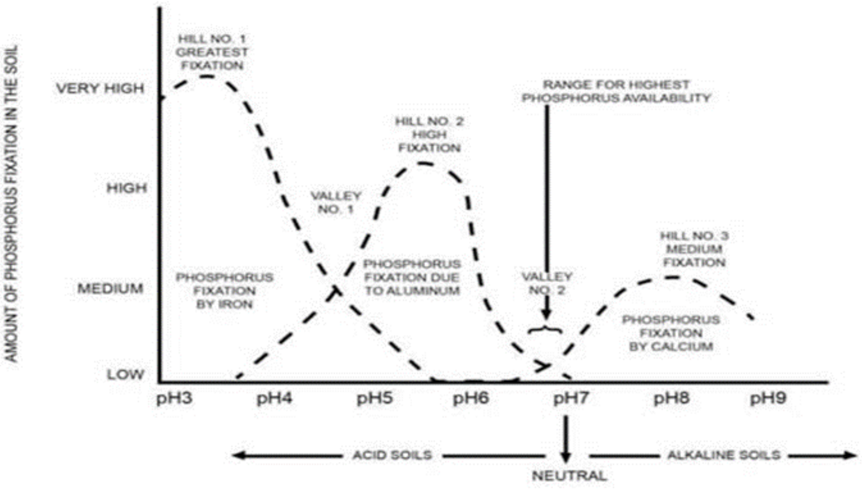


Figure 2.8.3 Phosphorus fixation at different pH value from Stevenson and Cole (1999).

In natural environments, pH value of the soil solution, water column and pore waters all significantly change on a diurnal basis as a result of photosynthesis and respiration. The pH values of the soil can be adjusted through application of lime and sulphur, in order to increase and decrease pH respectively. A lime requirement model is available (Goulding et al., 1989). Due to the increase in CO_2 levels, about

75 to 90% of the precipitated P can be remobilised in soil solution at the pH below 8 (Diazo et al., 1994) through the supports from buffering capacity. In the mild alkalinity, only 6% of the TP is removed by co-precipitation with high Ca^{+2}. The remainder is available for biological uptake (House, 1990)predominantly with metastable calcium phosphate species.

2.8.2 The role of plant roots

Significant quantities of P are required in the early stages of plant growth. For instance, within the first 40 days after the emergence of cereal crop, approximately 75% of plant necessary P has already been taken up by crops. Rapidly growing crops can absorb P with the rate of 1 kg ha^{-1} per day (www.agric.gov.ab.ca) during the development of sink organs, including fruits, seeds and tubers and flower formation (Brevedan et al., 1978). The root growth is influenced by the plant life cycle, genetic variability, nutrients, and water composition of soil, biotic and abiotic environmental stimuli. Beyond the mutualistic association (will be discussed in chapter 3), plant species appear to adapt in variable P conditioning through the operational adaptive functions of cells that can stimulate the nutrient mobilisation, uptake process, and root growth. The root is a highly plastic system to respond to local heterogeneity of soils through the spatial and temporal dynamic processes of (1) physiology: root exudation phosphatase activities, and phosphate transporter (Daram et al., 1998) and (2) morphology: root architectural structure, biomass partitioning for root investing, formation of cluster roots, root hair growth and root proliferation. The genetic expression of encoding gene serves a mediator, leading to two responses: (a) upregulation to enhance plant root growth under the nutrient impoverished soil (Lyons et al., 2008) and (b) downregulation to supress root growth under the excessive nutrient inputs (Shen et al., 2011).

Roots can increase capacity of P uptake within the soil volume via spatial availability of root size by branching, elongating, and proliferating root hair and foraging in the topsoil (Shen et al., 2011). Some plants can improve the surface area of nutrient absorption by forming cluster roots and root nodules through the plant hormones for sugar signalling and nitric oxide (Nacry et al., 2005; Vance, 2010; Wang et al., 2010). The best-known root clusters are the proteoid root, formed by the *Lupines albus* in Fabaceae family (Fig 2.8.4) and many native woody plant and herbaceous dicotyledonous taxa for example, non-mycorrhizal Proteaceae. Monocotyledonous plants are promoting a formation of root clusters to enhance P acquisition under the nutrient impoverished of soil including *dauciform* roots in *Cyperaceae* (Lamont, 2010) and capillaroid roots in *Restionaceae* (Lambers et al., 2006a). Upon these morphological changes, the cluster root stimulates a release of root organic exudates and carboxylates (Shane et al., 2004; Yiyong et al., 2005). Carboxylate-exudation cluster root would be useful agricultural strategies in the soil with a large fraction of sorbed P (Lambers et al., 2013).

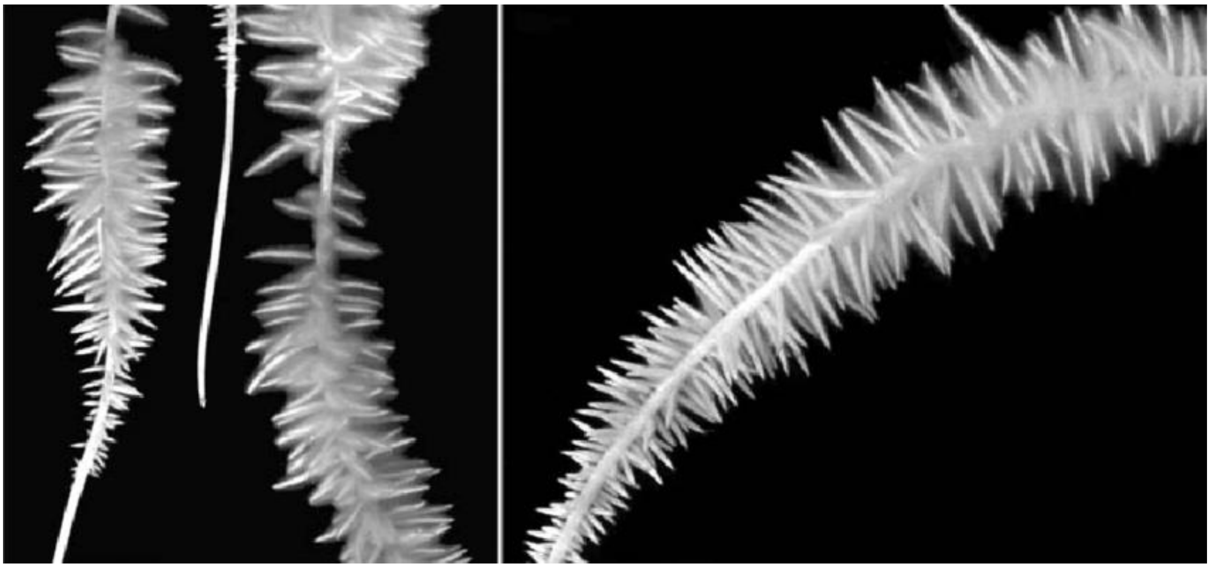


Figure 2.8.4 Proteoid cluster roots of hydroponically grown *L. albus* (Shane et al., 2003b).

The anatomy of root

The stem cell at the tip root is the main driver of root growth. The root apical meristem (RAM) generates new cells for the root elongation in their earlier growth through the active rapid mitosis. These cells are protected by the cap of surrounding tissue type cells, called a quiescent centre (Hodge et al., 2009). This centre can initially organise the direction of root growth (Jenik et al., 2007; Mitsuhiro et al., 2004). The cap and RAM form the zone of the distal root apex. Figure 2.8.5 provides an overview of the root structure. The certain distance away from the RAM region, the lateral roots are generated from pericycle meristem cells through the anticlinal and asymmetric divisions by forming a dome shape primordium from outer cell layers of parental root. Such root architectural development and lateral root formation are regulated by the distribution of phytohormones, auxin, and cytokines in the plant root. Auxin is a particularly important signalling molecule for further differentiation of roots secreted from the apical region. Auxin flux to hypophysis is carried out by active transport, activated by encoding genes of auxin efflux carriers and auxin transporter (Benková et al., 2003).

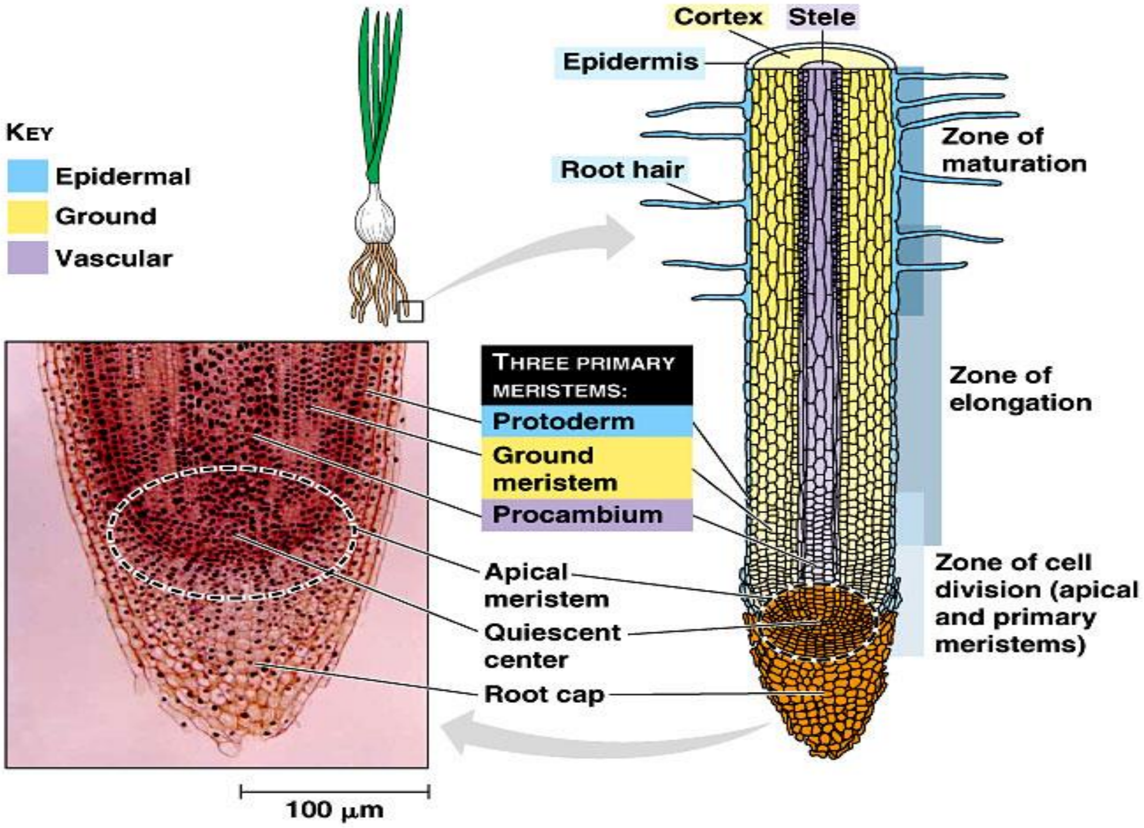


Figure 2.8.5 Schematic representation of overview of the root structure (www.pearsoned.co.uk)

The key indicator of the root is the root length density (Majdi, 2001), branching pattern, specific root length (SRL) and specific root area, and root shoot ratio (R/S). The changes of theses parameters are showing nutrition imbalance that is caused by large agricultural inputs and long-term climate change effects of leaching base cations (Ericsson, 1995). The observed decrease R/S ratio under the P deficiency leads to the starch accumulation of the leaves and reduction of the sugar export that is used for the synthesis of the new structure (Ericsson and Ingestad, 2010). However, the changes of

R/S ratio can happen naturally during the plant lifespan, for example decreased with the ageing processes. Ideally, SRL (ratio of root fragments to root mass) can be used to define the proportion of root system construction to resource acquisition characteristic (Eissensta and Yanai, 1997). Representation of increased SRL is an increased volume of exploited soil via fine root per unit biomass. Consequently, less expensive plants (Withington et al., 2006) having thin leaves are produced by the support from the long and thin root (Ostonen et al., 2007).

2.8.3 The evaluation of equilibrium relations of phosphorus transformation in the soil

The evaluation of P dynamics in the soils is most challenging (Sharpley et al., 2000), especially under the influence of climate change. The risks of P loss can be evaluated by laboratory simulation e.g., soil binding capacity in 24 hours, degree of P saturation (DPS), sorption maximum (Smax), equilibrium phosphorus concentration (EPC), P buffering capacity (PBC), solution P turnover (*Km*), M3 and ammonium lactate-extractable P (P-AL). Here, I will briefly discuss some methods. Firstly, PBC is one of the most practical ways to measure soil P dynamics (Barrow and Debnath, 2014; Bolland et al., 2003; Burkitt et al., 2002) through the concentration differences between P in soil solution and soil. PBC is an ability of soil to resist the changes in P concentration of soil solution (Holford, 1997), for example, against an increase after a fertiliser application and against a decrease during P uptake by plants. Earlier research studies exaggerated the importance of the soil PBC (Olsen and Khasawneh, 1980; Pypers et al., 2006), however, in these days the exchange rate of inorganic P (Helfenstein et al., 2018) known as soil solution P turnover (*Km*) has been used to determine the P dynamics in soil solution.

Secondly, the *Km* refers to a short-term experiment of isotopic exchange kinetics (IEK) to measure the mean rate of exchange of phosphate ion between soil solution and inorganic P in soil, by using radioisotopes (Frossard et al., 1994). Measurement of isotopic exchangeable P is more accurate way to calculate the bioavailable P source for most crops (Frossard et al., 2011). The rate of exchange ranges from 10^{-2} to 10^{6} min^{-1} according to the survey of 217 different soils across Ferrosols, Andosols, Cambisols and Podzols that have different P sorption capacity (Helfenstein et al., 2018). However, the IEK approaches do not consider the root-induced alteration of soil chemical properties that can trigger the weathering processes of poorly soluble P sources in the surrounding soil via the rhizosphere exudation (Narang et al., 2000) and etching of plant roots (Mottershead et al., 2003).

Thirdly, the EPC is to determine P sorption and desorption characteristics within the sorption isotherm through P binding bath experiment in 24 hours and degree of P saturation. The EPC represents P desorption indices that equilibrate the concentration of soluble reactive P (SRP) in solution over 24 hours in µmol l^{-}. Equilibrium P concentration zero (EPC_0) is no net sorption or desorption, related with quantity and intensity relations either Langmuir or Freundlich isotherms (Jarvie et al., 2005). On the contrary, the P *Smax* and bonding energy (*K*) in P binding bath experiment represent P sorption indices that fits to the Langmuir model. The calculation is used the amount of sorbed P on solid phase (mg kg^{-}) and remained P in solution (mg L^{-}). Soil with high *Smax* can release P into water, if P concentration in water and binding energy (*k*) of sorbed P are low (Belmont et al., 2009). However, it has a potential for high desorption under oxidized conditions (Patrick and Khalid, 1974). Therefore, to avoid overestimating adsorption, EPC is relatively reliable option to evaluate the value of P dissolution when adsorption and desorption processes are in equilibrium (Brand-Klibanski et al., 2007). If EPC is greater than DP in soils and sediments, P will release to water until a new equilibrium is reached. If EPC is less than DP in soils and sediments, P will adsorb from water, thus decreasing P concentration in water.

Water quality of the P binding bath experiment can be impaired before soil P sorption reaches the maximum. Therefore, DPS is used as a detection of simple index of P sorption capacity to estimate the ratio between occupied P sorption sites in soils and P sorption capacity (Schoumans, 2000) via different equations. Soils with greater DPS than the threshold will behave as sources of P mobilisation. Ammonium oxalate reaction (Eq. 1) of DPS varies with respect to the soil type, for example, by the empirical factor, $\alpha = 0.5$ in sandy soils (Nair et al., 2004) and $\alpha = 1$ in other soils (Sharpley, 2002; Zhang et al., 2005). Ammonium oxalate extraction *DPS-ox (Fe+Al)* is replaced lately by Mehlich-3 (Eq.2 and 3), as Mehlich reaction can be performed in a wide range of soil pH (Sims, 2009). *DPS-M3(Fe+Al)* is used in acidic soils, correlated well with *DPS-ox (Fe+Al)*. In alkaline soils, another version of DPS equation is used for the calculation Mehlich-3 extractable Ca (CaM3) (Ige et al., 2005). The threshold value of DPS is 20% in *DPS-ox (Fe+Al)* and 16% in *DPS-M3 (Fe+Al)* (Sharpley, 2002). The equations are as follows:

$$DP \quad \textit{Eq (1)} \quad + AL) = \frac{P_{\mathrm{ox}}}{\mathrm{α}(Fe_{\mathrm{ox}} + Al_{\mathrm{ox}})} x100$$

$$DP\text{:} \quad \textit{Eq (2)} \quad 'e + Al) = \frac{PM3}{(FeM3 + AlM3)} x10$$

$$DP \quad \textit{Eq (3)} \quad 'a) = \frac{PM3}{CaM3} x100$$

2.9 Delivery of phosphorus via hydrological transfer pathways

The P delivery can employ the hydrological pathways including subsurface and surface flows or leaching to groundwater. However, the catchment scale hydrology and hydrological factors play an important role in P transport (Heathwaite et al., 2005). The hydrology driven P transfer pathways with spatial and temporal dynamic appearance are indeed supported by topography (Beven, 2011; Lane et al., 2004; Page et al., 2005), landscape sources, land use and management (Mullan, 2013; 2012) and characteristics of sites (Heathwaite and Dils, 2000; Heathwaite et al., 2000). Once the forms of mobilised P are incorporated with climate changes (Kendon, 2014), it alters transport route, pathway dominance, speed of flow and P cycling across the land and water. However, the rate of predominant flow depends on soil hydraulic conductivity (McDowell et al., 2004; O'Neal et al., 2005), vegetation cover and soil characteristics (Haygarth et al., 2005; Vadas et al., 2005).

In flat regions, the surface runoff is less likely to occur. However, due to the sloping landscape in many regions of Europe, the dominant hydrological pathway is an overland P transport. The overland flow is surface pathways, including infiltration and saturation excess flow. The rate of overland flow is positively correlated with the degree, intensity and duration of precipitation (MartíNez-Casasnovas et al., 2002), ranging from rain splash to gully, and rill erosion on hillslopes (Mullan et al., 2012; Nearing et al., 2004). Under the prolonged dry spells, the soil surfaces will increase hydrophobicity, associated with a coating of soil particle with hydrophobic substances (Gilkes et al., 2002; Roberts and Carbon, 1972), providing the routing flow of surface runoff (Shakesby et al., 2000). The intermittent yet powerful nature occurrence of overland flow and surface runoff significantly contribute the particulate P loading to the water bodies (Sharpley et al., 2013) through the dispersion of particles (et al., 2004; 2005).

Lateral percolation close to the surface of soil can elevate soil water level then converge into hillslope drainage and stream water (hydrology.usu.edu). In the deep soils with permeable bedrock, the percolation to ground more commonly occurs than lateral flow. The dissolved P mostly transfers with lateral flow (Koopmans et al., 2007; Leinweber et al., 2010) via pipe drains or natural drainage into the receiving water bodies. The surface flow is the dominant P loss pathways than subsurface flow, as the P sorption process incorporated well within the depth of soil profile. However, under the heavy storms (Mullan et al., 2012) as particulate P transport via erosion ceased, the significant downward movement of soluble P appears throughout the soil profile (Beauchemin et al., 1998; Pierzynski and McDowell, 2005; Simard et al., 2000).

Generally, subsurface pathways (Haygarth et al., 2005) are the drainage network of groundwater and lateral flow, which forms the streams and rivers. The favourable conditions of subsurface flow are the concave topographical feature, relative impermeable shallow layer and hydraulic lateral conductivity. The subsurface includes matrix (piston) and preferential (macropore) flow. Preferential flow is rapid water transmission pathway with carrying PP particularly attached with colloidal material (Heathwaite et al., 2005) through macropore, channelling and cracking. Drying soil can enhance P losses under the rewetting by forming the cracking. Even wet soil can also form micro-cracks that are capable of transporting P.

2.9.1 Phosphorus delivery from various types of agricultural land

The anthropogenic P in waterbodies can generally be classified into point (discrete) and nonpoint (diffuse) sources (Edwards and Withers, 2007; Haygarth et al., 2005; Withers and Jarvie, 2008). The point sources are associated with urban and industrial areas. Due to the implementation of new efficient P removal technology, the contributions of point sources have decreased recently. Whereas nonpoint diffuse sources still remain the main contributor of P loading in water. Under the normal water flow, two thirds of total P (TP) loading derived from agricultural land with fertiliser application, stream bank erosion (www.pca.state.mn.us), atmospheric dust and wet deposition (Chadwick et al., 1999). However, the sources and sink capacity of P transfer are difficult to quantify individually under the influence of environmental factors. Here, I briefly explore the main representatives of sources by the land uses.

2.9.1.1 Cultivated land

The P transfer from cultivated lands is one of the fundamental contributors of diffuse water pollution from agriculture (DWPA). The large proportion of P moved from the cultivated soils are the sediment and associated P forms with clay and silt sized soil particles, minerals, and organic matter (Haygarth et al., 2000). The P movement from the cultivated land depends on the crop type, residue cover, soil characteristics, moisture condition, season, and management practices (Haygarth and Jarvis, 1999). During the primarily tillage and springtime manure application, the surface runoff often occurs with particulate P form. In the tillage practice, volume of this particulate P runoff decreased inversely proportional (Blevins et al., 1990) to the increased DP concentration through the infiltration (Wendt and Corey, 1980).

When studied using rainfall simulations, the loss of soluble reactive phosphorus (SRP) in conventionally managed plots closely resembled that of no-till practices (Mostaghimi et al., 1988). This similarity persisted despite conservation tillage endeavours, which aim to mitigate soil erosion through minimising the tillage, preserving crop residue on the surfaces, and reducing slopes via contour cropping. This conservation tillage may be inefficient to suppress the P transfer problems, it still looks increasingly likely to elevate in P leaching from plots with the surface application of fertilisers and manure (Torbert et al., 1996) and vegetative cover when 80% of nutrients can be released from senescing vegetation (Schreiber and Mc Dowell, 1985) under freezing and drying conditions (Timmons et al., 1970).

2.9.1.2 Grasslands

The overland flow is the dominant P movement pathways of grasslands with the subsequent rainfall, due to the soil surface compaction resulting from the presence of livestock and wildlife (Heathwaite, 1995; Van Keuren et al., 1979). The TP export to the watershed is proportional to the concentration of cattle (Mitchell et al., 1982). Half of TP are consisting of organic forms originated from the various grazing regimes and manure wastes of livestock (Dormaar and Webster, 1963). In grassland and uncultivated soils, surface runoff carries mainly dissolved P (DP) and particulate organic P (POP) forms (Heathwaite et al., 1990). The dense cover of graze can protect the soil against the formation of transfer pathways, however, overgrazed areas can be vulnerable to surface runoff, as the infiltration rates lessened by soil compaction associated with movement of livestock. After the occurrence of surface runoff, the runoff velocity further decreased when it passes through the grazing. As raindrops reduced the speed into the soil surface, the living or dead biomaterials can form an aggregate with the soil particles over a more extended period. In turn, it allows more sediment deposit to enter into the runoff and more P infiltrate to ground (Mcdowell and Sharpley, 2001).

The amount of P transferred from the under grazed area mostly higher than in grazed areas (Schepers and Francis, 1982), which is equivalent to TP losses (Jawson et al., 1982). More significant occurrences usually happen in the spring as snow-melt runoff having more than 80% of average TP at 0.11 kg ha^{-1} and total DP at 0.03 kg ha^{-1} (Timmons et al., 1977). However, the catchment-wide P losses of grassland depend on hydrological connectivity of P sources and watercourse (Edwards and Daniel, 1994), the intensity of rainfall (Preedy et al., 2001), the processes of deposition-suspension, adsorption-desorption, dissolution-precipitation, and biological uptake mineralization (Nelson et al., 1996), management of grassland, and the diversity of the natural landscape.

2.9.1.3 Irrigated land

Irrigated lands may lose P at greater rate than the corresponding dryland, due to the increased amount of DP and soil and water contact time (Sharpley et al., 1999) and enriched return-flow of irrigation by fertilisers, pesticides, sediment, salts and pathogenic bacteria (Cessna et al., 2001; F. Cuffney et al., 2000). Likewise, increased particulate P (PP) movement is also expected during the sediment loading associated with crop management and irrigation methods of field that include flood-irrigation (Ebbert and Kim, 1998) and furrow-irrigation (Carter et al., 1971; Lentz et al., 1998). Total P concentrations of flood-irrigated field elevated from two to five times more than inflow water (Sharpley et al., 1999), with 14 times increases of DP (Cessna et al., 2001). Sprinkler-irrigated areas are designated to minimize amounts of runoff and sediment loads as it is similar to natural rain; however, the presence of DP loads is still significant (Greenlee et al., 2000).

2.9.1.4 Wetland

Wetland is a land area saturated with water alongside waterways, varying widely the soil types, topography, climate, water chemistry, and vegetation. Constructive wetlands can be used for the effective means of remediating diffuse pollution acting as a buffer by reducing excess nutrients and pollutants before they can reach waterbodies through the process of accumulation within the sediment (Cooke et al., 1992). However, most soil suspension could potentially behave a long-term sink source of P losses to the waterways under the anaerobic condition, when the loading rates exceed for an indefinite period. Thus the P retention in wetland reservoir may be not sustainable over the time as particulate P species are dissolved (Richardson, 1985) and remobilised again in the sediments (Reddy et al., 1999). For example, P diffusion in wetlands may not occur from the water column into underlying soils if water column P concentration is lower than the pore water. P in water column can release rather than retain (Khalid et al., 1977; Logan, 1982; Sonzogni et al., 1982) and then transfer via runoff and drainage water (Shih et al., 1998; Wright and Reddy, 2001).

The removal process of biotic and abiotic retention in the wetland occurs through P sequestration. It incorporates with the underlying soil with initial filtration through macrophytes and other organisms. P is immobilised in their tissue. Indeed, the outgoing P concentration inflow from wetlands has been reduced by 22% at 2.8 g P m^{-2} $year^{-1}$ in receiving waterways (Mudroch and Capobianco, 1979). In the wetlands, oxalate extractable Fe and Al, and total organic carbon are highly correlated with the sorption maximum (Reddy et al., 1995), due to the sorption process between soil OM and minerals. Abiotic processes include the sedimentation, adsorption, precipitation, and exchange processes between sediment and the overlying water column. Biotic processes include the assimilation of organic P by vegetation, plankton, periphyton, and microorganisms. The microorganisms have attributed 60% P retention in wetland treatment systems (Lee et al., 1975; Sloey et al., 1978). In the wetland, the soil types and organic loading from external and internal sources have been a great effect on the proportion of organic P, including the deposition of dead algal cells and detrital tissue from aquatic vegetation. A higher proportion of organic P in peat dominated wetland is associated with humic and fulvic acid derived material from plant litter and recently deposited organic matter, with representing at 40% of TP (Brannon and Sommers, 1985; Swift and Posner, 1972).Phosphorus equilibrium in wetland depends on the soil types, physicochemical characteristics of the sediment-water interface, inflow concentration, load variations, hydraulic retention time, temperature, hydraulic efficiency, a dynamic of the wetland stream system, P concentration of the pore water, P concentration of the overlying water, and residence time (Portielje and Lijklema, 1993; Reddy et al., 1995). Various forms of wetland construction methods have been formulated to build up the concentrated solution. These methods include (1) Free water surface: it consists of plant and algae community from 0.1 to 2 m depth (2) Horizontal subsurface flow: an anaerobic and unsaturated aerobic zone with water, that flows horizontally beneath the rhizosphere through a permeable filter material with plants (3) Vertical flow: unsaturated aerated zone with water, that percolates through the rhizosphere. However, in future, wetlands have potential recover P for recycling through the innovative management, such as removing the accumulated P in decayed vegetation, sediment and concentrated solution.

2.10 Impact of phosphorus losses to the environment

Eutrophication is a naturally occurring process; however, it has become the biggest threat of the freshwater supply in many parts of the world (Powers et al., 2016). Only a small elevation of P concentration as low as 0.02 mg L^{-1} in water originating from agricultural land has a potential to disrupt the natural equilibrium, change receiving aquatic systems, including acceleration of aquatic plant and algal growth (Sharpley et al., 1999) and decrease the size and composition of the diversity of aquatic species (Fig. 2.10.1). Human-induced artificial enrichment of plant nutrient fluxes drive these water problems, which is related to population pressure, intensive agriculture, climate change, land-use change, modification of hydrological cycle, and an increase of water demand (Carpenter et al., 2011; Johnson, 2009). These can change the physical condition of the water, such as flow and runoff regime, water temperature, and light.

The Organization for Economic Cooperation and Development (OECD) suggested a classification of trophic status based on the P concentrations of 35-100 µg P L^{-1} for eutrophic and excess of 100 µg P L^{-1} for hypereutrophic (Vollenweider and Kerekes, 1982). However, the situation is different in running water. According to the water quality criteria of the Environmental Protection Agency (EPA) of the USA, P should not exceed 0.05 mgL^{-1} in streams discharge into lakes, 0.025 mgL^{-1} within a lake, and 0.1 mgL^{-1} in streams not discharging into lakes for the control of algal growth (USEPA, 1986).

The eutrophication processes have sequential steps with corresponding symptoms. Nutrients transferred from terrestrial landscapes primarily stimulate the growth of marine periphyton species and toxin-producing harmful algae such as blue green and cyanobacteria. These are dangerous to aquatic life and humans. However, one of the main problems of eutrophication is the depletion of the oxygen level in the water. Initially, the respiration of marine plants and species reduces dissolved oxygen level in water at the night-time, when photosynthesis ceases. After continued active phytoplankton growth, exhaustion appears in the concentrated dissolved nutrient sources in the epilimnion, and organic nutrients are then transported to hypolimnion with senescence.

The concentrated organic materials of algal decay, they stimulate the microbial decomposition and create more hypoxic conditions in the hypolimnetic area. Further, as organic materials sink down to gradually settle as aggregates into insoluble particulate organic P (POP) forms in the water column and they continue to bind with the sediments and water interface. Under anoxic conditions, the aggregates can remobilise P again to allow filterable reactive P to diffuse into hypolimnion, for further eutrophic symptoms (Bennion et al., 1996; Johannesse and Dahl, 1996). This process regenerates ammonium (Borowiak et al., 2010; Van den Meersche et al., 2004) that no organisms can survive. Consequently, there is a reduction in water transparency, undesirable odours, and the unsuitability of water for animal and human consumption.

Figure 2.10.1 Aerial view of an algae bloom on the Dnieper River in Kiev, Ukraine by Leonid Andronov taken from www.britannica.com

The acceleration of algal and marine growth has direct and indirect costs. For example, the presence of overgrown single celled algal organisms can increase water turbidity and lead an unappealing feature, deterring many swimmers and tourists from the area. Indirectly, algae and tiny marine growth beneath the waterline of ships can cause an increase in the rate of corrosion and fuel consumption, reduce a vessel's speed, and delay in freight travel time for goods (Edmiston et al., 2021). However, the global cost of accelerated eutrophication is very difficult to quantify. For example, in the UK, the annual costs of the National Eutrophication Monitoring Programme are estimated at 4.2 million pounds (defra.gov.uk).

2.11 Conclusion

This literature review was intended to provide context and theoretical basis of the separate elements and components of the PhD that follow, focussing on the soil P cycling of P at rhizosphere level. The P solubilisation and potential onward transfer to water are all considered relevant because they all originate in the rhizosphere from activities of soil microbes and plants. Understanding P cycling around the rhizosphere and how this will be affected by climate change includes many physical, biological, and chemical components of soil- rhizosphere- plant- water systems. To date, the understanding the role of climate change in the P solubilisation processes of the rhizosphere are poorly conceptualised. The naturally occurring DRW processes associated with climate change are highly likely to alter the characteristics of mobilisation and transport tiers of P transfer continuum, via increased solubilisation and detachment processes, and excess of flow under the rewetting of dried soil. Adding climate change and rhizosphere P solubilisation processes into the P transfer continuum can be helpful to synthesize the net effects of pathways nutrient losses on whole system and increase P use efficiency.

In the next chapters, I will be using experimental approaches to study this, and will include a more detailed discussion in plant physiological processes and rhizosphere engineering (chapter 3 and chapter 5) and *i*-DRW and *d*-DRW (chapter 4).

CHAPTER 3

Nutrient management approaches to rhizosphere and enhancement of plant phosphorus acquisition under direct application of low-grade phosphate rock

In the previous chapter, I reviewed a range of literature research studies on the various aspects of phosphorus (P) biochemistry, behaviour of P species in the soil, the dynamic natures of P cycling and, the implication of soil drying and rewetting (DRW) stresses that is associated with climate change. In particular, I focused mainly on the mobilisation tier of the P transfer continuum, in relation to the rhizosphere. In this chapter, I will be establishing a solid experimental foundation that attempts to link between amount of solubilised P within the root medium and associated solubilisation processes in the rhizosphere in order to test hypothesis number 1 and 2. Hypotheses 1 and 2 were that (1) different types of P fertilisers have differing resulting effects on the P solubilisation in the rhizosphere; and (2) the emission of organic acids in the rhizosphere at near neutral soil pH increases the P solubilisation of soils with different levels of P exposure. The key objectives to test these hypotheses are shown on page 19-20 of Chapter One. Two sets of controlled pot experiments (A and B) were conducted in the greenhouse to determine the processes of P solubilisation within the rhizosphere, using various types of P amendments. The low-grade phosphate rock (PR) used in these experiments was directly applied to the soil under the root induced changes of soil, whilst considering plant physiological and root morphological responses.

The experiment A was presented in **6th symposium on Phosphorus in Soils and Plants, PSP6**, Leuven, Belgium, 2018.

3.1 Abstract

The processes in the rhizosphere provide a strong linkage between plant and soil and can increase P solubilisation of the soil particularly treated with agricultural inputs by acidifying the soil around the root zone, de-phosphorylating and chelating metal ions. Currently, the sustainable type of new low-grade P supply come into the market, as a result of the increasingly scarcity of resource of high-grade PR material over time. For this reason, assessing the behaviour of different phosphate fertiliser types, specifically widely available low-grade PR, and their potential efficiency, was critical. The efficiency of this P supply material can be manipulated by rhizosphere engineering approaches, which has the potential to provide further insight of P solubilisation and enrich this study for crop benefit.

The main aim of the research in this chapter was therefore, to gain a better understanding of how to optimize nutrient management in the rhizosphere. The working hypotheses of this chapter are: (1) different types of P fertilisers have differing resulting effects on the P solubilisation in the rhizosphere; and (2) the emission of organic acids in the rhizosphere at near neutral soil pH increases the P solubilisation of soils with different levels of P exposure. The objectives were: (1) to assess the link between behaviour of P dynamics and P solubilisation processes in the rhizosphere, with corresponding plant performance under various levels of different P exposure using different types of PR, calcium perphosphate monohydrate and urea phosphate (2) to study the effects of rhizosphere P solubilisation processes in combination with different types of root zone acidification in soils treated with low-grade PR.

This study was designed to test the P solubilisation processes in the rhizosphere with corresponding plant performance into two separate experiments with mono and mixed cropping systems under the variable P conditioning via the different types of P fertiliser application at different rates, as a foundation for later chapters. The low-grade raw material was intentionally applied directly to the soil to determine how much of PR was solubilised within the rhizosphere to be available for plant uptake. I conducted the controlled experiments in the small pot for a duration of the period after planting: 50

days (Experiment A); and 36 days (Experiment B). The Olsen P in soil was used as key indicator of P solubilisation. The biomass, plant P concentrations, root morphology and other rhizosphere parameters were also determined.

The results highlighted a positive relationship between plant growth and soluble P in the soil. The plant growth performed well under increased rate of solubilised P in the rhizosphere associated with types and rates of P supply. The P solubilisation of directly applied low-grade PR increased when it integrated with root acidifying conditionings via various forms of chemical application and inter-cropping. For example, the low-grade PR application in combination with sulphuric acid produced the most significant level of soluble P than the controlled pots including with and without PR addition on its own where it showed a weaker P solubilisation effect with slower plant growth rate. Taken together, the outcomes of these experiment support to accept the Hypothesis 1 and 2. Evidencing that, under the influence of rhizosphere engineering, the utilisation of direct application of low-grade PR could help to build a viable case for sustainable use of P in the future.

3.2 Introduction

Phosphorus is an essential plant nutrient for cellular function and plant growth. Significant quantities of P are required in the early stages of plant growth (Brevedan et al., 1978). In addition to the plant P demand, many agricultural soils need more than one P application per growing season, due to the depletion of soil soluble P at different levels. Phosphate rock (PR) is used as major P source to maintain soil fertility for target crop productivity. This is a primary raw phosphate bearing mineral, coming from the mineral apatite (further detailed discussion in the literature review at chapter 2 on page 40-41). To understand the behavior of different types of P fertilisers in the rhizosphere, particularly low-grade PR is key focus of this chapter.

3.2.1 The phosphate rock application

The phosphate compounds of PR may not be ready for the plant available, since it is not easily soluble in water. Without a prior ignition procedure, phosphate compounds of PR are only extracted by H_2SO_4. However, various chemical-extracting solutions are capable to solubilise the phosphate compounds of PR such as neutral ammonium citrate (NAC), 2% citric acid, and 2% formic acid. The association of Official Agricultural Chemists (AOAC) categorizes PR into: (1) water soluble; citrate soluble, (2) water insoluble; neutral ammonium citrate soluble, and (3) citrate insoluble. However, almost 89% of the world’s PR reserves containing P compounds in medium solubility (according to the United States Geological Survey), which may be suitable for direct application on the soil. The direct application of phosphate rock (DAPR) would be an alternative option of fertiliser application in many developing countries, particularly where resources are suitable and widely available. The DAPR helps to avoid labour intensive and energy consuming manufacturing procedure of producing soluble fertiliser associated with the thermal process of carbonate removal. This method is also not generating harmful by-products in the mining sites. Directly applied PR can be solubilised P within the soil through biochemical processes in the rhizosphere (Prochnow et al., 2001) derived from plants and microbes.

The level of solubilisation will depend on the chemical, physical, mineralogical, and crystallographic characteristics of particular apatite (please visit literature review in Chapter 2).

3.2.2 Role of mutualistic association in the phosphorus solubilisation around the root zone

The root-induced acidification decreases the rhizosphere pH by 2 to 3 units originating from proton released by the organic acid exudation and the inhibition of nitrate uptake processes surrounding mutualistic association (Shen et al., 2005). The soil pH has a strong impact on P solubilisation processes, as the rhizosphere processes can promote the dissolution of P bearing minerals, alter kinetics of orthophosphate adsorption- desorption reactions and increase solubility of Ca phosphates, especially in alkaline soils (Hinsinger, 2001; Neumann et al., 1999; Neumann and Römheld 1999; Shen et al., 2005).

Symbiotic relationships between plants

The co-cropping for example, combination of cereal and legume is a common agricultural phenomenon, which is employed as a tool to improve phosphorus use efficiency (PUE) and P uptake (Hauggaardnielsen et al., 2001) and practiced in many parts of the world. In cropland, legume species can nourish neighbouring plants (Li et al., 2014) by adding carboxylates and nitrogen compounds (e.g., NH_4^+ and amino acids) into the zone of root intermingling. The root medium acidification can ultimately increase the level of soluble P in the soil (Li et al., 2008) by stimulating acid phosphatase activities in the rhizosphere. Under the companion of different types of crops in the field, plant P acquisition can be increased as much as 20%, even there is an increase in the fields without adding any P supply (Li et al., 2008). In parallel to underground processes, the co-growth of crops in the agro-ecosystem has a wide range of benefits on the over-ground, including, structural support, suppression of weeds, partial shading from too much sunlight, reduction of pests (plant combination with marigolds) and increase in biodiversity and land equivalency ratio by planting the deep-rooted crops with shallow-rooted crops, tall with shorter, fast growing with slow growing crops and legume with cereal. However, in the crop yield, the companion of different types of crops growing in close proximity can cause heavy shading that may lead the detrimental negative impact on plant morphology (Wu et al., 2017), physiology (Liu et al., 2016) and carbon production (Feng et al., 2018).

Multiple types of plants form a mutualistic association of saprophytes and symbionts (Barea, 2000). In the co-cropping systems, the biodiversity of the crop species always encourages the habitats, insect and soil microorganisms, which may not be present in a sole crop environment. As shown in Figure 3.2.1, in the intercropping system of legume and cereal, the maize root exudates the flavonoid genistein (Coskun et al., 2017) the nodulation and N_2 fixation factor that contribute the expression of *nod* genes in diazotrophic bacteria and increase in synthesis of nodulation (Nod) factors and lipochitooligosaccharides via the root of faba bean (Begum et al., 2001). Such network signalling flavonoid exudates produced by legume root e.g., genistein, naringenin, and hesperetin (Erik et al., 2015) orchestrates the endosymbiotic relationship between plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) through activation of Ca^+ dependent signalling cascade of nodule formation (Laurent et al., 2013).

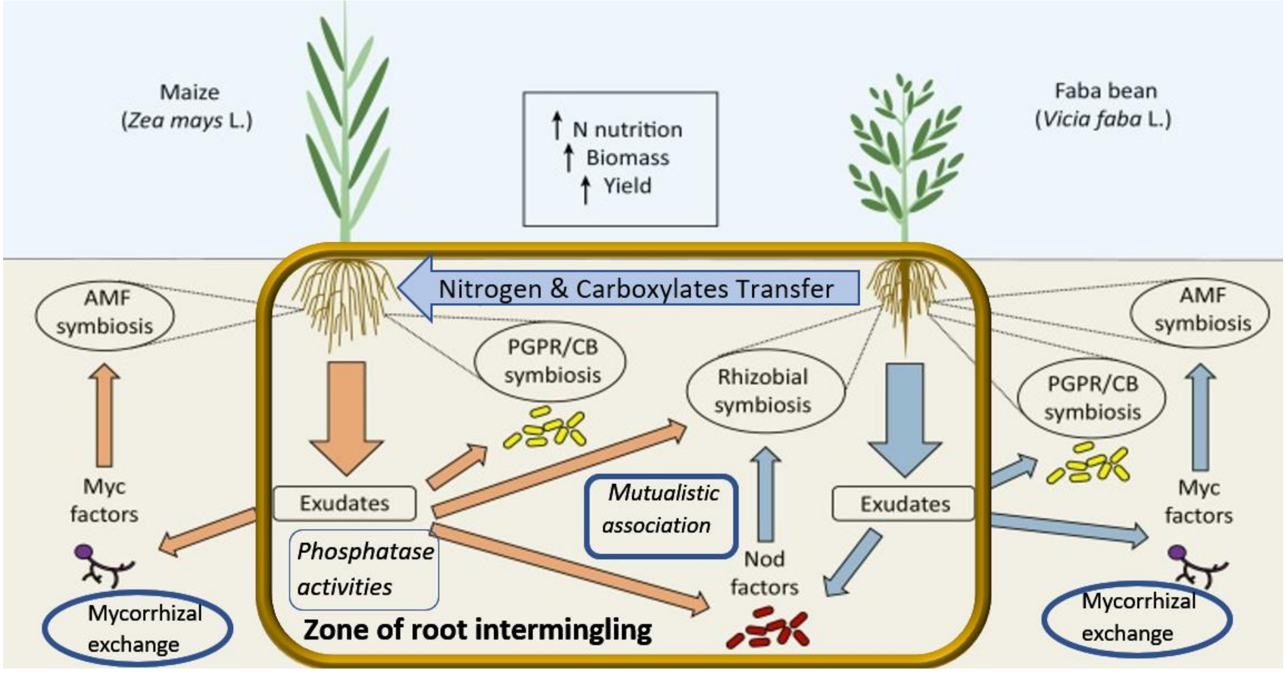


Figure 0.1 Schematic representation of the endosymbiotic relationship between plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi in intercropping system, which was taken from Coskun et al. (2017) adjusted by the author of this thesis.

Root association with symbionts

Plant root and microorganisms including saprophytes and mutualistic symbiont association are the natural allies in exchanging nutrients (Barea, 2000) through an ingenious trade-off within the immediate area of soil around the root. The bacteria and fungi can usually take up carbon from the sugars and sugary products of photosynthesis from the plant root (Smith et al., 2003), in turn they extract a range of vital nutrients into the soil matrix. In addition, fungal hyphae are efficiently support the plant nutrient uptake by different mechanisms, including enlarging spatial availability of root surface area, forming an underground network for the connection of multiple plants and exploiting soil pores. This allows the plant roots to access vast range of nutrients at a distance of several cm where nutrient patches may not be directly accessible (Begum et al., 2019). The most prevalent plant symbiotic associations represented as arbuscular mycorrhizal fungi (AMF) endosymbiosis (Fig 3.2.2), accounting for 74% (Brundrett, 2009). The length and density of fungal hyphae are always positively correlated with the plant growth rate, shoot biomass, and P uptake. At the same time, the hyphal exudates can affect the soil aggregates and the bacterial community around the root (Filion et al., 1999; Toljander et al., 2010).

Prior to the root colonization, the spores are germinated in the suitable soil condition under the appropriate P concentration (Zobel et al., 2007; 2005). The several stages of AMF development include recognition of host, the formation of appressorium, and hyphal growth. In the low P level of soil, mycelium of AMF can form a long-scattered branch (Nagahashi et al., 1996) that is controlled by the host root exudates, known as strigolactones (Kohki et al., 2005). If the concentration of these growth promoting root exudates are high, the mycorrhizal branches are tightly clustered. However, disruption of extra-radical hyphal network often occurs during the tillage practices and excessive agricultural P input, which can reduce the inoculation potential of soil mycorrhizae.

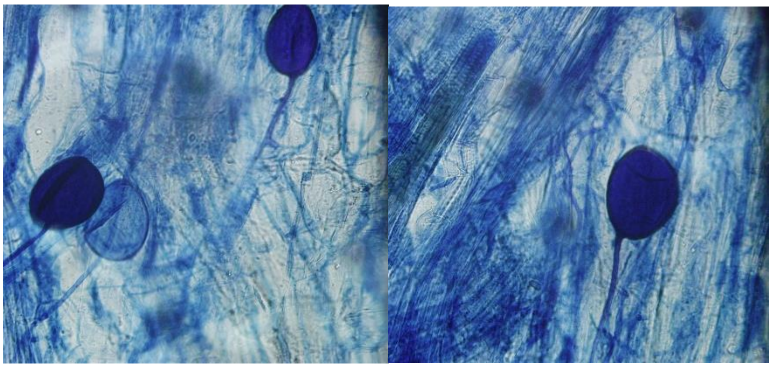


Figure 0.2 The arbuscular mycorrhizal fungi colonization of maize root, grown in soil added phosphate rock, observed by the author during the experiment A.

To improve P acquisition is a great challenge specifically plant growing in the soil treated with low-grade PR supply. It requires the constructive strategies for manipulating plant and soil to enhance plant productivity. Some strategies directly target rhizosphere processes that impact on plant growth and components of the rhizosphere. For instance, the rhizosphere can be engineered to modify the root medium pH and to interfere with the synthesis of exudates that improve P availability of the soil.

3.2.3 Aims and hypothesis

The rationale of this chapter is, therefore, to gain better understanding of the optimization of PR uptake for maize in the rhizosphere. This research aims to investigate how the processes in the rhizosphere enable their manipulation to increase P use efficiency under the behaviour of different types of phosphate fertiliser. Moreover, this work is also interested in assessing potential efficiency of new low-grade PR under the scenario of root zone acidification associated with various form of acidifying chemicals and legume addition in relation to the rhizosphere engineering, where the sustainability of P resources is in considerable critical attention. The P solubilisation in the root medium of maize is further studied in the mixed cropped system.

The objectives of this chapter, and their associated hypotheses are:

1. To assess the link between behaviour of P dynamics and P solubilisation processes in the rhizosphere, with corresponding plant performance under various levels of different P

exposure using different types of PR, calcium perphosphate monohydrate and urea phosphate

Hypothesis 1: Different types of P fertilisers have differing resulting effects on the P solubilisation in the rhizosphere

2. To study the effects of rhizosphere P solubilisation processes in combination with different types of root zone acidification in soils treated with low-grade PR.

   Hypothesis 2: The emission of organic acids in the rhizosphere at near neutral soil pH increases the P solubilisation of soils with different levels of P exposure.

3.3 Materials and methods

This chapter consists of two experiments in the greenhouse undertaken on monocropped (A) and intercropped (B) maize performance in order to determine the behaviour of different types of phosphate fertiliser and their potential efficiency on the rhizosphere. The same amount of solid phosphate rocks (PR) used for each pot in both experiments A and B. The PR was grounded to a powder, being able to pass through a mesh at the size of 100. Two different types of PR were directly applied to the soil: (a) one was the low-grade PR obtained from Jinning, Yunnan province used as a centre of interest in both experiments A and B, and (b) another PR was only used in experiment B and bought from online purchase that is commercially available for the general public. The abbreviation of CPR shall be used when referring to latter commercial PRs throughout this study and any graphs herein. Both PR and CPR samples were sent to the laboratory analysis prior to the experiment in order to confirm P composition. The contents were (a) low-grade PR: 18.15% of P_2O_5; 4.36% citrate soluble P and 0.06% water soluble P and (b) CPR: 15.39 % of P_2O_5; 14.12 % citrate soluble P and 11.78 % water soluble P according to the results obtained from the laboratory at Yuntianhua group in Yunnan province, China.

Olsen P was used for the representative of P solubilisation. The focus crop was maize (*Zea mays* L. cv. ZD958) in both experiment A and B. Whilst, three different types of crop seeds were included in the experiment, including the alfalfa (*Medicago sativa* L.), faba bean (*Vicia faba* L.) seeds at the weight of 0.003g and 1.20g respectively. The maize seeds used in this experiment was weighting between 0.45 to 0.50g. All seeds were obtained from Plant and Nutrition Lab, CAU.

Soil description	Experiment A	Experiment B
Soil sampling sites	Unfertilized native vegetation sites in Taian, Shandong	Fallow land, Qingdao, Shandong
Soil texture	Silt loam	Sandy loam
Olsen P content (mg kg^{-1})	9.74	22.3
Soil acidity (pH)	6.5	7.3

Table 3.3.1 Types of soil used in experiments A and B, collected from different sampling sites of Shandong province, China.

The soils were used in this chapter, sampled from two different locations of Shandong province of China. The silt loam textured brown soils used in the mono-cropping experiment A were collected from unfertilized native vegetation sites in Taian regions of Shandong with containing Olsen-P at 9.74 mg kg^{-1} and pH at 6.5. Whereas sandy loam textured brown Cambisol soils were used in the intercropped experiment B, collected from fallow land, Qingdao, Shandong, China (Latitude: 29^{o}03’N, Longitude: 106^{o}11’E 170m) by Prof, Ding (Qingdao University experiment station) with containing Olsen P of 22.3 mg kg^{-1} and soil pH 7.3.

3.3.1 Experimental design

These experiments were designed to show a multi model exercise of rhizosphere in exploring the morphological and physiological performance of maize grown in the soil with the different types of P supply at a different rate. In order to support the experiment, four distinctive P fertiliser applications were selected to represent the behaviours of different phosphate fertiliser types on the rhizosphere, including superphosphate, urea phosphate and two different types of PR. Each treatment was replicated in four samples. The direct application of low-grade PR into the soil was intentionally mixed with the root medium acidifiers: ammonium, sulphuric acid, and exogenous citrate, and legume species in order to optimise low-grade PR application. The outcomes enabled me to cross compare P solubilisation of soils with non-P added, PR added on their own, and PR application under the root-induced acidification conditioning.

The rates of P supply were used in both experiments, ranged from 0 to 500 mg kg^{-1}, particularly, I chose a high dose value of 500 mg kg^{-1} to the soil by a single instance, prior to the planting. I opted to apply larger quantities of P fertiliser supply following reasons. Firstly, P bioavailability of pot soil continuously depletes during the planting period over the duration of the 50-day and 36-day. Secondly, poor solubility of low-grade PR materials is assumed unlikely to maintain the optimal plant available P concentration in the rhizosphere to meet the P demand of faster growing plant. As compared to field growth, the natural root adventure can be restricted by the size of pot, which may require a considerably higher concentration of P in the soil. Thirdly, to observe growth differences of maize among the different P treatment within the relative shorter period of time extension during these experiments, the wide range of P rate can be introduced.

3.3.1.2 Experiment A: Optimisation of phosphate rock (PR) uptake to maize in the rhizosphere

This pot grown mono-cropped maize experiment was conducted in the greenhouse between 09th July and 28th August 2017. This controlled experiment consisted of eight different treatments e.g. different types soluble P fertilisers and low-grade PR in combination with other chemicals including control without P addition. In all treatments, soils were prepared prior planting by adding P fertiliser equivalent to 500 mg P kg^{-1}, except for treatments UP and SP250. The amount of P applied to the UP and SP250 treatments were at the rate of 75 mg P kg^{-1} in the urea phosphate and 250 mg P kg^{-1} in the calcium perphosphate monohydrate respectively. The table below demonstrated the eight treatments including abbreviations, fertiliser types, chemical formula, rate and amount of P applied to the pot soils and illustrates the calculation of each treatment by the explanatory note.

The explanatory note of table below is as follows:

***In the treatment of PR-SA, the calculations of amounts of sulphuric acid were made on the basis of manufacturing ratio to proceed PR that is equivalent with 75 mg P kg^{-}.

** Manufacturing procedure to produce soluble P from PR requires 98% sulphuric acid at the ratio of 25:14.5-PR to H_2SO which means the extraction of 25 tonnes PR requires 14 tonnes of sulphuric acid per hour.

* An amount of basal nutrients added to the potted soils were exactly the same as stated in Wen et al., in 2017. Equal amounts of calcium nitrate $Ca(NO_3)_2.4H_2O$ and ammonium sulphate $(NH4)_2SO_4$ were supplemented to all pots at the rate of 100 and 100 mg kg^{-1} as mentioned (Mollier and Pellerin, 1999), with the sum of 200 mg kg^{-1} N supply at all. However, in the treatment PR-AS, the ammonium sulphate was only applied by their own at the rate of 200 mg kg^{-1}.

	Abbreviation of treatments	Phosphorus supply and contents	Chemical formula	Phosphorus application rate	Amount of P per 2 kg soil	Calculation is based on
1	***P0***	Control	No additional P			
2	***SP250***	Calcium perphosphate monohydrate	$Ca(H_2PO_4)_2$ $CaSO_4 \cdot H_2O$	250 mg kg^{-1}	3.1 g	Molecular mass
3	***SP500***	Calcium perphosphate monohydrate	$Ca(H_2PO_4)_2$ $CaSO_4 \cdot H_2O$	500 mg kg^{-1}	6.2 g	Molecular mass
4	***PR500***	Low graded Jinning phosphate rock	Phosphate rock (PR)	500 mg kg^{-1}	12.62g	P_2O_5 contents at 18.15%
5	***PR-UP***	1. Urea phosphate	$CO(NH_2)_2 \cdot H_3PO_4$	75 mg kg^{-1}	0.7g	Molecular Mass
		2. Phosphate rock	Phosphate rock (PR)	425 mg kg^{-1}	10.72g	P_2O_5
6	***UP***	Urea phosphate	$CO(NH_2)_2 \cdot H_3PO_4$	75 mg kg^{-1}	0.7g	Molecular mass
7	***PR-SA***	1. Phosphate rock	Phosphate rock (PR)	500 mg kg^{-1}	12.62g	P_2O_5 contents at 18.15%
		2. Sulphuric acids	98% H_2SO_4	equivalent to process PR at the rate of 75mg P kg $^{-1}$ ***	1.1ml	Manufacturing procedure ratio** PR: H_2SO_4 is 25:14.5
8	***PR-AS***	1. Phosphate rock	Phosphate rock (PR)	500 mg kg^{-1}	12.62g	P_2O_5 contents at 18.15%
		2. Ammonium sulphate	$(NH_4)_2SO_4$	200 mg kg^{-1}	0.4g	Basal nutrient supplements*

Table 3.3.2 Calculations of phosphorus and other chemicals used in this study with the explanatory note in the page above.

3.3.1.2 Experiment B: Behaviour of mixed crops in the soil on the uptake of phosphate rock. Testing for alternative approaches in rhizosphere

The co-cropped pot experiment B was conducted in the greenhouse between 1st May and 6th June 2018. The schematic representation of experimental design shows in figure 3.3.1, (a) the planting combinations in the cylindrical shaped cells and (b) the P treatments of each planting combination in the oval shaped cells coloured in grey. The experiment consisted of 8 treatments: five different P treatments were isolated in three planting combination treatments (a) the monocropped maize (***M&M***) with two maize (*Zea mays*. L) per pot; (b) intercropped maize and faba bean (***M&F***) with one maize (*Zea mays*. L) and one faba bean (*Vicia faba*. L) per pot; and (c) maize and alfalfa (***M&A***) with one maize (*Zea mays*. L) and eight alfalfa (*Medicago sativa*. L) per pot. The mono-cropped maize was used as a control. Citric acid monohydrate ($C_6H_8O_7H_2O$) solution was used in this experiment to modify the rhizosphere pH. The amount of citric acid was calculated on average of 4.5 mg kg^{-1} on the basis of the molar mass (210.14). It was diluted in 20 ml deionised water to make a solution at the concentration of 2 mM. During the growing period, this dilution was sprayed into the soil and repeated in five times in the days of 6, 12, 18, 24 and 30 after planting. This method was modified from Hu et al. (2016). Citric acid treatment was subcategorized into two groups, such as (a) ***cit*-P0** and (b) ***cit*-PR**. The P treatments of this experiment including the abbreviation were:

- ***P0***: Control without any additional P
- ***cit*-P0**: Citric acid only treatment, without adding any P supply.
- ***cit*-PR**: Combination of citric acid and PR with P concentration at the rate of 500 mg kg^{-1}.
- ***PR***: The low-grade PR treatment with P concentration at a rate of 500 mg kg^{-1} (calculation please see PR in Tab 3.1).
- ***CPR***: The commercial phosphate rock treatment, used 14.8g PR per pot of 2kg soil, was calculated P_2O_5 concentration at 15.39 %.

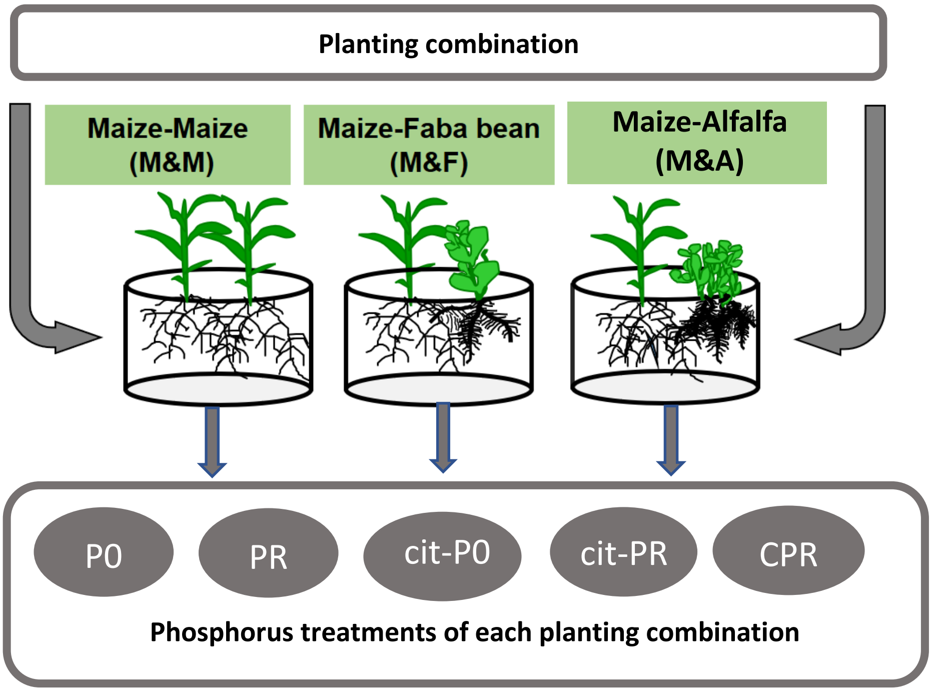


Figure 0.1 Schematic representation of intercropping experiment with three different plant combinations and five phosphorus treatments.

3.3.2 Experimental procedure

Both experiments were conducted in a naturally lit greenhouse at the China Agricultural University (CAU) within the different periods and duration of time, consisting of four steps: preparation, planting, data collection and data analysis.

Preparation for the experiment:

All soils were air dried for 72 hours, then sieved through a 2 mm sieve in order to separate soil particles prior to the addition of the different P treatments and basal nutrients. The basal micronutrients were adequately calculated as a supplement in the soil prior to plant placement according to methods modified from Mollier in 1999. Basal nutrients were added at the following rates (mg pot^{-1}): $Ca(NO_3)_2\cdot 4H_2O$ 3374; K_2SO_4 670; $CaCl_2$ 252; $MgSO_4\cdot 7H_2O$ 86; EDTA-FeNa 11.6; $MnSO_4\cdot 4H_2O$ 13.4; $ZnSO_4\cdot 7H_2O$ 20; $CuSO_4\cdot 5H_2O$ 4; H_3BO_3 1.34 and $(NH_4)6Mo_7O_{24}\cdot 4H_2O$ 0.52. Two layers of filter papers were lined on the bottom of appropriately labelled pots to cover the drainage hole. Each pot was filled with 2kg of thoroughly mixed soil with the appropriate amount of rock phosphate and basal nutrients.

Seeds were surface sterilised by 10% H_2O_2 for 30 minutes, then rinsed by distilled water several times before being imbibed in well-aerated saturated $CaSO_4$ solution in dark room for 8 hours. All seeds were germinated for 3 days (1 day for Alfalfa) in paper towels, which are placed on the tray soaked with distilled water, in the dark and humid environment at 22^{o}C (Mollier and Pellerin, 1999). The tray of was covered with a loose-fitting lid in order to allow some gas exchange. Two uniformly germinated seedlings were then transplanted into each pot within the depth of 2 cm from surface by keeping the same spacing distance from neighbouring transplant. The size of the pot was 18 cm in height with a diameter 18.5 and 14.5 cm at the top and bottom respectively. Each treatment was replicated 4 times with the control. Pots in both experiments were randomly designed.

The planting periods:

Greenhouse temperature in experiment (A) was maintained at around 20-28^{o}C in the daytime and 16-20^{o}C in the night, with 14-15 hours for the daytime throughout the growth. Whereas in experiment (B) 22-26^{o}C in the daytime and 13-17^{o}C in the night, with 13-14 hours for the daytime. Pots were watered via deionised water with the frequency of every other day in order to maintain the weight at 75% field capacity whilst controlling infection. I kept observing the aerial parts of maize with periodical observation in every 12 days for the detailed plant growth performance and photosynthetic activities including leave number, leaves area, height of stem, shoot diameter and chlorophyll content of leaves. The leaf chlorophyll concentration was measured by chlorophyll SPAD-502 Plus meter (Konica, Minolta). The shoot diameter and length including leaf length and width were collected by tape measure and caliper. Then total leave area was calculated by sum of all individual leaf areas ($A=F \times L \times W$) through the multiplication of length (L) and width (W). F is equivalent to 0.75 in all fully expanded leaves, 0.5 is other leaves.

The data collection:

Plants were harvested at the day of 50 in experiment (A) and 38 in experiment (B) after planting, when growth differences of plants were visible among the P treatments. The list of the measures made for the analysis as follows:

1. Soil analysis
    i. Olsen P (bulk and rhizosphere soil)
    ii. pH (bulk and rhizosphere soil)
    iii. Dry weight of rhizosphere soil
    iv. Total P
    v. Water soluble P
    vi. Acid/Alkaline phosphatase activity (rhizosphere soil)
    vii. Organic acid determination (rhizosphere soil)
2. Plant analysis
    i. Shoot and root dry mass
    ii. Plant P concentration/ P content
    iii. The root image analysis for total root length
    iv. Specific root length
3. Mycorrhizal analysis
    i. Intensity of colonisation
    ii. Frequency of colonisation

Plant shoots were cut just above the soil surface and then oven dried for 30 minutes at 105^{o}C and then 3 days at 70^{o}C for weighing biomass. Roots were removed carefully from soil and shaken in 50 ml 0.2greenhouse temperature mM $CaCl_2$ solution to collect the rhizosphere solution modified from the published methods (Pearse et al., 2007). All samples were kept at 4^{o}C for 3 days prior to further analysis of acid phosphatase activity. The remainder of rhizosphere extraction subsamples was stored at -20^{o}C for carboxylates HPLC determination with adding microbial inhibitor Micropur (Sicheres Trinkwasser, Germany) at 0.01gL^{-1} and 2 drops of concentrated phosphoric acids.

Rhizosphere pH value was measured on the day of harvesting. The arithmetical measure of pH value of rhizosphere solution was measured by using Seven-Compact S210 pH meter, whilst measurement of bulk soil was suspended in deionised water at the ratio 1:2.5 in soil to water after the air-drying of 7 days (www.globe.gov).

Determination of the acid phosphatase activity of the intact roots was performed through (Alvey et al., 2001) the colorimetric analysis of the *p*-nitrophenol. 0.4 mL of 0.04M sodium acetate buffer (pH 5.2) and 0.1 mL 0.15 mol L^{-1} *p*-nitrophenol phosphate (PNP) were added to 0.5mL rhizosphere suspension. After incubation of soil with at 28^{o}C for 30 minutes, the reactions were terminated of by 0.5 M NaOH. The absorbent substance released by phosphatase activities was measured spectrophotometrically at 405nm. One unit of acid phosphatase activity was defined as the activity per gram soil that produced 1 µmol p-nitrophenol per hour.

Carboxylates were analysed by a reversed-phase high-performance liquid chromatography (HPLC) system modified from Shen et al., 2003 and Wang et al., 2010. In prior, soil suspension was filtered through a 0.22-µm to avoiding large particle to block a machine. The chromatographic separation was conducted on a 250 × 4.6 mm reversed-phase column (Alltima C18, 5 µm; Alltech Associates, Inc., Deerfield, IL, USA). The mobile phase was 25 mmol L^{-1} KH_2PO_4 (pH 2.3) with a flow rate of 1 mL min^{-1} at 31 °C with detection of carboxylates at 214 nm.

The remaining part of rhizosphere extraction in the tube was dried in open air in order to measure rhizosphere soil weigh and further soil P testing. Washed roots were frozen until root image analysis with EPSON scanner at 400 dpi (Epson Expression 1600 pro, Model EU-35, Japan) following software Win-Rhizo (Regent Instruments Inc., Quebec, Canada) for calculation of total and specific root length. Specific root length is length of root per 1g dry weight via m g^{-} (Jing et al., 2010). It can be used as indicator of environmental changes (Ostonen et al., 2007). As soon as completion of root scanning, oven dried roots were used to measure dry weight, R/S ratio, and root P concentration.

Plant P was determined by 50 mg of dried plant material through the digestion method of the wet (exp A) or heat (exp B) process, due to the equipment availability. This method uses concentrated sulphuric acid-hydrogen peroxide (H_2SO_4 and H_2O_2) for wet or furnace at 550^{o}C for heat method. Digested samples were diluted to a final volume of 50 mL with milli-Q water and stored at room temperature before determination of orthophosphate. The P concentration of plant was determined by vanadomolybdate method using spectrophotometry at 440 nm (Johnson and Ulrich, 1959).

Soil samples were analysed at the beginning before planting and at the harvesting to allow comparison of the change soil P availability over the growth period. In brief, soil P fraction were designated to represent “soil soluble P” was determined by the Olsen (1954) methods from the air-dried soil extracted with 0.5 M $NaHCO_3$ at pH 8.5. In addition, water soluble P was determined by the method is modified from Juan et al (Feng et al., 2011) by using malachite green oxalate salt with polyvinyl alcohol, added after the molybdate acidifying reaction in soil solution, with absorption of wavelength in spectrophotometric measurement at 610 nm. Soil TP was also analysed by using digestive and colorimetric method based on a reaction with acidic molybdate in the presence of phosphomolybdate complex and measured spectrophotometrically at 880 nm.

Mycorrhizal colonization was determined by methods modified from the published paper (Trouvelot et al., 1986). One cm long cut root pieces were heated for 60 minutes at 90 ° C within the bath of 10% KOH and then washed by tap water for 3-5 times. After washing, rinsed with 2% HCl for 5 minutes, citric acid was added and then roots were stained by trypan blue in 90 ℃ with water bath for 30 minutes. After overnight incubation at 1: 1 ratio of lactic acid and glycerol solution at room temperature, 30 root pieces were placed on the slide for microscopic examination of mycorrhizal infection rate. The root colonization parameters were calculated by *MYCOCALC.EXE* software.

Data analysis

Statistical analyses were performed using the software SPSS version 23 (IBM, SPSS 2014). In all cases, a confidence threshold of 0.05 was applied. A one-way analysis of variance was performed on all datasets and treatments with significance of differences between treatments (significance reported as $p < 0.05$) by using SPSS statistical software. The data are reported as mean ± standard deviation. Standard error of four replicates was calculated throughout the experiments. The mean differences of soil were assessed based on the least significant difference (LSD) at the $P \leq 0.05$ probability under

Duncan's *post-hoc* multiple range analysis for multiple comparisons. The exponential equation analysis was performed to explore relationship between root/shoot ratio, soil pH and shoot P concentration or soil Olsen-P (Li et al. 2008). Empirical polynomial (inverse third order) equations were used to establish the relationship between root morpho-physiological traits (i.e. total root length, specific root length or proportion of fine roots, the organic acids and acid phosphatase activity of root medium) and rhizosphere Olsen-P through the SigmaPlot (SigmaPlot 10.0, USA) modified from published method (Deng et al., 2014).

3.4 Results

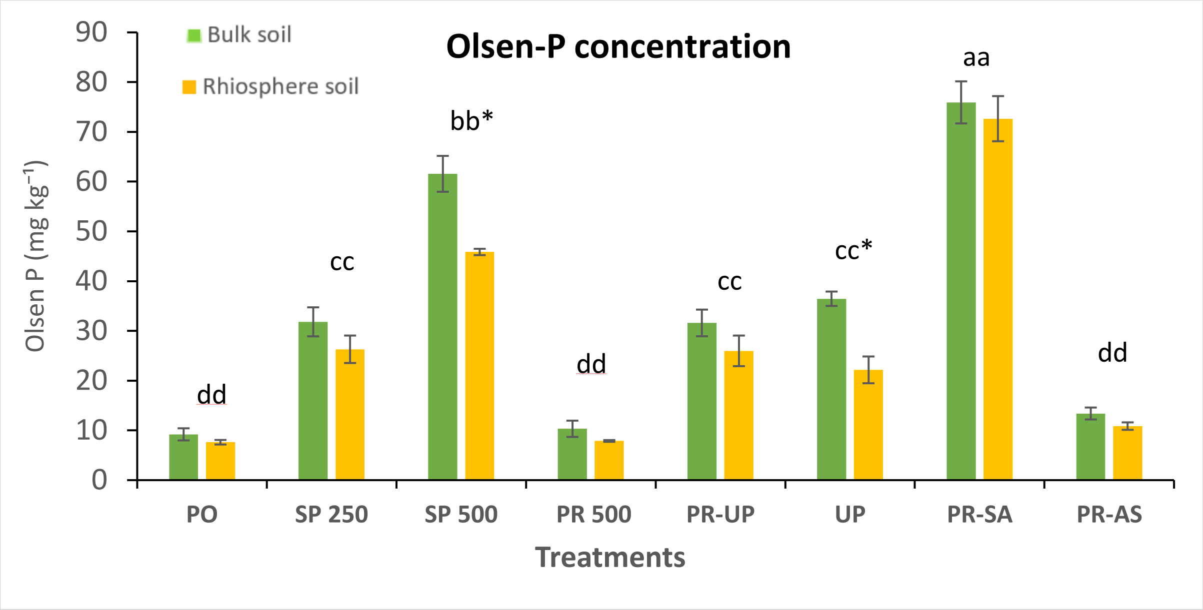
The result obtained from both experiments A (monocropping) and B (intercropping) was generally indicated a strong linkage between soil P dynamics and processes around the root zone. Finding of the experiments can be grouped into the following categories: (a) soil P dissolution; (b) plant P uptake and growth; (c) root morphological structure and mycorrhizal symbiosis; and (d) rhizosphere excretions and pH. The properties of soils sampled from two different locations of Shandong soil were outlined in the Materials and Methods section. Analyses were carried out in Plant and Nutrition Lab, CAU.

3.4.1 Experiment A: Optimisation of phosphate rock uptake to maize in the rhizosphere

The silt loam textured brown soils used in the mono-cropping experiment (A) were collected from the Taian regions of Shandong with containing Olsen-P at 9.74 mg kg^{-1}; water soluble P at 0.104 mg kg^{-1} and TP at 167 mg kg^{-1} total N 0.013%, total carbon 0.11% and pH at 6.5.

3.4.1.1. Soil phosphorus dissolution

The main characteristics of soil P solubilisation represented as an Olsen P and detected in bulk and rhizosphere soil. The figure below illustrates the comparisons of effects of the P amendments through an overview of the analysis of soil Olsen P concentration of each treatment group.



* The mean difference is significant at the 0.05 level.

Figure 0.1 The Olsen-P concentrations of the bulk soil (i) and their changes in the rhizosphere (ii) of maize (*Zea mays*. L) that grown in variable phosphorus supply including statistically significant differences (*) of Olsen P between bulk and rhizosphere.

The data sets above are based on the means of four replicates and error bars shown on standard error. The different letters indicate the difference by one-way ANOVA, a Duncan *post hoc* test. The statistically significant difference between groups (i) and (ii) were determined by the T test and the outcome was that there was no statistically significant difference found between the variable phosphorus treatment groups ($p \leq 0.05$).

The substantially different levels of P solubility showed in Figure 3.4.1 depend on the degree of root medium acidification, and effects of P source and concentrations. The addition of variable chemical substances, among all treatment groups, made a clear impact on P solubilisation processes of the soil specifically when it treated with low grade PR. For example, the highest concentration of Olsen-P was observed in the PR application being combined with sulphuric acid, with 8-fold increase from 9.7 to 76 mg kg^{-1}. It is followed by superphosphate application (SP500) at 61 mg kg^{-1}. In contrast, the Olsen-P concentration of control (P0) and PR only (PR500) pots were similarly low at the end of experiment at 9 and 10 mg kg^{-1}, respectively. The statistically significant difference ($p \leq 0.05$) between Olsen-P in the bulk and rhizosphere soil was observed in SP500 and UP treatments under closer inspection. Indeed, the Olsen-P in bulk and rhizosphere soil was at 61.6 and 45.9 mg kg ^{1} in SP500 and 36.5 and 22.2 mg kg ^{1} in UP, respectively.

The level of water-soluble P considerably varied in this experiment, ranging from 0.51 mg kg $^{-1}$ in PR-SA to 0.178 mg kg $^{-1}$ control. It follows a similar pattern to the Olsen-P. However, unlike Olsen P, the concentration of water-soluble P in calcium phosphate (SP 250 and SP 500) appeared almost equal to PR-UP and UP treatments at around 0.29 and 0.3 mg kg $^{-1}$, respectively. This may be associated with the behaviours of P fertilisers in the rhizosphere. In this experiment, amount of total P in soils was also observed that elevated in all treatment at the end of the experiment, except for the unfertilized control soil with a concentration of 154 mg kg^{-1}. The highest total P concentrations were detected, particularly, in the soils treated with phosphate rock only (PR500) and PR in combination with ammonium sulphate (PR-AS) at 266 and 244 mg kg $^{-1}$ respectively in the harvest.

3.4.1.2 Plant indicator parameters

The entire plant indicator parameters of eight treatments are presented in Table 3.4.1, including plant P contents and the relative plant responses to different levels of soil soluble P concentration.

Table 3.4.1 Summary of mono-cropping maize experiment including the soil phosphorus availability and plant total phosphorus concentration under the different level of phosphorus supply. The plant shoot and root morphological parameters such as dry biomass weight, specific root length and proportion of fine root with the diameter below 0.2 cm are shown below. Plants were sampled at the day 50 of planting.

Rate of P supply per treatment (mg kg^{-1})		**Rhizosphere Olsen P (mg kg^{-1})**	**Total P content of plant (mg g^{-1})**	**Shoot P concentration (mg g^{-1})**	**Plant P uptake (mg plant^{-1})**	**Dry weight (g plant^{-1})**		**Root length (m plant^{-1})**	**R/S ratio**	**Specific root length (m g^{-1})**	**Volume of fine root (%)(%)**
						Shoot	**Root**				
PO	0	7.6 (0.46) d	2.3 (0.33) d	1.33 (0.16) d	11.6 (1.62) e	8.7 (0.22) d	2.42(0.02) b	139.6 (0.14) cd	0.28 (0.01)	57.63 (5.4)	58 (0.9)
PR	500	7.9 (0.17) d	2.9 (0.5) d	1.87 (0.33) cd	16.7 (2.9) de	9 (0.84) d	2.00 (0.02) c	81.5 (0.14) d	0.23 (0.02)	40.74 (6.7)	55 (2.7)
PR-AS	500	10.9 (0.75) d	3.1 (0.25) d	2.0 (0.19) cd	22 (1.67) de	11.1 (0.26) c	1.93 (0.03) c	103.6 (0.07) cd	0.17(0.006)	53.61 (3.8)	58 (1.7)
UP	75	22.2 (2.69) c	3.4 (0.32) cd	2.64 (0.28) bcd	33.6 (5.57) cd	12.5 (1.3) bc	2.45 (0.05) b	145.5 (0.25) bcd	0.2 (0.02)	59.37 (9.3)	65 (3.3)
PR-UP	500	25.9 (3.08) c	5 (1) bc	3.79 (0.8) b	51 (10.56) bc	13.5 (0.75) ab	2.37 (0.02) b	113.5 (0.14) cd	0.18 (0.01)	47.94 (5.7)	56 (1.4)
SP250	250	26.3 (2.8) c	4.9 (0.32) bc	2.74 (0.17) bc	39.9 (3.34) c	14.5 (0.36) ab	2.85 (0.03) a	204 (0.24) ab	0.2 (0.005)	71.71 (8.04)	68 (1.3)
SP500	500	45.9 (0.64) b	6.7 (0.43) b	4.0 (0.3) ab	59.6 (4.87) ab	14.9 (0.46) a	2.87 (0.03) a	216.8 (0.11) a	0.19 (0.01)	75.50 (4.5)	69 (1.4)
PR-SA	500	72.7 (4.53) a	8.5 (1)a	5.17 (0.7) a	76.4 (8.77) a	14.9 (0.57) a	2.40 (0.04) b	160.6 (0.35) abc	0.16 (0.004)	66.87 (14.3)	66 (2.9)

Note: The column arranged in ascending order of Olsen-P concentration. Values are based on means of four biological replicates. Different letters in each column indicated statistical differences (*$P \leq 0.05$*) between different P variables.

Plant phosphorus uptake and growth

The results suggest that the plant growth positively correlates with a concentration of bioavailable P in the soil. It is noticeable that the concentration of bioavailable P in the soil tends to support the plant biomass with being consistent with the plant P concentration and P uptake. The simultaneous upward trends appeared over the enhanced level of Olsen P in rhizosphere. An improvement of P use efficiency can be observed when implementing the rhizosphere engineering techniques to acidify the soil, particularly in the context of maize grown on soil supplemented with the low-grade PR addition. For example, a 6-fold increase of plant P uptake occurred at 76 mg kg^{-1} in PR-SA treatment when a remarkable elevation of Olsen P was detected at 72.7 mg kg^{-1} in the rhizosphere soil at the same treatment. In contrast, the plant P uptake exists as 11.6 mg kg^{-1} in the control pot with non-P addition, when Olsen-P concentration was at 7.6 mg kg^{-1} at the harvest.

In the shoot P, the concentration range was 1-5 mg g^{-1}, from non-P added control soil (PO) to PR combined with sulphuric acid (PR-SA) and a mean concentration of 2.9 mg g^{-1}. Shoot P concentration of all treatments is listed at Table below 3.4.1 in order of ascending level of Olsen P for 2 mg g^{-1} in PR-AS and PR, 3 mg g^{-1} in SP250 and UP and 4 mg g^{-1} in SP500 and PR-UP. The noticeable changes of shoot P concentration were again proportional to the biomass level, ranging from 8.7g (PO) to 14.9g (PR-SA and SP500) with 67% of increase. The mean maize biomass was 12 g of dry weight. The shoot biomass was divided into two groups- (1) below 12.5g: PO, PR, PR-AS, UP, and (2) above 12.5g: PR-UP, SP250, SP500, PR-SA. The shoot biomass is then used for the determination of the root shoot ratio (R/S) by comparing it with the root dry weight. Unsurprisingly, the highest root dry mass is likely to result the higher R/S ratio. The highest R/S in this experiment was found in control pot at 0.28 (PO). In all treatments, R/S ranged between 0.18 and 0.21, apart from a marked decline in the treatments of PR-SA and PR-AS at 0.16 and 0.17 respectively.

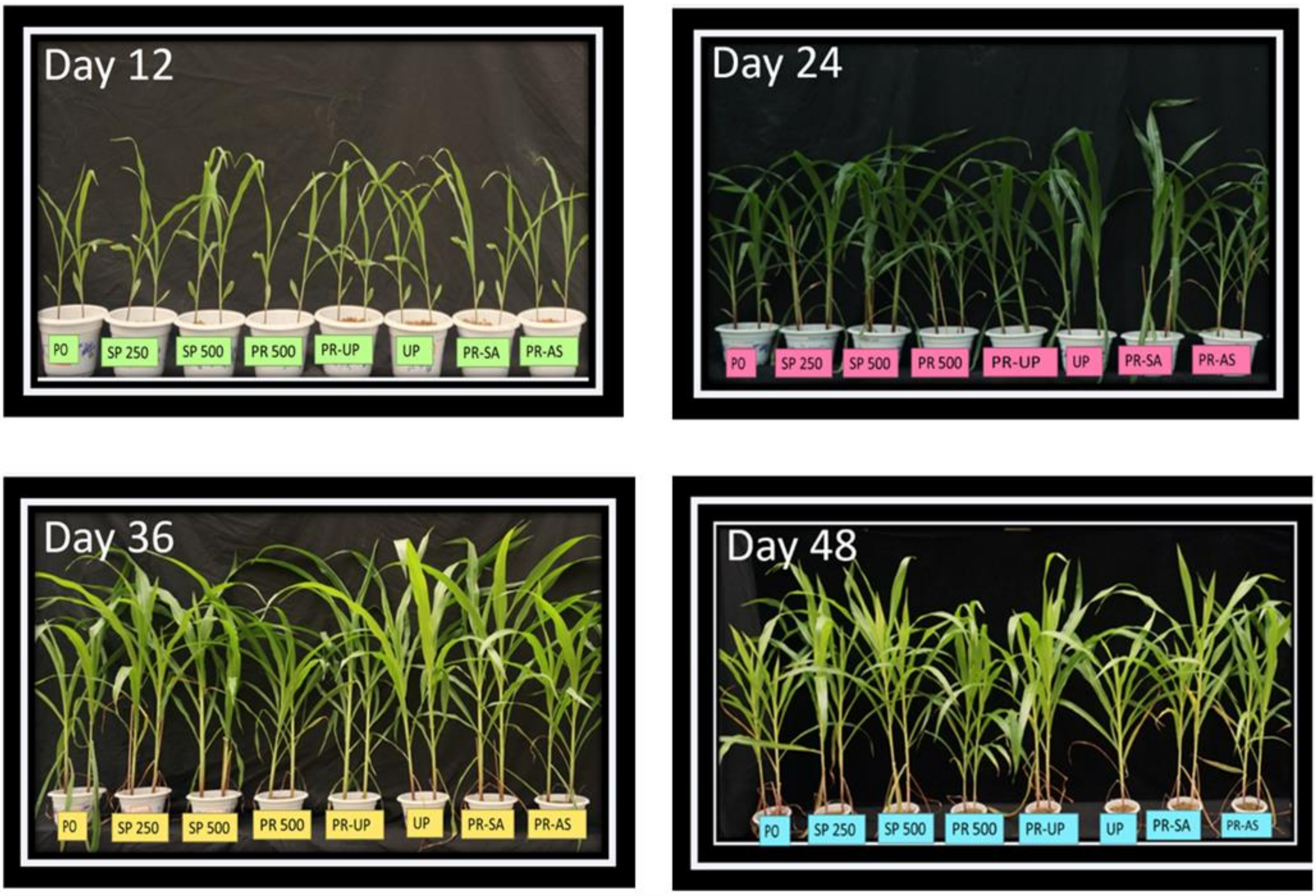


Figure 0.2 Periodic observation of aerial parts of maize grown in the eight different phosphorus treatments including a control, for a duration of 12 days. The abbreviations for each treatment are displayed underneath their respective pots each pot.

The plant aerial growth was assessed in every 12 days by measuring the number of leaves, leave area, height of stem, shoot diameter and chlorophyll content of leaves (measured by chlorophyll Meter of SPAD-520 via the Soil Plant Analysis Development). All these measurements did not produce enough data to support the conclusions statistically. A small occasional effect of plant growth appeared sporadically among the groups, yet no statistically significant differences in aerial parameters of plants were detected. It implies that plant shoot morphology might not be significantly affected. However, the overarching growth pattern of shoot observed throughout experiment revealed, an initial rapid increase in shoot length was displayed during the first two observation periods, with a steady average growth rate of 15 cm per period. In the subsequent observation phases, the average shoot extension exhibited a pattern of deceleration, for example, by 7 cm and 11 cm during the 36th and 48th of DAP, respectively. The tallest plant shoot at the harvest were 52 and 53 cm in the treatment of SP500 and PR-SA applications respectively, whilst the shortest shoots were being present in the soils with P0 and PR-AS application at 35 and 36 cm long. The shoot extension of plant corresponded proportionally to the enlargement of leaf area, with the largest leaf area of 2126 cm² in SP250, 2669 cm² in SP500, and 2408 cm² in the PR-SA treatment at the end of 48th DAP.

Root morphological distribution and mycorrhizal symbiosis

The outcome of root analysis is set out in Table 3.4.1 by providing root parameters of root dry mass, length, R/S ratio, SRL and volume of fine roots. The total length of roots calculated per one plant varied from 82 to 217 m with a mean measurement of 146 m. Overall, the root length was consistent with corresponding shoot biomass and soil soluble P concentration, except in treatments of PO, PR, and PR-SA. The root length of these plant did not affect a shoot biomass, for instance, the relatively longer root with 139 m in length was observed in the plant, grown on the control pot (PO), having with the lowest shoot biomass at 8.7g. This is longer than roots of the plant grown in soils treated with the PR treatments combined with urea phosphate (PR-UP) and ammonium sulphate (PR-AS) with measurements at 113.5 m and 103.6 m respectively. On the contrary, the root growth was suppressed noticeably under the PR only treatment, with the shortest plant roots with the length of 81.5m with the inclusion of SRL of 40.7 m g^{-1} and fine root volume of 55% with diameter less than 0.2 cm. In the PR application integrated with sulphuric acid (PR-SA), the roots of the plants only be extended to 160 m in the soil where the greatest amounts of rhizosphere Olsen-P was observed at 72.7 mg kg ^{1}. Further, the root length of plants grown in soils treated with calcium perphosphate were much longer at 204 m and 217 m at the rate of P application at 250 and 500 kg mg^{-1} respectively and following the greatest SRL 71.7 and 75.5 m g^{-1}, even shoot biomass of both was similarly high in treatment of PR-SA.

The analysis of mycorrhizal colonization did not show any statistically significant differences among the treatments, which may limit the strength of conclusion drawn. However, the intensity of mycorrhizal colonisation exhibited a noticeable pattern that was inversely related to bioavailable P concentration in the soil. Comparatively higher level of mycorrhizal colonisation was associated with the lower bioavailable P concentration of the soil. For example, the treatments of PO, PR, UP, and PR-UP, the intensity mycorrhizal colonization displayed similarly ranging between 25-34%. Conversely, in the treatment of SP500 and PR-SA, considerably lower rate of colonisation was identified at 7%, coincided with higher levels of soluble P in the soil was observed. This observed pattern suggests, AMF may play a role in P acquisition of plants grown in the soil with lower level of soluble P.

3.4.1.3 Rhizosphere excretion and pH value

The fundamental assessment of the activities in rhizosphere often incudes the evaluation of rhizosphere acidity. Numerical representation of the acidity of a root medium is obtained through a measurement rhizosphere pH by using a pH meter (mentioned in methodology section). The arithmetical mean of measure of rhizosphere pH at the harvest was ranging from 5.3 to 6.4, with demonstrating pH value from maximum reduction of 1 unit to minimum reduction level at 0.1 in the PR treatments. Plant root can alter the pH via organic acid and ion exudation, depending on plant species. The most significant decline of pH occurred in the control pot with soil without any additional P supply. The highest level of trans-aconitic and fumaric acids was recorded here at 303 and 32 µmol carboxylate g $^{-1}$ soil respectively. Chemical acidifier did not impact the pH value a lot. There was no obvious change of pH recorded between bulk and rhizosphere soils as well, except the significant difference that was identified in the treatment of with PR and ammonium sulphate addition.

The physiological responses of maize to the different level of Olsen P concentration in the rhizosphere (excretion of organic acid and phosphatase) are represented in figure 3.4.3 amongst all treatments. The results set out therein includes the emission of organic acids and acid phosphatase activities. The trans-aconitate and fumarate were the two detectable predominant organic acids in across all P treatments with considerably variable quantities. Other carboxylates including malate, citrate and succinate, for example, were presented in trace quantities (data not shown), yet they are all considered to be essential acids to maintain the optimal soil pH that was required to solubilise P compounds. In the PR added soil, the slight changes of rhizosphere pH were detected at around 6.4, where the lowest level of organic acid presented including 134 µmol trans-aconitic and 2µmol fumaric carboxylate g $^{-1}$ soil.

The soil acid phosphatase activities within the maize root medium varied between 142 and 474 µg PNP h^{-1} g^{-1} with an average of 297 µg PNP h^{-1} g^{-1}. The acid phosphatase activity in the rhizosphere was greater in the maize grown in the soils with the treatments of PR, PR-UP and UP, with just below 475 to 446 µg PNP h^{-1} g^{-1}. Whereas acid phosphatase activities in the treatments of SP250, PR-SA and PR-AS recorded seemingly lower within the range from 142 to 174 µg PNP h^{-1} g^{-1} with three times lower than above mentioned treatment group. In the remaining treatments, including PO and SP500 acid phosphatase activities appeared at 255 and 283 µg PNP h^{-1}g^{-1}.

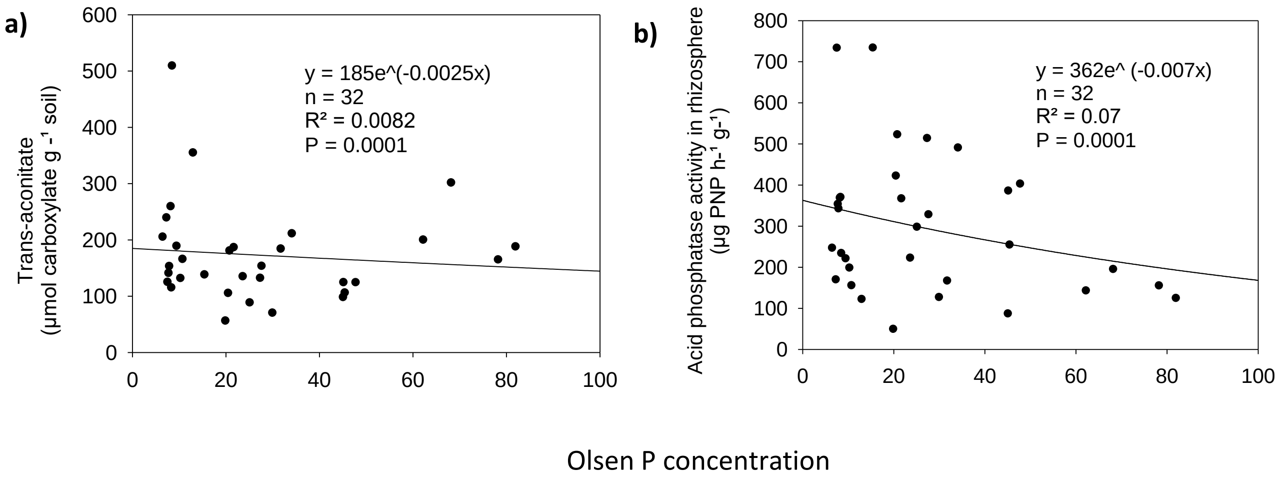


Figure 0.3 Maize root physiological responses to the different levels of solubilised P, the relationship between Olsen P concentration in the rhizosphere soil and (a) an emission of trans-aconitic organic acid and (b) acid phosphatase activity.

3.4.2 Experiment B: Behaviour of mixed crops in the soil for the uptake of phosphate rock, testing for alternative approaches in rhizosphere

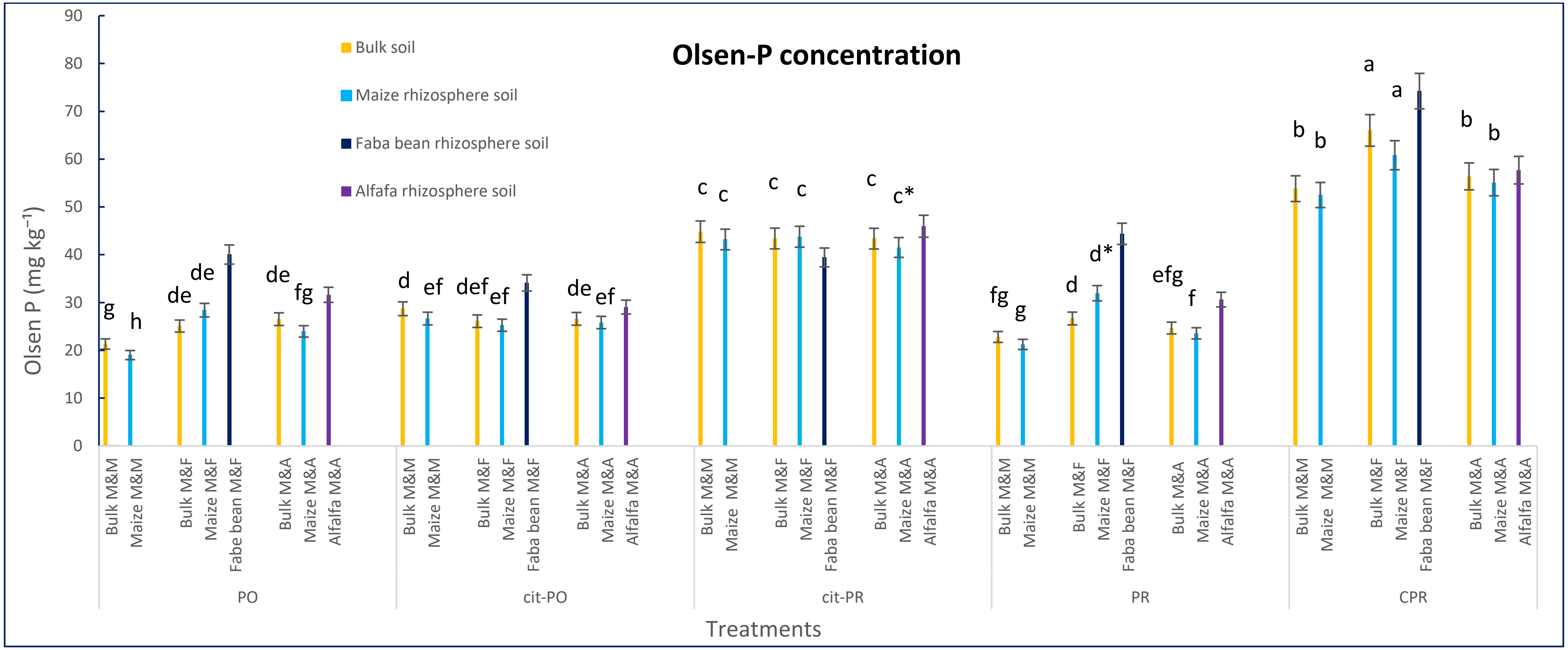
Sandy loam textured brown Cambisol soils were used in the intercropped experiment B, collected from fallow land, Qingdao, Shandong, China (Latitude: $29^{o}03'$N, Longitude: $106^{o}11'$E 170m). It contained Olsen P of 22.3 mg kg^{-1}, water-soluble P 0.12 mg kg^{-1}, total P (TP) 389 mg kg^{-1}, total nitrogen (TN) 0.03%, organic carbon 11.5 g kg^{-1}, total carbon (TC) 0. 28% and the ratio carbon to nitrogen (C: N) 9.06 with soil pH 7.3

3.4.2.1 Soil phosphorus dissolution

The P solubilisation within the root medium ranged differently across all P treatments and combination of plant species. The results of analysis showed a noticeable increase in P solubilisation in the rhizosphere of maize in both commercial PR application (CPR) and PR application coupled with citric acid by demonstrating the greatest changes of Olsen-P concentration with more than doubled at (a) 53 and 43 mg kg^{-1} in the mono-cropping maize; (b) 61 and 44 mg kg^{-1} in mixed cropping maize with faba bean, and (c) 55 and 42 mg kg ^{1} with alfalfa CPR and cit-PR respectively. In the control treatment of non-P added soils with mono-cropped maize, the rates of Olsen P in the rhizosphere were at the lowest at 19 mg kg^{-1}. However, the marked differences of Olsen P concentration in the maize root medium between mono-cropped and diverse co-cropped system can be visible in the control P0 treatment, as Olsen P in the maize rhizosphere was 28 mg kg^{-1} and 24 mg kg^{-1} grown with faba bean and alfalfa respectively. The demonstration of data in the figure showed the marked differences of rhizosphere P solubilisation performances between the plant combinations. In figure 3.4.4, the horizontal axis represents the number of varying cropping combinations in each treatment.

The statistically significant differences (*p*≤0.05) of Olsen-P appeared between bulk and rhizosphere soil across intercropped maize, which were 43 and 41 mg kg ^{1} in the *cit*-PR-M&A and 29 and 31mg kg ^{1} in the PR-M&F treatments of bulk and rhizosphere respectively. Regardless of this fluctuation, there were surprisingly different Olsen P levels in the soils between mixed cropping treatments. The most striking rhizosphere P solubilisation performance of maize throughout all treatments was observed in the combination with faba bean expect for the treatment of *cit*-PR. Olsen-P concentration varied widely between 40 mg kg^{-1} in non-P added control and 74 mg kg^{-1} in CPR added pots.

Generally, the pattern of water-soluble P was very similar to Olsen P, showing an upward trend in accordance with increased P application rate. Overall, the rate of water-soluble P was lower in all non-P added treatments approximately from 0.1 to 0. 2 mg kg $^{-1}$ and higher in all *cit*-PR and CPR treatments from 0.7 to 0.9 mg kg $^{-1}$. The water-soluble P concentration of bulk soil was ranging broadly from 0.1 mg kg^{-1} (PO) to 0.9 mg kg^{-1} (*cit*-PR) particularly in the intercropped M&A pots. However, as compared with the pattern appeared in Olsen-P, the elevated level of water-soluble P was observed in the treatments of PR only added soil for solo and co-cropping system, ranges from 0.3 to 0.4 mg kg $^{-1}$ at harvest stage, which made this pattern noticeably different than the general P pattern.



*. The mean difference is significant at the 0.05 level.

Figure 0.4 The bulk and rhizosphere soil Olsen P concentrations of the mixed- and mono-cropping maize (***Zea mays*. L**) with faba bean (***Vicia faba*. L**) and *alfalfa* (***Medicago sativa*. L**), grown in five different treatments including two types of phosphorus rock applications with control and citric acid addition.

All data are based on the mean figures of four replicates and error bars show standard error. The statistically significant difference ($p \leq 0.05$) between bulk and rhizosphere soil was performed only on the maize root medium by *t*-test. Different letters indicate the significant difference by one-way ANOVA, a Duncan post hoc test. There was no statistically significant difference between the treatment groups. Abbreviations for mono and mixed crop pots: **M&M**: the monocropped maize (*Zea mays*. L). **M&F**: intercropped maize (*Zea mays*. L) and faba bean (*Vicia faba*. L). **M&A**: maize (*Zea mays*. L)and alfalfa (*Medicago sativa*. L). Abbreviation for P treatments: **P0**: control without any additional P. **PR:** The low-grade phosphate rock addition. Citrate treatment: (a) ***cit*-P0**: citric acid only; and (b) ***cit*-PR**: combination of citric acid solution and PR. **CPR**: The commercial phosphate rock addition.

3.4.2.2 Plant indicator parameters

The entire plant indicator parameters of intercropping and monocropping maize under five treatments are presented in Table 3.4.2, including plant P contents and the relative plant responses to different levels of soil soluble P concentration.

Table 3.4.2 Summary of mixed cropping maize experiment including the soil Olsen P and plant parameters of *Zea mays* grown their own (M&M) and with the different legume species (M&F) and (M&A) under different types of phosphate rock supply with and without citric acid addition. Plants were sampled at the 38 days of planting.

	Phosphorus supply rate per treatment (mg kg^{-1})		*Rhizosphere Olsen P (mg kg^{-1})*	*Total P content of plant (mg g^{-1})*	*Shoot P concentration (mg g^{-1})*	*Plant P uptake (mg plant^{-1})*	*Dry weight (g plant^{-1})*		*Root length (m plant^{-1})*	*Root shoot ratio*	*Specific root length (m g^{-1})*	*Fine root volume (%)*
							Shoot	Root				
Maize and Maize (M&M)	**P0**	0	19 (0.94) h	4.3 (0.07) g	2 (0.08) f	2.7 (0.1) d	1.4 (0.02) ef	0.23 (0.03) cd	31.8 (5.44) a	0.16 (0.02)	155 (40.9)	64 (2.3)
	***cit*-P0**	0	26.7 (2.42) ef	5.1 (0.27) fg	2.6 (0.26) f	3.9 (0.33) d	1.5 (0.03) ef	0.2 (0.01) d	19.5 (1.11) abc	0.14 (0.01)	93.6 (3.9)	72 (2.7)
	***cit*-PR**	500	43.2 (0.49) c	6.8 (0.19) def	4.1 (0.05) de	7 (0.36) cd	1.7 (0.08) def	0.2 (0.01) d	19.1 (0.79) abc	0.13 (0.01)	91.5 (7.4)	73 (0.4)
	PR	500	21.2 (0.86) gh	5.2(0.26) fg	2.6 (0.1) f	3.4 (0.22) d	1.3 (0.05) f	0.02 (0.02) cd	22.1 (3.06) abc	0.17 (0.02)	102.4 (12.8)	70 (1.2)
	CPR	500	52.5 (0.45) c	17(1.18) b	12.7 (1.1) b	46.6 (2.9) b	3.7 (0.1) c	0.3 (0.03) abcd	16.2 (1.76) bc	0.07 (0.01)	59.8 (8.5)	63 (2.7)
Maize and Faba bean (M&F)	**P0**	0	28.4 (2.1) de	4.9 (0.55) fg	2.4 (0.15) f	3.9 (0.27) d	1.6 (0.02) def	0.3 (0.03) abcd	24.3 (4.42) abc	0.17 (0.02)	88.4 (15.2)	60 (4.2)
	***cit*-P0**	0	25.3 (2.55) efg	5.3 (0.34) fg	3 (0.41) ef	5 (0.93) cd	1.6 (0.12) def	0.2 (0.03) cd	20.2 (2.88) abc	0.14 (0.02)	97 (25.3)	48 (6.0)
	***cit*-PR**	500	43.8 (0.84) c	7.7 (0.57) de	4.2 (0.16) de	7.6 (0.4) cd	1.8 (0.08) de	0.3 (0.01) abcd	20.7 (2.25) abc	0.15 (0.01)	77 (6.4)	63 (3.7)
	PR	500	32 (0.74) d	8.3 (0.74) d	4.7 (0.02) d	7.9 (1.1) cd	1.7 (0.23) def	0.3 (0.03) bcd	26.8 (8.46) ab	0.17 (0.04)	103.2 (28.4)	62 (2.2)
	CPR	500	60.8 (1.93) a	19.5 (1.3) a	14.5 (0.89) a	66.4 (5.3) a	4.6 (0.12) b	0.3 (0.01) ab	15.1 (1.23) bc	0.07 (0.004)	47.6 (3.6)	52 (7.2)
Maize and Alfalfa (M&A)	**P0**	0	24 (0.49) fg	4.8 (0.32) g	2.2 (0.28) f	3.7 (0.4) d	1.7 (0.06) def	0.2 (0.02) cd	20.5 (5.44) abc	0.14 (0.01)	90.2 (22.9)	56 (4.0)
	***cit*-P0**	0	25.8 (1.5) ef	5.8 (0.35) efg	3 (0.08) ef	5 (0.09) cd	1.7 (0.03) def	0.2 (0.01) d	20 (2.69) abc	0.13 (0.01)	96.4 (15.7)	58 (6.9)
	***cit*-PR**	500	41.5 (0.2) c	7.7 (0.45) de	4.5 (0.37) de	9.2 (1.23) cd	2 (0.17) d	0.2 (0.02) bcd	21.4 (1.7) abc	0.12 (0.01)	90.2 (8.2)	62 (1.2)
	PR	500	23.6 (1.15) fg	10.4 (0.52) c	6.8 (0.31) c	12.1 (0.56) c	1.8 (0.04) de	0.3 (0.03) abc	31.2 (5.67) a	0.17 (0.02)	105.7 (11)	64 (4.2)
	CPR	500	55 (1.02) c	17.4 (0.57) b	12.4 (0.59) b	66 (6.41) a	5.3 (0.38) a	0.4 (0.05) a	12.2 (1.04) c	0.07 (0.01)	35.3 (5.5)	46 (3.7)

Note: Only *Zea mays* parameters were measured on the basis of the calculation in one maize. The column separately arranged in accordance with the planting variation. Values are means of four biological replicates. Different letters in each column indicated statistical differences ($p \leq 0.05$) between different P variables. Abbreviation for phosphorus treatments: **P0**: control without any P addition. **PR:** The low-grade phosphate rock. Citrate treatment: (a) ***cit*-P0**: citric acid only; and (b) ***cit*-PR**: combination of citric acid solution and PR. **CPR**: The commercial phosphate rock addition.

Plant phosphorus uptake and growth

Most results from this experiment, particularly solo cropped maize performance, were consistent with the previous experiment A. All parameters isolated from biomass accumulation provided further evidence for the impacts of soil acidification, rates and types of PR. However, the increasing biomass and plant P uptake was associated with neighboring legume species in each P treatment, showing simultaneous upward trends. Biomass accumulation was always higher in shoots than roots. The level of shoot biomass and P uptake varied widely among different plant combinations, with the significant elevation from control to CPR treatments: in the mono-cropping maize at 1.3-3.7 g and 2.7- 46.6 mg; in the intercropping maize with faba bean 1.6- 4.5 g and 3.9- 66.4 mg, and with alfalfa 1.7- 5.3 g and 3.7-66 mg, respectively. Increasing P uptake by 1-2 mg appeared across all maize grown with legume species than their own, for example in CRP treatment, plant P uptake has increased dramatically, from 47 in sole cropped maize to 66 mg plant^{-1} intercropping maize.

According to the information provided, the P accumulation in the shoot of maize plants increased as the plant grew, corresponding to elevated level of P concentrations in soil. This relationship is outlined in Table 3.4.2. Total P content was found to be higher in shoots than that in the roots. The shoot P concentration of maize was related shoot biomass production, indicating that a mean concentration of shoot P at 5.5 mg g^{-1}. Under the CPR application, the highest rates of shoot P accumulation appeared, ranging between 12 and 15 mg g^{-1} in solo and intercropping maize. This was followed by the intercropping maize grown in the PR application with alfalfa at 7 mg g^{-1} and with faba bean at 5 mg g^{-1}, which was an incremental double increase of shoot P concentration of mono-cropping maize in control at 2.6 mg g^{-1} at the harvest. Interestingly, this plant P content in control shows twice as much as P in the plant of the same P treatment at the first experiment. Perhaps, it may depend on plant growth stage associated with the different harvesting dates of 50 (Exp. A) and 36 (Exp. B). As mentioned in the previous experiment, the general trend of the root to shoot ratio was an opposite of the biomass cumulation and soil soluble P pattern, that means the higher soluble P is in the soil, the lower root shoot ratio will be. The highest value of ratio at 0.17 was detected in the mono-cropping maize (PR) and intercropping maize with alfalfa (PR) and with faba bean non-P added control pots. The root shoot ratio declined substantially to 0.06 in all combination of CPR treatment.

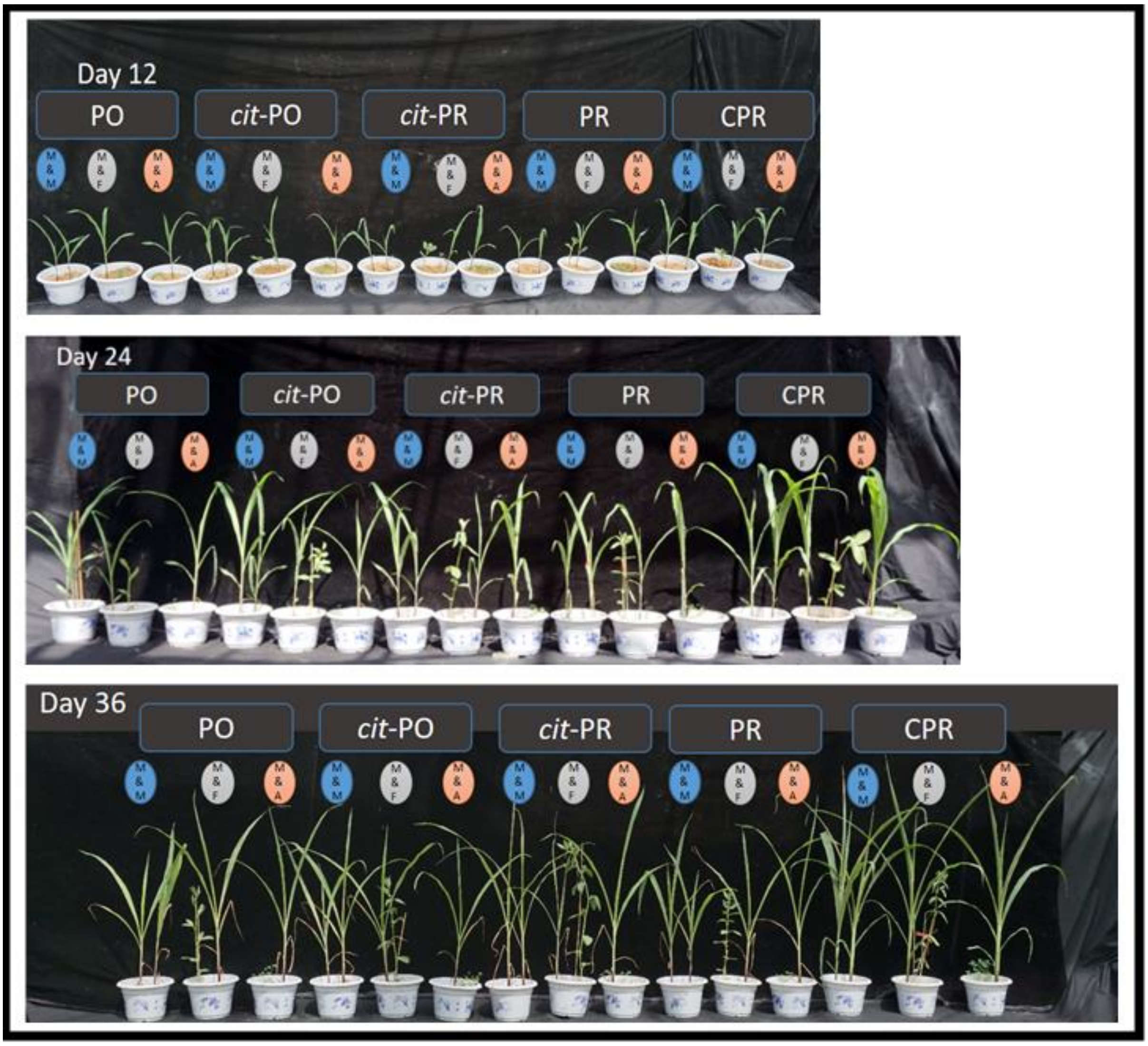


Figure 0.5 Periodic observation of aerial parts of plants grown in the five different phosphorus treatments under the monocropped and intercropped maize with faba bean and alfalfa for the duration of 12 days, with the abbreviations displayed on top of potted plants.

In the figure above, the treatments include with their abbreviation used in this experiment that has been drawn, incorporating with highlight in different colours: (a) box representing the phosphorus and citrate treatments , including **P0** (control without any additional P), **PR** (low-grade phosphate rock) and **CPR** (commercially available phosphate rock), ***cit*-P0** (citric acid only), and ***cit*-PR** (combination of citric acid solution and PR) and (b) oval shaped circle representing for planting combinations under each P treatment **M&M**: the monocropped maize are in blue (two maize in each pot). **M&F**: intercropped maize and faba bean are in grey (one maize and one faba bean in each pot). **M&A**: intercropped maize and alfalfa are in orange (one maize and multiple alfalfa for 8 in each pot).

Root morphological distribution

In this experiment, the measurement of root length focused solely on the maize roots, particularly calculated in one maize. The root length parameters of other co-cropping plants were not included for the analysis of this experiment. To obtain the root length of maize and maize (M&M) treatment, the mean value was calculated for the data analysis by summarizing average root length that represents the tendency of root length data for each maize. The total length of maize roots per plant varied from 12 to 32 m, with a mean of 21.4 m. The root length was proportional to corresponding shoot biomass and soil soluble P concentration, except for treatments CPR. During this experiment, the shortest root length was observed between 12 m and 16 m in the plants grown under the CRP supply in all planting combination with the lowest SRL 35 and 60 m g^{-1}, where the highest shoot biomass was produced at the same time around 4 and 5g of dry weight. These figures were more than half the root length of maize grown in the mono-cropping P0 control and intercropping PR with alfalfa at 32m. In the treatment PR integrated with citric acid, the roots of plants were prolonged to 19m in mono-cropped maize and 21m in intercropped maize in both combinations, where amounts of Olsen-P in the rhizosphere observed in the second greatest at 42-44 mg kg ^{1}. The value of plant P acquisition, the sum of P concentration in the rhizosphere and plant parameters, including total and specific root length are presented in Table 3.4.2.

3.4.2.3 Rhizosphere excretion and pH value

The true pH average of maize refers to the arithmetical means for the pH values observed in the soil solution. This average provides a representative value for the pH of the soil solution. The rhizosphere pH was measured on a log scale, ranged from 6.3 to 6.7 at the harvest stage, indicating pH level observed. The difference of represents the arithmetical pH values indicates a uniform decrease of 1 unit across all treatments. Within the P treatment, the rhizosphere pH of each treatment was gradually dropped from mono-cropping to intercropping plant except for the citric acid added pots. In the citric acid treatments, pH decline of the rhizosphere depends on P addition, which dropped slightly higher in *cit*-P0 with intercropped maize than *cit*-PR with sole cropped maize, by 0.3 and 0.1 respectively. However, the significant changes of pH between bulk and maize rhizosphere soils were determined in the all solo and co-planting combination of citric acid added P control pots (*cit*-P0), intercropping maize with alfalfa and faba bean pots with PR application and *cit*-PR treatment with alfalfa.

As compared with the previous experiment, many forms of organic acids including trans-aconitic, tartaric, malic, fumaric, citric and succinic acids were presented at sufficient level of determination, apart from the intercropping maize with alfalfa in the *cit*-PR. The predominant carboxylate in all crop combination amongst all treatments was the succinic acid with average rate of 1507 µmol carboxylate g $^{-1}$ soil and followed by tartaric acid with 713 µmol carboxylate g $^{-1}$ soil with random isolation. Citric acid was determined in citric acid treatment with an average of 209 µmol carboxylate g $^{-1}$ soil. Generally, all types of carboxylates were excreted into the root medium of intercropping maize in the P control, PR, and CPR treatments at greater amounts, which was more than the average detected level.

The acid phosphatase activity varied between 433 and 1312 µg PNP h-$^{-1}$g^{-1} within the maize root medium with average 780 µg PNP h^{-1} g^{-1} (Fig 3.4.6). The lowest level of acid phosphatase activity occurred in the mono-cropped maize in P0 treatment, while the significantly highest level of acid phosphatase activity more than 850 µg PNP h^{-1} g^{-1} occurred in treatments of intercropping *cit*-PO and mono-cropped maize PR. It was followed by all planting combination of *cit*-PR treatment from 720 to 815µg PNP h-$^{-1}$g^{-1}.

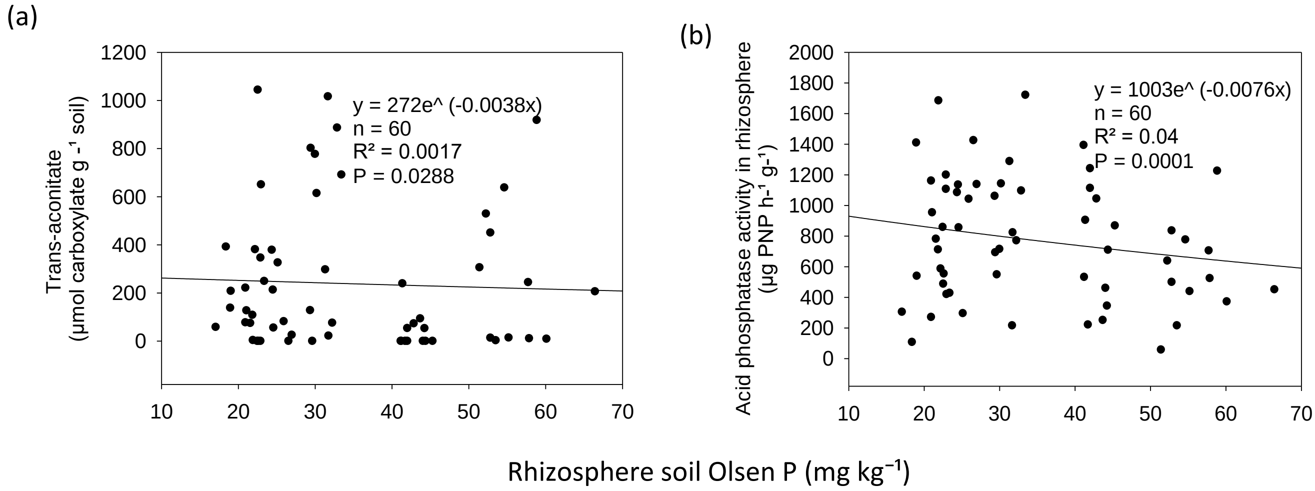


Figure 0.6 Mono and intercropping maize root physiological responses to the different level of Olsen P concentration in the rhizosphere, the correlation between soluble P content in the soil and (a) trans-aconitic organic acid emission (b) acid phosphatase activity.

3.5 Discussion

Both studies in this chapter set out with aim of assessing the importance of the various rhizosphere approaches that can improve the P utilisation by maize, leading to enhanced P use efficiency of P application. In this discussion, I am only focusing the low-grade PR application in context of root zone acidification. Overall, the level of plant available P of the soil enhanced under the direct application of PR (DAPR) as applied PR can be optimised in the rhizosphere via the rhizosphere engineering techniques employed to modify the pH of the soil surrounding plant roots. In these small pot experiments, the rhizosphere was designed to be manipulated via the emission of organic acids under these soil amendments that specifically influence neutral soil pH. The P use efficiency and plant performance, particularly *Zea mays* can be consequently improved in the soil, as supplemented low-grade PR addition served as a source of P. However, the measure of true efficiency of elevated Olsen P in the soil to indicate the P utilisation of plant of these experiments has not been calculated, as the complete picture of P dynamics in the rhizosphere include microbes and potential leaching.

In this chapter, it was found that direct comparison between findings of experiment A and B was not possible due to the several factors, that has a potential to limit the ability to compare results of these studies, such as (1) the complexity of soil consistency: two different types of soil with different characteristics and P composition, collected from completely different places of Shandong, were used in experiments A and B, since an amount of soil in storage used for experiment A was insufficient to carry out experiment B (2) selection of growing season: one set in from July to August and other set is in May (3) duration of planting period: 50-day for experiment A and 36-day for B and (4) equipment availability of the lab during the analytical period: digestion method of plant P analysis was changed from wet to heat procedure in experiment A and B, as mentioned earlier in method section. In addition to the limitations mentioned, other factors are important to acknowledge such as light, temperature, other nutrients and plant hormones, which were possibly involved in intercropping condition (Li et al., 2014; Vandermeer, 1992). It leads to both experiments experience in different condition.

3.5.1 Experiment A: Optimisation of phosphate rock uptake to maize in the rhizosphere

The vast majority of evidence in P dissolution of the soil found here agreed with the finding of earlier studies carried out the different types of PR (Bolan and Hedley, 1990; Halder et al., 1990; Hedley et al., 1995; Hedley et al., 2010; Mackay and Syers, 1986; Prochnow et al., 2001). However, the results indicated that the quantity of solubilised P in the soil treated only low-grade PR application was very similar to non-P added control soil, even in the maximum P supply dosage at the rate of 500 mg kg^{-1}. Suggesting that weak P solubilisation of low graded rock materials through direct application might not meet the demand of high yield to sustain mono-cropping maize systems as compared with many other P fertiliser types. Therefore, it has been assumed that low-grade PR having less available P on its own will not improve the level of P bioavailability unless the rhizosphere is manipulated to change soil acidity through rhizosphere engineering approaches. The rhizosphere engineering approaches are included in the methods to induce the proton release into the root medium to change soil acidity.

The soil-acidifying chemicals have the potential to induce in the root medium changes, which can affect the availability and solubility of P and degradation of PR at different level. The amount of soluble P in the soil is indeed directly related to the plant biomass throughout this experiment via increased nutrient uptake through the improved root development and plant physiological processes, agreed already with others, Rengel and Marschner (2005), Brady and Weil, Hinsinger (2001). However, one of the key findings of this experiment suggests that the significant increase in Olsen P appeared in the treatment of PR in combination with SA is not consistent with the observed general trend of plant biomass, as amount of root material produced in this treatment was noticeably declined. It can be explained by the shoot P concentration that was detected the highest in this experiment. When plant shoot P concentration reached a certain threshold, plants no longer uptake P effectively by producing more root (Lambers et al., 2006b; Shen et al., 2011; Vance et al., 2003). This can be an important interpretation to understand how P availability and solubilisation affects plant growth.

Regardless of these soil acidification attempts, soil pH of all treatments did not decline at the harvest even in the sulphuric acid and UP addition. The maximum pH value drop was observed in control soil with the reduction of 1 pH unit, where adaptive plant strategies were developed with the highest level of carboxylate exudation determination. The findings, in that adaptive plant strategies evolve to cope under a range of P conditionings, were consistent with previous research findings; however, the exception was with ammonium sulphate (Hinsinger, 2001; Lambers et al., 2006a; Neumann et al., 1999b; Neumann and Römheld, 1999; Rengel and Marschner, 2005; Zhang et al., 2010). To produce a desired level of soluble P for plant uptake, ammonium sulphate stimulates root physiological activities to release proton that can lower pH value in the rhizosphere soils (Hinsinger, 2001; Neumann et al., 1999; Neumann and Römheld, 1999; Shen et al., 2005). Conversely, cereal crops fail to resolve the P acquisition by physiological activities, due to the role of strong root morphological responses under limited availability of soluble P in the soil (Bennetzen and Hake, 2009; Calderónvázquez et al., 2009; Carvalhais et al., 2011; Lyu et al., 2016; Pearse et al., 2010).

The general pattern of this study confirms that the biomass accumulation was directly influenced by soil soluble P concentration and plant growth phase, as mentioned in earlier literature review. Particularly, the significance in root morpho-physiological activities along with mycorrhizal colonisation was always associated with a lower-level of Olsen-P concentration in the soil. Increased total root length, specific root length, and level of root exudation further support the P acquisition and plant activities such as elongation of shoot, development of leaves, plant photosynthesis, and other plant enzyme activities (Daram et al., 1998; Eissenstat and Yanai, 1997; Shen et al., 2011). However, in this experiment, the areal plant growth parameters revealed no statistically significant distinctions among the treatments, potentially diminishing the robustness of the conclusions in terms of soil soluble P concentration. The data produced by the periodic observation is not enough to be deemed a regular pattern of areal elongation of the plant. Whereas the root extension was directly influenced by soluble P concentration in the soil, except the demonstration of growth regression in PR only treatment (including the shortest total and specific root length) that is contradicting the expected phenomenon. The root length was markedly suppressed in PR treatment in comparison with control P0 treatment with no P supply, where similarly low amount of soluble P in the soil with relatively longer root was identified.

Yet, the total P value of soil was detected higher in both treatment of PR only application and PR with ammonium sulphate addition, as compared with other treatments used in this experiment. The observed finding of the greatest rate of acid phosphatase activities with intensity of mycorrhizal colonisation under the in PR treatment can be exclusively attributed to the subsequent soil condition that can facilitate the improvement of P solubilisation processes. However, no correlation was found between the soil total P concentration level and plant performance. One concern is that the PR only supplied soil may facilitate the suitable environment of AMF invasion showing with the greatest amount of symbiotic relation during the observation. Therefore, these results suggest that maize grown in PR only supplied soil mainly responded to lower level plant available P through a stimulation of physiological function and symbiotic relation rather than morphological changes, which did not agree with the statement of cereal crop morphological responses by many studies in this field.

The intensity of mycorrhizal colonisation exhibited a noticeable pattern that was inversely related to bioavailable P concentration in the soil, for example comparatively higher level of intensity of mycorrhizal colonisation was associated with the lower bioavailable P concentration of the soil as mentioned above in PR treatments. The observed pattern suggests that AMF may play a significant role in assisting plants to acquire P when grown in soils with lower levels of soluble P. However, the analysis of mycorrhizal colonization did not show any statistically significant differences among the treatments, which may limit the strength of any conclusions drawn. Statistical non-significance can be explained partly by seed sterilisation (please see in Methodology), as this process can eliminate surface microorganisms and destroy beneficial fungal spores and hyphae.

3.5.2 Experiment B: Behaviour of mixed crops in the soil on the uptake of phosphate rock, testing for alternative approaches in rhizosphere

The results of this experiment are consistent with experiment (A), which indicate that there is an increased level of P bioavailability in accordance with various ways of root zone acidification and types and concentration of P sources. Even if the soil used this experiment (B) had a slightly higher Olsen P concentration than experiment (A) with the concentration Olsen-P at 9.74 mg kg^{-1} and 22.3 mg kg^{-1} respectively. Apart from citric acid addition, the improved P solubilisation within their root medium of each P treatment was associated with soil acidification triggered by neighboring legume species. The findings of increased Olsen P concentration and production of biomass with corresponding P uptake were very similar to the results from previous research studies (Inal et al., 2007; Li et al., 2008; Li et al., 2010; Xiao et al., 2004). The superiority of plant combination particularly maize and legumes further supports the P solubilisation in the root medium and the level of maize performance.

The relationships of plant species in the intercropping systems resulted in positive interaction in terms of maize growth, contributing to produce simultaneous upward trends of Olsen P concentration and biomass with corresponding P uptake. For example, rhizosphere Olsen P concentration of non-P added treatment was 19 mg kg^{-1}, 28 mg kg^{-1} and 24 mg kg^{-1}, whereas P uptake rate 2.7 mg plant^{-1}, 3.9 mg plant^{-1}, 3.7 mg plant^{-1} in the maize monocropped M&M, and intercropped with M&F and M&A planting combinations, respectively. The finding of the P accumulation in above-ground parts of maize and direct relation between shoot P concentration and shoot biomass production imply the translocation of significant portion of absorbed P in the shoot that can serve as an indicator of the overall plant productivity of maize. To highlight the importance of P accumulation of the shoot may suggest that optimization P availability and uptake can facilitate the improved growth and yield of maize crops.

In the earlier stage of growth, the length of maize in the intercropping system was characterised by a strong increase, although after the 33 DAP legume plant grew faster, with the changing trend of plant length visualisation during the observation periods shown in Fig.3.4.5. Then a reduced leaf elongation of maize was appeared, with no clear evidence of the recession effects. However, it is highly likely to associate with competitive interaction of plant species (Dunea, 2018; Li et al., 2016), beyond mutualistic association in the same pot, due to the availability of soluble P concentration to meet the demand of legume growth. Depletion level of available P have not been measured periodically in the middle of this study and the analysis of faba bean **(*Vicia faba*. L)** and alfalfa **(*Medicago sativa*. L)** was intentionally excluded in this experiment, as maize is used as a focus crop. This result might have been different in field conditions, as different plant species speculate different microbial diversity in their rhizosphere (Marschner et al., 2001; White and Hammond, 2008) and occupy their own resource niches (Li et al., 2008; Silvertown, 2004).

In this experiment, the performance of maize was also compared within the different types of PR application with and without citric acid addition. One interesting finding was that the low-grade PR dissolution could be greatly improved by root zone acidification through citric acid, which was consistent with experiment (A) PR-SA. Although citrate has been used in many studies for different purposes (Mallhi et al., 2019; Tusei, 2019), it is not common choice in practice to improve plant growth, due to the cost effectiveness. Therefore, there is a limited literature sources for the uses of application of citrate in relation to the agricultural practice. Multiple applications of citric acid via watering can surprisingly increase the soluble P concentration in all cropping types even sole cropped maize when the PR applied soil. However, responses of maize to solubilise P within the root medium is not improved under citric acid addition for the soil without any P addition (*cit*-P0). It can be exemplified through the similarity of Olsen P concentration between P0 treatments with (*cit*-P0) and without (P0) across all mono- and intercropped maize (M&M, M&F and M&A). The evidence highlighted that P solubilisation in the rhizosphere would not produce any difference under citrate application unless PR was added.

This study also found no difference of soluble P between monocropped and intercropped maize in the soil without adding PR, when applying citrate treatment (*cit*-PO) as shown in Figure 3.4.4. It seems, citric acid can possibly control the rhizosphere processes of legume species within the root medium of the intercropping pots in a way to supress a secretion of organic acids. The level of carboxylate exudation was generally less detectable than average observation. The trace amounts of tartaric and malic acid were detected in all cropping combination in both with and without PR. Whilst suppressing the organic acid exudation, the second highest elevation of acid phosphatase activities was observed in the intercropping pots of citrate addition treatments without PR, from 870 to 916 µg PNP h-$^{-1}$g^{-1} in the *cit*-PO M&F and *cit*-PO M&A. This finding of increase subsequent dephosphorylating activities of phosphatase (Hayes et al., 2000) suggested that the citrate can solubilise the P bounded with humic-metal complex (Gerke, 1993).

The similarities of soluble P concentration in the rhizosphere between control and low-grade PR only treatment of experiment (B) is consistent with the first experiment. As compared PR only treatment with the previous experiment (A), noticeable suppression of root growth was not identified in this experiment. For example, root length of mono-cropped maize grown in the PR only supplied pot was 22m, whilst the average root extension was 21 cm. This inversely increased root length was accompanied with the high level of carboxylates exudates and acid phosphatase activities in all planting combinations of maize. These patterns related to the PR treatment allows the experiment (B) to differ from the previous experiment. However, the total root length of maize in the experiment were shorter than experiment (A), which may be associated with the different harvesting dates (50 versus 36 days) and soil sampling locations.

3.6 Conclusions

The working hypotheses of this chapter are accepted: (1) different types of P fertilisers have differing resulting effects on the P solubilisation in the rhizosphere; and (2) the emission of organic acids in the rhizosphere at near neutral soil pH value increases the P solubilisation of soils with different levels of P exposure. The root zone acidification and emission of organic acids can increase P solubilisation at near neutral soil pH associated with processes of rhizosphere that enable their manipulation to P use efficiency. The strong linkage between soil P dynamics and rhizosphere processes along with plant performance in the mono- and intercropping systems will be helpful to understand the underlying mechanisms of soil P solubilisation under variable P exposure. Both experiments emphasised the potential importance of growth response of maize under different rhizosphere management approaches to improve the potential efficiency of new sustainable types of low-grade PR and its uses in agriculture. Despite the varying growth response, carboxylate concentration and acidic phosphate activities in the rhizosphere was modulated by the P availability of soils. More studies, particularly with longer growth periods with low-grade PR supply in the field observations coupled with potential P losses from the rhizosphere by collecting field leachate are needed to clarify the rhizosphere role in whole systems under real environmental pressure and direction for further research studies.

CHAPTER 4

Immediate effects of phosphorus solubilisation with varying drying and rewetting stresses, under four contrasting soils from different regions of China

This thesis has two experimental components: the controlled pot experiments carried out in the previous chapter 3 were the first component, which explored the processes of the rhizosphere and their effects on the efficient use of phosphorus (P), particularly under the application of low-grade phosphate rocks. The second experimental component of this thesis is dealt with here in Chapter 4 and continued in 5. The second component focuses on the immediate and delayed effects of soil drying and rewetting (DRW) on the rhizosphere processes by conducting lysimetric experiments with two sequential steps: Step One (here in Chapter 4) – introduces a cycle of four DRW (+) events on different types of soils. Simultaneously, it includes the DRW (-) controlled soil samples that maintained consistent soil moisture throughout the experimental period; and Step Two (Chapter 5) – later to be introduced the subsequent plant growth to all used soil samples in order to assess delayed drying and rewetting effects (d- DRW) in the subsequent chapter.

The immediate effect of soil DRW (*i*-DRW) on P mobilisation (via solubilisation) undertaken the laboratory study of the Step One will explore the amount of soluble P in the rhizosphere that has potential to penetrate through and show in the leachate water during the DRW events. The five working hypotheses of this chapter are listed in page 20 from Chapter One. In these experiments, I used strongly contrasting soil types with significantly varying bio-physiochemical contents in order to fully understand the role of soil types under the occurrence of a series of DRW events. The different rate of P supply was also used for the further detailed assessment of P solubilisation analysis; however, detailed information on the previous history of soil P fertility management was not considered.

4.1 Abstract

The experiment in this chapter tested the five hypotheses under the broad objective specifically to study the *immediate* responses of soil to the effects of soil drying rewetting (DRW). The list of the 5 hypotheses are: (1) DRW increases the P solubilisation (and thus the potential for leaching); (2) The amount of solubilised P has a potential for leaching, being proportionate to the rate of P applied; (3) Dephosphorylating enzymes and organic acids affect the P solubilisation in the rhizosphere after DRW; (4) The soils with different physiochemical contents respond differently to DRW (5) The forms of P in leachate water will vary depending of DRW event cycles and dynamics. The four diverse types of soils sampled from Shandong, Chongqing, Heilongjiang and Beijing (sieved <2 mm) were intentionally treated with four varying cycles of DRW events in order to establish the Olsen P in the soil and dissolved P forms in the leachate water.

Hypothesis 1 was rejected because there was no statistically significant difference in Olsen's P level between the DRW and stable moistened soils. However, the remaining hypotheses were accepted. The prevalent pattern of each soil type with upward trends of concentration of soil Olsen P and detectable P forms in the leachate clearly corresponds with the increased rates of P supply which directly support hypothesis (2). Furthermore, the different types of soils with different physicochemical appearance can differentially contribute to the performance of rhizosphere processes to solubilise P through their biological function. Consequently, the soil P solubilisation processes, which may potentially reflect great credit on quantities of P penetrated through the soil into the leachate, allow hypothesis (3) and (4) to be true. Ultimately, the frequency of a cycle of DRW events affects the concentration gradients of P in the leachate water with a noticeable pattern of following each DRW event, which showed a positive correlation between P concentration level against the number of frequencies of the DRW events. This outcome of the P leaching supports an acceptance of the Hypothesis (5).

4.2 Introduction

The natural occurrence of drying and rewetting events on the P cycle is commonly accepted phenomena in the rhizosphere (Blackwell et al., 2009; Gordon et al., 2008), but these changes are forecasted to change with climate change (Forber et al., 2018). The frequent DRW events can dramatically alter the normal distribution level of soil solids and pore space (the volume composition of three-phase of ideal soil system illustrated in the Figure 4.2.1, thereby increasingly disrupting the soil biotic and abiotic properties with respect to soil hydrology. Ultimate impacts of this climate driven disruption may subsequently orchestrate the underlying processes of P movement via the alteration of P forms and timing of fluxes through the changes in speed of natural underground activities, patterns of soil P mobilisation, and ability of soil to retain P. All these processes are included in the second and third tiers as described earlier in the P transfer continuum (Haygarth et al., 2005a) such as (a) the mobilisation: amount and form of P are initially mobilised via solubilisation processes of the rhizosphere or alternatively via detachment processes under the soil hydrological events, and (b) the delivery: mobilised P then transported from land to water throughout the soil hydrological pathways either via infiltration across the soil matrix or percolation by macropore and cracking particularly under the drying soil where water is removed from pore space. The formation of macropores with diameter of 0.3-0.5 mm or more appears under the soil structural cracks and root channelling associated with faunal activity and dense aggregates (Jarvis et al., 2009) shown in Figure 4.2.1.

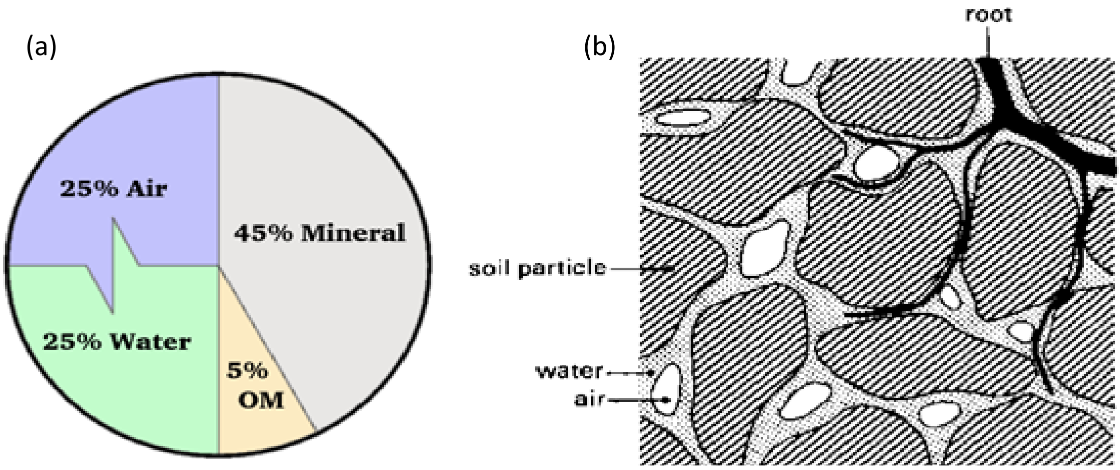


Figure 0.1 (a) The proportion of solids and pore space distribution of the ideal soil with (b) schematic representation of key components of soil system including root channelling taken from www.fao.org.

State of water in soil

The pore space of loam soil ideally consists of 40-60 percent of volume composition to the optimal plant growth, which are filled with equal parts air and water, as can be seen from the figure above. However, this type of distribution rarely occurs in the field setting, as a water storage of the soil is a highly dynamic property with spatial and temporal change, due to the extreme weather interruptions with long dry period followed by intense rainfall events (www. soilhealth.cals.cornell.edu). The state of water in soil is described by the amount of water (water content) and energy status of soil water movement (water potential), based on the soil characteristics including the particle types, arrangement of texture and structure (Brady and Weil, 1999) and sticky substances excreted by root and soil microorganisms. The distinctive methods of measurements are used in (a) water content and (b) potential assessment. Soil water content is the gravimetric or volumetric ratio of water and soil, known as water holding capacity (WHC). Whereas soil water potential is the sum of gravitational, matric, pressure and osmotic force expressed as potential energy per unit volume. The maximum WHC in the field for the optimal plant growth is known as the field capacity, which is equivalent to a water potential at -0.033 MPa. The schematic representation of water storage distribution is illustrated in Fig.4.2.2

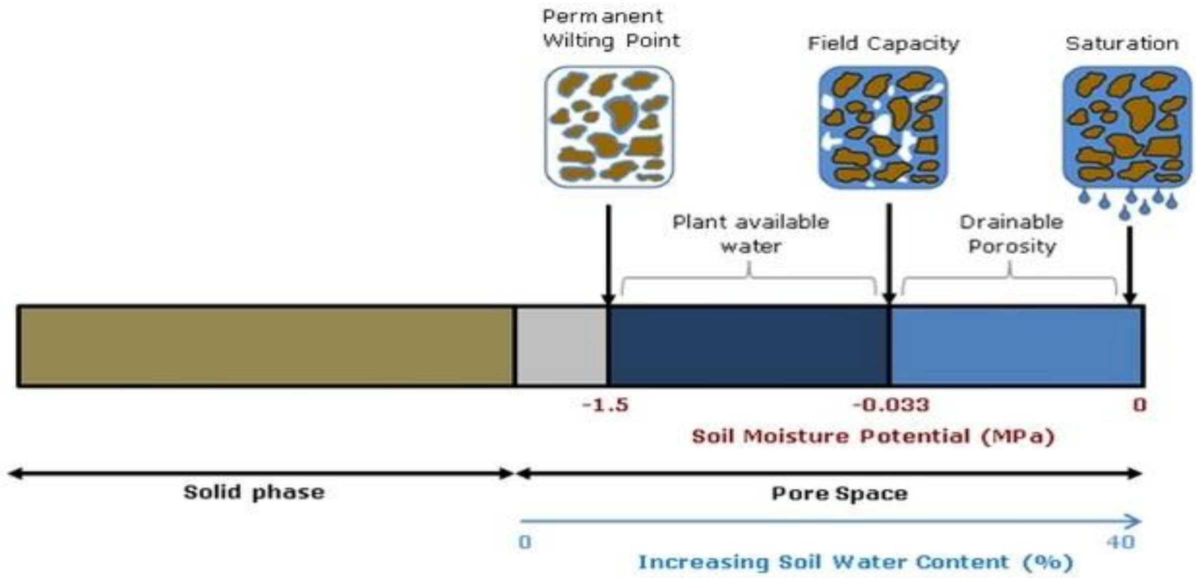


Figure 0.2 The conceptual model of soil moisture storage taken from O'Geen (2013).

The sticky organic substances in the soil form the aggregates that makes soil resistant to DRW effect and raindrop penetration. However, the large pore spaces created between dense aggregates (Jarvis, 2007) are prone to dry soil out, due to the lack of ability to holding water against the gravitational force. Arid land commonly occurs in the land area filled with single grained loose sandy soils, with large round particles 0.05-2mm in size. Likewise, the clay soils with thinner and flatter shaped particles in the size of less than 0.002mm, having fewer pore space, are capable of holding water with greater WHC. Under the *i*-DRW and freezing and thawing, the clay soil has a potential to transfer P via preferential flow by forming the macropores and cracks and building up uniformly or unevenly distributed network (Svanback et al., 2014). This is perhaps associated with the abilities of the clay to shrink and swell under the moisture fluctuation (Farewell et al., 2012; Harrison et al., 2012), thereby detaching soil colloids and increasing all forms of P in leachate (Blackwell et al., 2009; Chepkwony et al., 2001).

Phosphorus releases during the drying and rewetting

Under field conditions, a substantial amount of total P transport presents in the organic form, since the considerable proportion of water-soluble molybdate-unreactive P compounds was identified in the leachate at 90% (Blackwell et al., 2010; Blackwell et al., 2009; Koopmans et al., 2007; Turner and Haygarth, 2000; 2001). The majority of P sources passing through the soil during the *i*-DRW consists of microbial biomass P (Blackwell et al., 2010; Gordon et al., 2008; Turner et al., 2003), microbial osmoregulatory compounds (Halverson et al., 2000) and soil non-living organic material (Blackwell et al., 2009; Wu and Brookes, 2005) that can be mineralised later (Blackwell et al., 2013).

Soil non-living organic material

The macromolecular structure of soil aggregate is generally protected from physical breakdown (Unger et al., 2012) through the accumulation of soil osmolytes, amino acids and carbohydrates (Bouskill et al., 2016). During the drying period, these agents are absorbed with soil as protective complexes. However, the occluded SOM can be released from soil aggregates under the very hot and dry weather conditions (Denef et al., 2001) via disintegration of protective agents from the associated materials (Lal and Shukla, 2013). Under the intense wetting, the clay and soil particle are extracted through the dispersion over a wide area (Fierer et al., 2003), the pulse of carbon dioxide (CO_2) emitted into the atmosphere from soil surface coatings (Bartlett and James, 1980), known as flush of soil respiration. The sudden fluctuation of soil moisture then immediately leads to the subsequent P pulse events under this flush of soil respiration (Bünemann et al., 2013) through further desorption of organic and inorganic P from specific surface sites of the non-living organic material (Buenemann et al., 2013, Chepkwony et al., 2001) via mineralization (Blackwell et al., 2013; Styles and Coxon, 2006).

The soil microbial responses to DRW

The pulse changes of soil water state can create a challenging environment for resident microorganisms to fight to survive (Schimel et al., 2007). Under the completely drying soil, the process of lysing can occur very strongly in the bacterial cells (Butterly et al., 2011; Fierer et al., 2003; Pailler et al., 2014; Wang et al., 2016), accounting for 17 - 58% of the microbial community (Kieft et al., 1987; Salema et al., 1982). This results in the expulsion of cellular contents with substrate diffusion into the soil (Turner and Haygarth, 2001) and further supports of MBP via death cell with presenting more P content upon rewetting (Chowdhury et al., 2011). However, the bacterial communities are highly likely to survive in the significant fluctuation of soil moisture (Pailler et al., 2014) resulting from *i*-DRW, as frequency of events increase (Butterly et al., 2009) through: (1) formation of biofilm, whereby bacterial cells are embedded within a self-synthesised matrix of extracellular polymeric substances on the soil surface (Bouskill et al., 2016) or inoculated in the intact soil aggregates (Navarro-García et al., 2012) and (2) adaption of dormant status for a long period of time where the bacterial cells remain at the lower level of metabolic functions (Schimel et al., 2007), based on the upregulation of gene associated with the bacterial and archaeal phylogenetic marker 16S rRNA and *amo A* genes (Blazewicz et al., 2013; Jones and Lennon, 2010). As compared with bacteria, the fungi are not affected by sudden soil moisture changes (Bapiri et al., 2010), due to the extended networks of hyphae (Meisner et al., 2018).

Although, there are many existing studies in the broader literatures, which have examined an effect of frequent DRW interruptions on soil P solubilisation and potential leaching, which is potentially predicted under *i*-DRW during changing pattern of seasonal rainfall associated with the climate change. It is believed, a more in-depth analysis of the rhizosphere contribution is required to verify over a range of soil types and variation of P supply to validate the true impact of *i*-DRW concerning the biochemical make-up of the soil its own. Further, this may enable me to study an appropriate management action that can be adopted by the farm sites to intervene in increased levels of soil soluble P associated with *i*-DRW within the root medium, through crop utilisation. In turn, it potentially assists in mitigating P transfer. The rationale of this research is, therefore, to gain a better understanding of how *i*-DRW sudden soil moisture fluctuations can influence the efficiency of P solubilisation processes in the rhizosphere and the amount of P leached into water from remarkably diverse soil types. The broad objective of this chapter is to study the immediate effects of DRW on the dissolved P forms in both soil solution and leachate water, using a further detailed assessment of P solubilisation processes within four diverse types of soils at different rates of P application.

It will test the following five hypotheses:

Hypothesis 1. DRW increases the P solubilisation (and thus the potential for leaching).

Hypothesis 2. The amount of solubilised P has a potential for a leaching, being proportionate to the rate of P applied.

Hypothesis 3. Dephosphorylating enzymes and organic acids affect the P solubilisation in the rhizosphere after DRW.

Hypothesis 4. The soils with different physiochemical contents respond differently to DRW.

Hypothesis 5. The forms of P in leachate water will vary depending of DRW event cycles and dynamics

4.3 Materials and methods

Four contrasting sites of arid agricultural areas in the distant location ranging from southwest to northeast regions of China were chosen to represent an example of the soil sampling in the diverse types (Figure 4.3.1.1), with particular the selection of soil with a variable soil bio-physiochemical content. It will allow me to compare the responses of different type of soils to the immediate DRW in respect of P solubilisation (and potential leaching) under varying P application rate. The analysis of soil diversity is shown in the Table 4.4.1. included Olsen P, TP, water-soluble P, pH, water holding capacity, texture, and infiltration rate. This experiment was conducted between 09th July and 03rd October 2018 in a naturally lit greenhouse at the China Agricultural University. The greenhouse temperature ranged between 17-28° C during the experimental period.

4.3.1 Preparation of soil and lysimeter tube

As mentioned, the soils were collected from four distinct locations of China (shown in the map 4.3.2.2), and sampled upper 30 cm depths of three randomised points in a single field within the inter spaces of 100 metre. The soil samples were then kept in a soil storage room of the greenhouse at 10° C for less than six-month period prior to the experiment under the field moist condition between 30-40%. Before the commencement of the experiment, the soil samples were air-dried indoors for ten days at the room temperature and further sieved through a 2 mm filter to remove visible non-soil material. On the 10th day of air- drying process 0.3 kg of each soil sample was loosely packed in the open-ended conical tubes.

After this experiment, this soil will continue to be used in the experiment described in chapter 5. Therefore, soil sampling for soil analysis can be done before next experiment by using small core from the 3 cm of surface, without disturbing the soil.

The lysimeter tubes, which were designed by myself, had a diameter of 6 cm at the top and the gradual sliding sides at the bottom to collect the leachate. A spout with 2.5 cm diameter was plugged with a

10x10 cm cotton mesh. 100 g coarse sand was placed in the lower half of the lysimeter tube to resemble a natural structure of soil layering for water retention purposes (Figure 4.3.1). The composition of sand is unknown, which may affect the results.

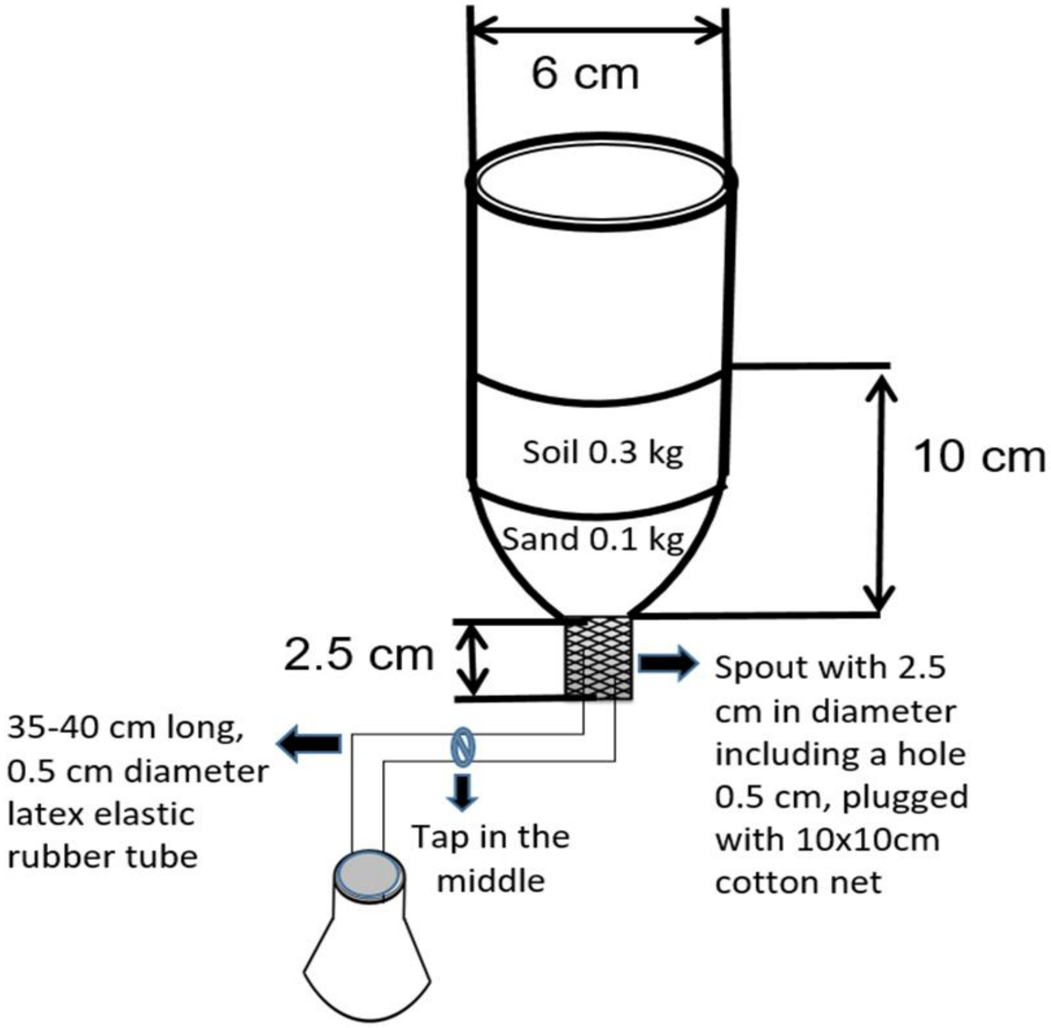


Figure 0.1 Lysimeter tube design specification

4.3.2 Experimental design

The treatments used in this immediate DRW experiment were of varying nature, in terms of the level of soil moisture (DRW+) and (DRW-), the frequency of DRW occurrence (DRW I, II, III and IV), P supply rate (P0, P250 and P500), and soil types. The figure 4.3.2 illustrates a schematic representation of this experiment including several key characteristics. Each treatment was replicated four times including control. Phosphorus fertiliser application was added at an equivalent rate of 0, 250 and 500 mg kg^{-1} soil prior to the first wetting cycle, supplied as monopotassium phosphate (KH_2PO_4). The amount of P was calculated based on molar mass.

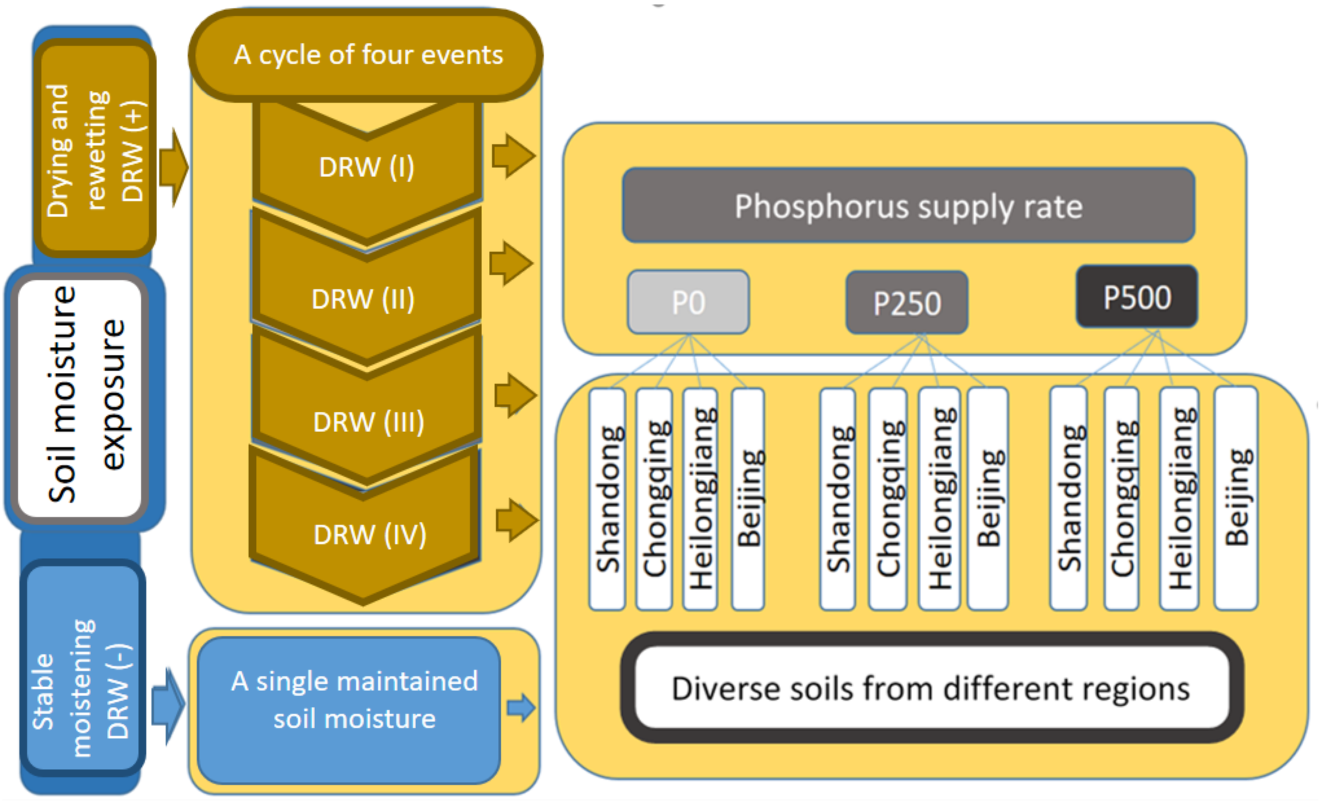


Figure 0.2 The schematic representation of experimental components including the soil moisture treatments that are denoted in (a) blue: control and (b) deep yellow: moisture fluctuation.

4.3.2.1 Soil Sample Types

The following four contrasting soil samples collected from different agricultural lands located in distant regions of China (Figure 4.3.3) were used in this experiment:

1. Sandy loam textured brown Cambisol soils collected from the fallow land in Qingdao, Shandong, China (Latitude: 29°03’N, Longitude: 106°11’E 170m) by Prof, Ding of Qingdao University experiment station. It contains Olsen P 22.3 mg kg^{-1}, TP 389 mg kg^{-1}, organic carbon 11.5 g kg^{-1}, and soil pH 7.3.

2. Fine and coarse loamy texture red Lithosol soils were collected from Huangzhuang village, Yong Xing town, Jiang Jin, Chongqing, China (Latitude: 29°03’N, Longitude: 106°11’E, 266 m). The land is used for the crop rotation of winter wheat with summer maize or wheat with rice. The soil contains Olsen-P 7.85 mg kg^{-1}, TP 270 mg kg^{-1}, organic carbon 15.5 g kg^{-1}, and pH 4.7.
3. Clay loam textured black Haplic Phaeozems soils were collected from Heilongjiang Province (Latitude: 45°40’N, Longitude: 126° 35’E, 151 m). The land is utilised for a single crop rotation of wheat- soybean-maize. Soil contains Olsen-P 34 mg kg^{-1}, TP 448 mg kg^{-1}, organic carbon 24.5 g kg^{-1}, and pH 5.1.
4. Silt loam textured calcareous Luvic Kastanozems soils with low P availability were collected from the fallow Shangzhuang field, long-term fertiliser CAU experiment station, Beijing, China (Latitude: 40°01’N, Longitude: 116°16’E, 130 m). Soil contains Olsen P 2.6 mg kg^{-1}, TP 288 mg k^{g-1}, organic carbon 12 g kg^{-1}, and pH 8.9.

Table 4.3.1 Fundamental chemical and physical properties of soils used the DRW experiments collected from four different location of China.

Soil sampling location	Shandong	Chongqing	Heilongjiang	Beijing
FAO classification	Cambisol	Lithosol	Haplic Phaeozems	Luvic Kastanozems
Soil sampling site details	Fallow land in Qingdao, Shandong	Winter and summer crop rotation land, Huangzhuang Chongqing	Single crop rotation (wheat-soybean-maize) land, Heilongjiang	Fallow land in long-term experiment station of CAU Shangzhuang
Texture	Sandy loam	Fine and coarse loamy	Clay loam	Silt loam
Olsen P (mg kg^{-1})	22.3	7.85	34	2.6
Total P (mg kg^{-1})	389	270	448	288

Organic Carbon (g kg^{-1})	11.5	15.5	24.5	12
Soil acidity	7.3	4.7	5.1	8.9
Nitrogen (%)	0.03	0.05	0.07	0.03
Carbon (%)	0.28	0.42	0.68	0.22
Infiltration rate (cm h^{-1})	4.4	3.5	3.2	3.9
Water holding capacity (%)	23	39	41	33

All analyses were conducted at Plant and Nutrition Lab, CAU.

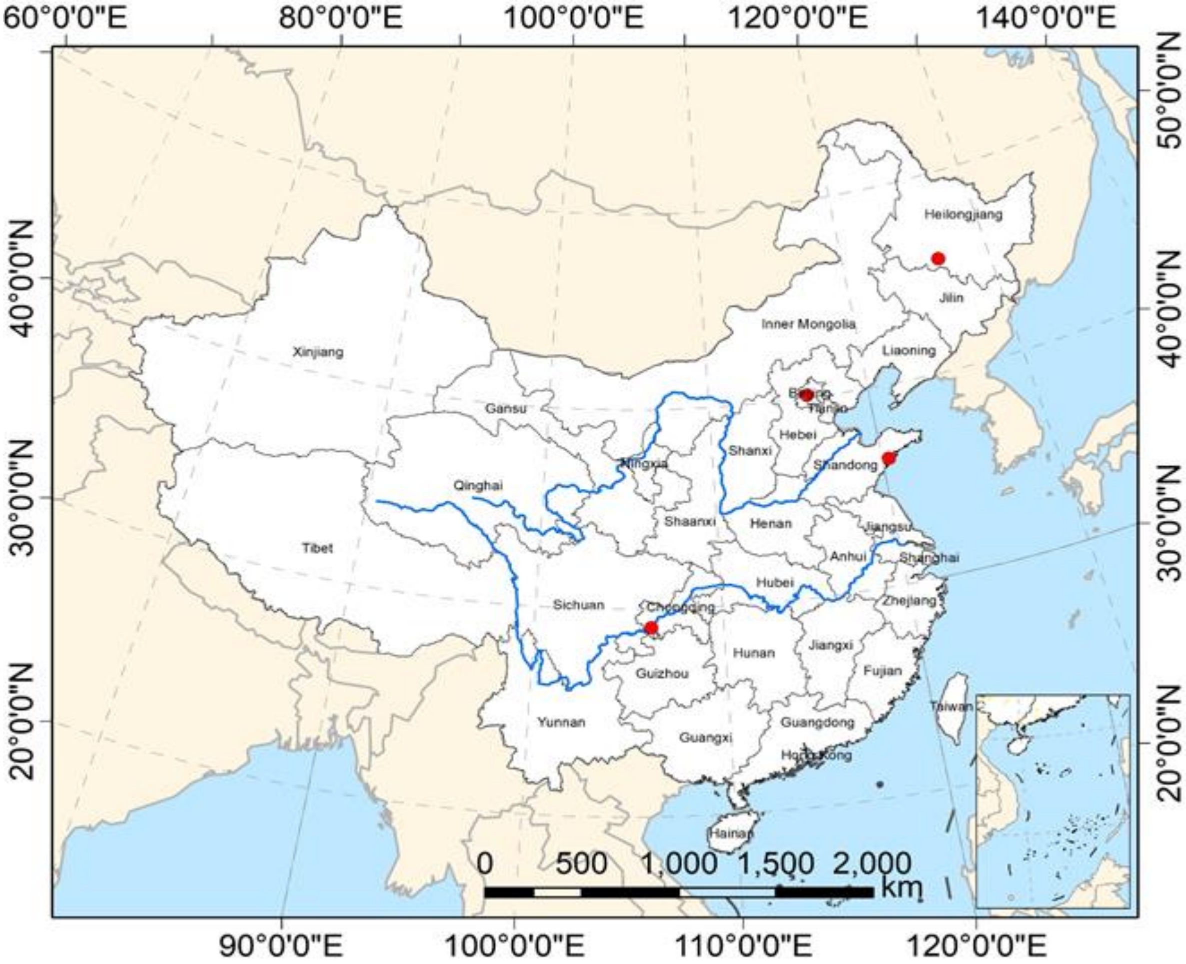


Figure 0.3 The four different soil sampling locations of China (illustrated by red dots).

4.3.2.2 Drying and Rewetting Approach

The DRW treatment was split into two groups (Figure 4.3.4). First, one treatment had a single maintained moisture: DRW (-), which acted as the control. Second, other treatment was exposed the immediate soil moisture fluctuation effect via a cycle of four rewetting events DRW (+) that was simulated as the soil reached at 30% of water holding capacity. In this particular chapter, the WHC was intentially reduced under the experimental setup without the presence of plants to see the immediate responces of non-planted soil properties under the scarcity of water availability, which may be predicted in future climate change. In this experiment, the percentage of WHC was determined by myself, as a fraction of the 100% WHC found on the basis of the ratio of weigth of soil with maximum amount of water retained after 24-hour drainage (Saxton et al., 2006, Reynolds et al., 2000) to the dry soil mass. The method used in this experiment is a modified version based on the methodology described by Bajwa et al. in 2017.

Figure 0.4 The four different soil sampling locations of China (illustrated by red dots).

The following steps and actions were undertaken as party of each DRW cycle. The weight of the tubes was monitored every other day. The drying period varied among the soil types. The total amount of water irrigated to dry soil was equivalent to 175% field capacity, which is enough to generate a minimum of 20-35 ml leachate water for the measurement of TP and RP in both the filtered and unfiltered leachate. A single piece of filter paper was placed on the topsoil surface to facilitate an even distribution of irrigation water, a modified version of the method by Blackwell et al., (2009). Water was added slowly in one application on the filter paper. The taps remained closed for the first 30 min after wetting in order to prevent the egde effect of water pouring down directly through the gap between wall and soil. At the same time, the soil moistures of the DRW (-) Controlled sample tubes were maintained at 70% of water holding capacity throughout the experimental period by deionised water. The water holding capacity of 300 g soil at the rate of 100% is equavalent of 68ml water in Shandong, 124ml in Chongqing, 117ml in Heilongjiang and 98ml Beijing soil.

After each DRW cycle, the leachate was collected into the 40 ml plastic centrifuge tubes under 24 hours incubation and was stored at 4°C, prior to P analysis in accordance with the monitoring protocols of Enviromental Protection Agency (paragraph 5.6). Within 24hours of storage, the leachate collection was divided into two halves for the filtration. The one half of the leachate was filtered through Whatman 0.45µm cellulose nitrate membrane filter using a syringe, whilst the other half was kept in unfiltered. The filtered and unfiltered leachate was further divided into two equal parts to create two sets of filtered and unfiltered samples for reactive P analysis. The first set of both filtered and unfiltered leachate samples were immidiately analysed for RP, whereas the remaining set of filtered and unfiltered leachate water was stored for 7 days at 4°C for TP analysis.

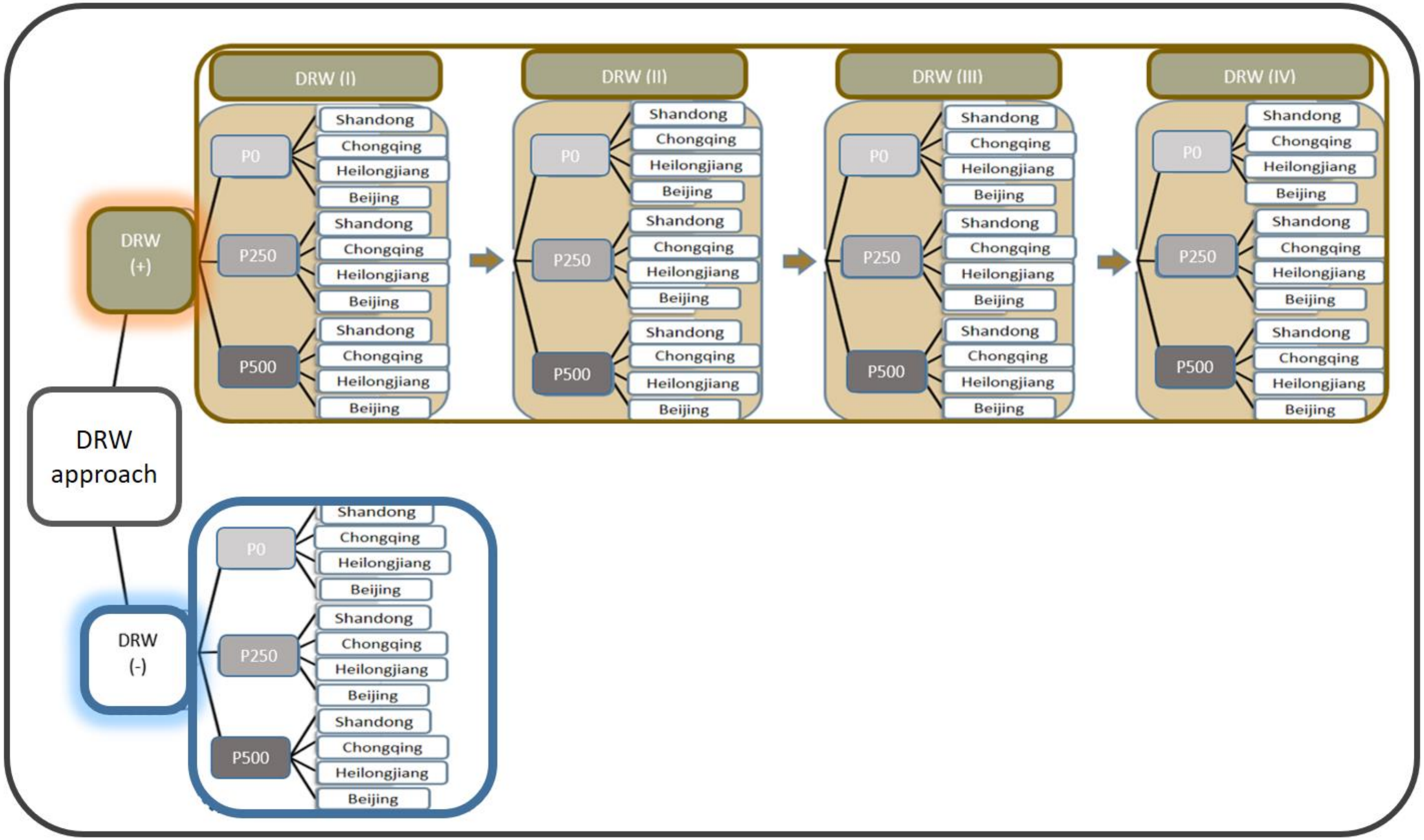


Figure 0.5 The overview of the DRW leaching experiment in the greenhouse, including a cycle of four immediate drying and wetting events and types of soils, from four different regions: Shandong, Chongqing, Heilongjiang, and Beijing.

4.3.3 Laboratory analyses

The soil and leachate water analyses are the key components of this experiment, which includes the test of soil phosphorus and biological characteristics and phosphorus species in the drained water.

4.3.3.1 Analysis of soil characteristics

A. Soil phosphorus

In this experiment, I have determined Olsen-P, water-soluble P and total P concentration of the soil. Soil P availability was determined through the Olsen method (1954) by using the air-dried soil extracted with 0.5 M $NaHCO_3$ at pH 8.5. Malachite green oxalate salt with polyvinyl alcohol is used for the determination of water-soluble P in the soil solution through the reaction with additional molybdate acidification, with spectrophotometric measurement at the absorption wavelength of 610 nm. This method is modified from Juan et al version (Feng et al., 2011). Soil TP analysis was performed by using the digestion and colorimetric method based on the acidic molybdate reaction for the presence of the phosphomolybdate complex and measured spectrophotometrically at 880 nm.

B. Soil enzymatic activities and carboxylate analysis

The determination of acid and alkaline phosphatase activity (Alvey et al., 2001; Neumann, 2006) was performed under the colorimetric analysis of the *p*-nitrophenol. 0.4 mL of 0.04M sodium acetate buffer at pH 5.2 was used for acid phosphatase activity, and Trizma buffer at pH 7.4 for alkaline phosphatase activity. 0.1 mL 0.15 mol L^{-1} *p*-nitrophenol phosphate (PNP) was diluted in sodium acetate buffer for acid phosphatase, and in Trizma buffer for alkaline phosphatase activity then added to 0.5 mL rhizosphere suspension. After incubation of soil at $28^{o}C$ for 30 minutes, the reactions were terminated by adding 0.5 M NaOH. The wavelength of absorbance for phosphatase activities was spectrophotometrically measured at 405nm. One unit of acid and alkaline phosphatase activity was defined as the activity per gram soil that produced 1 µmol p-nitrophenol per hour.

An assessment of phytase activity was using the methods modified from previous research studies (Richardson et al., 2000). 2 g of soil was used for the preparation of the soil suspension with 8 ml deionised water. 0.5 mL of soil solution was mixed with 0.4 ml InsP6 MES buffer at pH 5.5, including 30mM MES, 2mM EDTA, and 4mM InsP6. After incubation of soil at 37^{o}C for 1 hour, the reactions were terminated by 1ml 10% trichloroacetic acid (TCA). The sample was subsequently centrifuged at 12000 r.p.m for 10 min to remove soil particles. The orthophosphate concentration in the supernatant was determined by colorimetric assay at wavelength 882 nm using the molybdenum-blue reaction (Murphy and Riley, 1962). One unit of phytase activity was defined as the activity per gram soil dissolved in 1ml $CaCl_2$ that liberated 1 mg P per hour. Carboxylates were analysed by a reversed-phase high-performance liquid chromatography (HPLC) system in prior filtration of soil suspension through a 0.22-μm filter to prevent blocking the machine. The chromatographic separation was conducted on a 250 × 4.6 mm reversed-phase column (Alltima C18, 5 μm; Alltech Associates, Inc., Deerfield, IL, USA). The mobile phase was using 25 mmol L^{-1} KH_2PO_4 at pH 2.3 with a flow rate of 1 mL min^{-1} at 31 °C with the detection of carboxylates at 214 nm.

4.3.3.2 The analysis of leachate water

Total and reactive P in the leachate were determined through the method set out by the EPA in protocol 365.3 in 1978 (www.epa.gov). Forms of P have been classified into categories based on (1) physical separation via 0.45 μm filter membrane: dissolved P (DP) and particulate (PP); and (2) chemical interaction via an acid-Mo based colorimetric technique of Murphy and Riley (1962): reactive P (RP) and unreactive P (UP) with subsequent digestion methods (Haygarth and Sharpley, 2000). A schematic representation of the details on this experimental protocol is shown in Figure 4.3.2.1. The filtered P is the dissolved P fraction, which is exceedingly small molecule at a weight less than 10,000 Da (Haygarth and Jarvis, 1997), including orthophosphate ($H_2PO_4^{-}$, HPO_4^{-2}, $PO4^{-3}$). The difference between the filtered and unfiltered sample is the particulate P fractions, which are as great as 0.45 μm (Blackwell et al., 2009b). The particulate P fractions can be a deposit made up both inorganic and organic P species, which is being composed of P mineral apatite mixed with clays, clay- organic complexes, metal oxides and hydroxides (Maher and Woo, 1998).

The forms of P are further classified into reactive and unreactive by using the ammonium molybdate blue spectrometric methods. The unreactive P mostly derives from organic origin (Haygarth et al., 1998; 1997; 1997) including the sugar P, inositol P, ATP, phospholipids, phosphoproteins, phosphoramides and phosphonates (Turner et al., 2002) and condensed P such as pyro-, meta- and polyphosphates (Jarvie et al., 2002). An appropriate method of digestion was needed, when TP was determined in the filtered and unfiltered sample. In an autoclave digestion method (heating at 121^{o}C for 30 min), an aliquot was diluted to 50 ml by adding 0.4 g ammonium persulphate and 1 ml 0.3M sulphuric acids. After the digestion, the dissolved total and reactive P were determined through the molybdate colorimetric method by using ammonium molybdate antimony potassium tartrate and ascorbic acid with spectrophotometric absorbance at 650 nm, following five minutes. Then the calculation of P species was used the method adapted from Haygarth and Sharpley (2000) shown in the Figure 4.3.6. The proportion of dissolved unreactive P (DUP) form in the leachate was calculated by following formula for mass percent.

$$\text{Percentage composition of DUP in the leachate } = \frac{\text{mass of UP } (<0.45)}{\text{sum mass of total UP } (<0.45) \text{ and } (>0.45)} x100$$

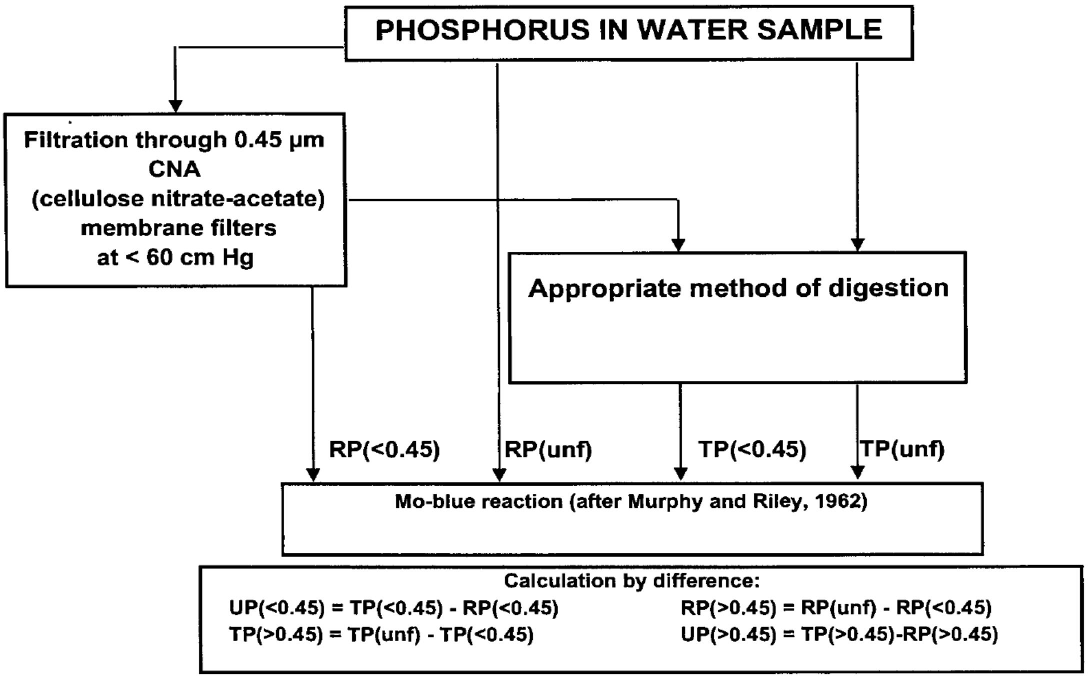


Figure 0.6 Schematic diagrams of all phosphorus forms in water that is used for the calculation of P species in leachate of this experiment (adapted from Haygarth and Sharpley, 2000).

4.3.3.3 Data analysis

The statistical analyses were performed by using the software SPSS version 23 (IBM, SPSS 2014). The data are reported as mean ± standard deviation with a confidence threshold of 0.05 applied in all cases. In the soil analysis, the significant differences in mean concentration of soil soluble P between moisture treatments were assessed using two-way ANOVA by employing univariate of linear regression analysis. The concentration of soil soluble P was divided into soil types based on the different rate of P supply to characterise the relationships and their significance. In each soil sampling group, the moisture effect of the soil was compared by t-test to determine the statistical significance of P solubility between DRW treatments ($p \leq 0.05$). The mean differences of P treatments were assessed using a one-way analysis of least significant difference with $p \leq 0.05$ by Duncan's *post-hoc* range analysis for multiple comparisons. In the leachate, the three-way analysis of variance was performed on datasets collected from the one type of soil, including P species and TP in the water. Empirical polynomial (inverse third order) exponential equation analysis was performed to explore the relationship between soil physiological traits (i.e., organic acids and enzymatic activities of soils) and soil Olsen-P by using SigmaPlot (SigmaPlot 10.0, USA).

4.4 Results

The findings of this experiment associated with the immediate DRW effect will be grouped into categories of (a) soil analysis and (b) leachate water analysis.

A. Soil analysis

The soil analysis will be subcategorised into three distinct sections (1) the soil P analysis, (2) the biological indicators of the soil associated with rhizosphere processes, and (3) the characteristic of the soil drying performance.

4.4.1 Soil phosphorus analysis

The summary of soil soluble P analysis of four distinct types of soils after fourth DRW events was shown in figure 4.4.1. There were no statistically significant differences of Olsen-P concentration between the moisture treated and non-treated soils, particularly when these are directly compared against each other. The P solubilisation process under immediate effects of DRW appeared to vary with the soil types and the P supply rate. Noticeable changes of P solubilisation were observed at the rates of P applications of 250 and 500 mg kg^{-1} (P250 and P500 treatment respectively) within the soil itself. At the end of the experiment, the concentration of Olsen P reached at 139, 177, 255 and 99 mg kg^{-1} under the P500 treatment from 20, 10, 37 and 4.6 mg kg^{-1} in the Shandong, Chongqing, Heilongjiang, and Beijing soils, respectively. When comparing the results between P250 and P500 treatments at doubling rate, the actual concentration level of Olsen-P recorded were not materially different despite the market difference between these two P applications.

Phosphorus solubilisation after the DRW pressure was appeared to related to the soil type and characteristic, rather than the overall rate of P supply itself. For example, the results show that the most striking increase of P solubilisation resulting from DRW process was observed in the silt loam textured calcareous Luvic Kastanozems soils with low P availability (pH 8.9) from Beijing with the increase of 2.6 to 99 mg kg^{-1}. The Olsen P of equally low bioavailable coarse loamy textured red Lithosol soil from Chongqing with pH 4.7 having Olsen-P at 7.9 mg kg^{-1} increased to 134 mg kg^{-1} in response to 250 mg kg^{-1} P application and then further increased to 177 mg kg^{-1} in 500 mg kg^{-1} P application. Whilst the lowest increase of Olsen P was observed in the Cambisol soils from Shandong from 22.3 to 139 mg kg^{-1} and Haplic Phaeozems soils from Heilongjiang from 34 to 255 mg kg^{-1}, where the soils are more clayey and sandier basis. Notwithstanding the fact that no statistically significant Olsen-P differences were noted, when assessing the water-soluble P concentration level in the same soil sample, statistically significant differences were noted between soil moisture treatment.

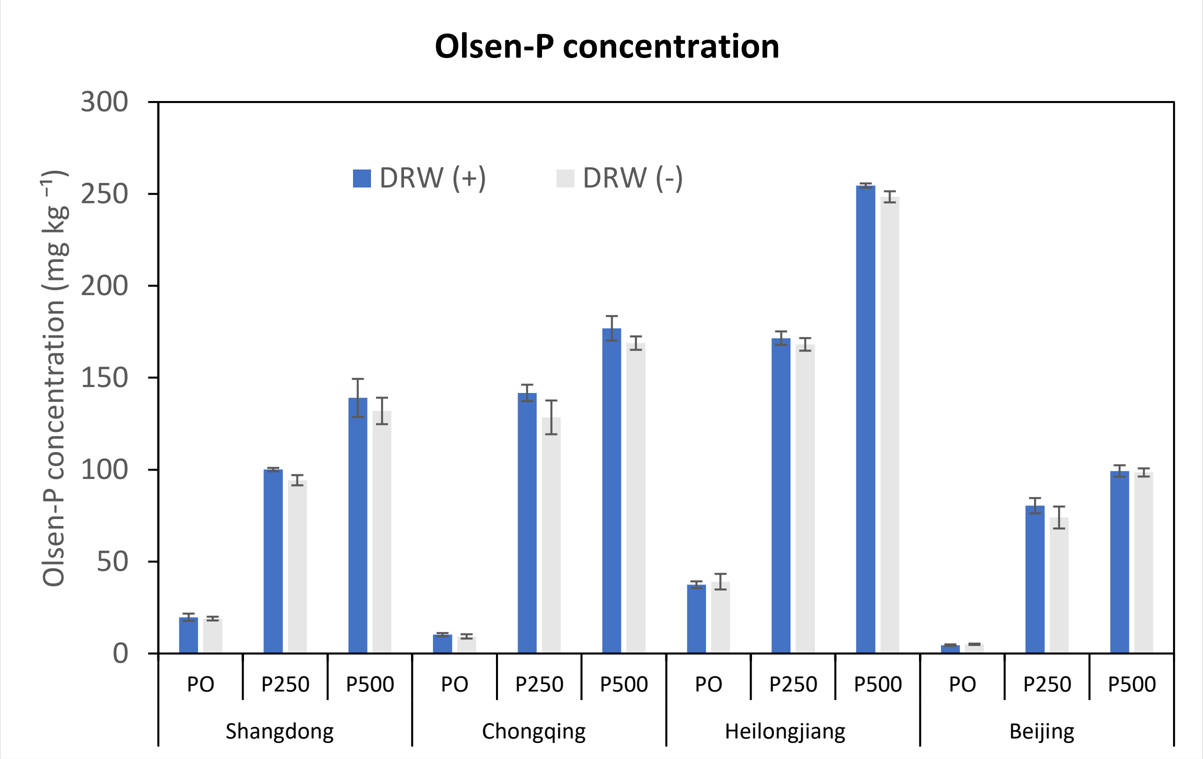


Figure **0.1** The solubilised phosphorus (Olsen-P) level compared in four distinct soil types under two different moisture conditions: the drying and rewetting DRW (+) and stable moistening DRW (-) soils, with taken the three phosphorus application rates. The four different types of soils having diverse physiochemical composition were collected from various sampling locations of China. The horizontal axis represents the number of varying soil types with a combination of P treatment, providing insights into distribution of Olsen P levels across the soil types, moisture conditions and P supply amendments.

The statistically significant differences of water-soluble P concentration between soil groups under the DRW treatments were determined in the soils from Shandong and Chongqing particularly with P treatments of P250 and P500, and with P250 respectively. Overall, the level of water-soluble P concentration in the soil followed the same pattern of Olsen P, with the upward trend in correspondence with the intensity of P loading. However, in most soils, the concentrations of water-soluble P were remarkably like each other ranging from 0.2 to 0.4 mg kg $^{-1}$, except the noticeable difference in the soil from Beijing. The lowest level of water-soluble P in the soil from Beijing at the rate of 0.02 mg kg $^{-1}$ elevated dramatically by the 3- and 9-fold increase at the end of fourth DRW cycles, reaching approximately 0.19 mg kg $^{-1}$ in P500 treatment.

The amount of total P amongst all observed soil types appeared to be elevated at the end of the experiment, except for (I) moisture treated and control soil groups without P addition; and (ii) the moisture treated soil from Shandong at the P 250 treatment. Regardless of the soil types, the highest amount of total P was always detected in soil samples with P500 treatment to 437, 385, 638 and 400 mg kg $^{-1}$ in the Shandong, Chongqing, Heilongjiang, and Beijing soils, respectively. This result suggests a direct influence of the rate of P application on the subsequent soil P condition (Table 4.4.1) and possible leaching consequences (Figure 4.4.1).

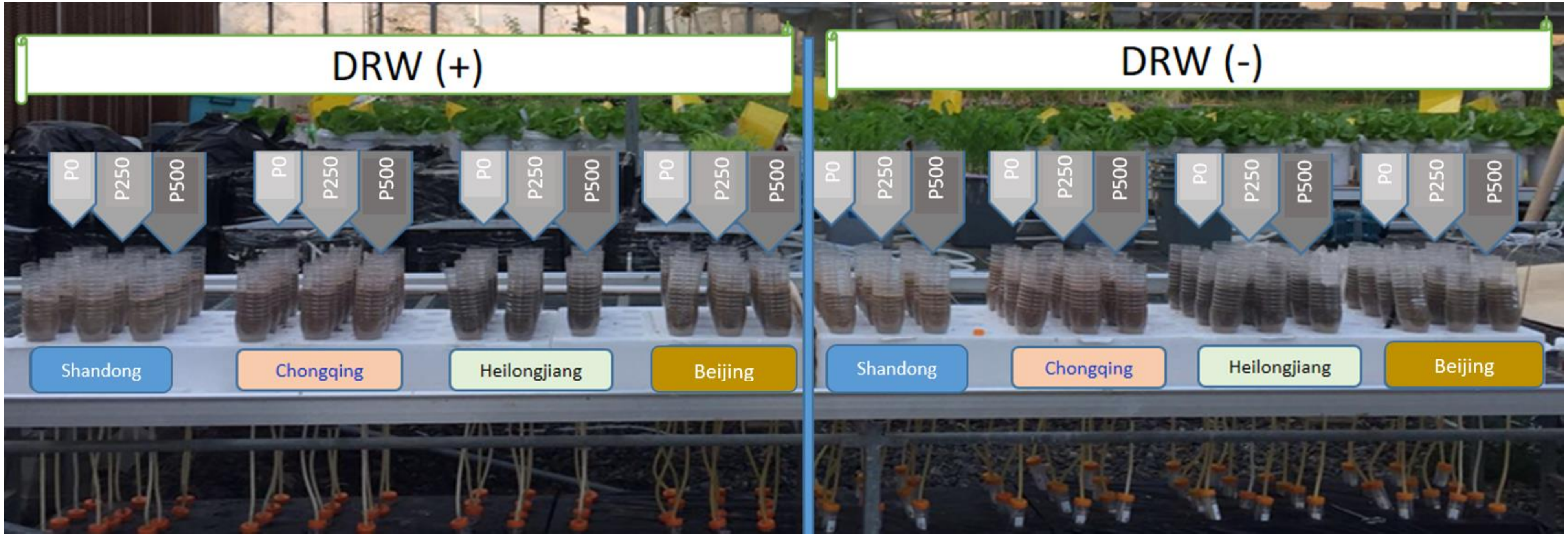


Figure 0.2 Photo of DRW experiment in the greenhouse

This experiment was split into two groups. Each group was made up the four different types of soil. The first sampling group was exposed by a cycle of four drying and rewetting event (DRW +), and the second group was maintained constant soil moisture level (DRW -) at 70% of water holding capacity (WHC) to be used as a control. In DRW (+) group, as the soil sample dried up at 30% of WHC, it was simulated by the deionised water equivalent to 175%. Depending on the soil type, the duration of each drying periods varied between 14-24 days.

Table 4.4.1. Summary of phosphorus analysis of the soil including the total P, Olsen-P, and water- soluble P of the four contrasting soil types in China under the different level of phosphorus supply with and without drying and rewetting cycles. The soils were sampled at the end of the 4th drying and rewetting cycles.

Sampling location FAO classification				**Shandong** **Cambisol**			**Chongqing** **Lithosol**			**Heilongjiang** **Haplic Phaeozems**			**Beijing** **Luvic Kastanozems**		
Phosphorus analysis				Total P	Olsen-P	Water Soluble P	Total P	Olsen-P	Water soluble P	Total P	Olsen-P	Water soluble P	Total P	Olsen-P	Water soluble P
Concentration of P before treatment ($mg\ kg^{-1}$)				389	22.3	0.115	270	7.85	0.15	448	34	0.196	288	2.6	0.015
Soil moisture treatments	DRW (+)	P supply rate ($mg\ kg^{-1}$)	0	321.9	19.7 (2)	0.2 (0.03)	248.4 (5.3)	10.3 (0.9)	0.22 (0.16)	428.6 (67.6)	37.4 (1.8)	0.2 (0.05)	276.9 (5)	4.6 (0.4)	0.1 (0.01)
			250	361.7 (18.2)	100 (0.9)	0.28 (0.02)*	289.9 (6.7)	141.8 (4.5)	0.3 (0.01)*	510.8 (178)	171.6 (3.7)	0.3 (0.06)	325.5 (23.2)	80.4 (4.1)	0.13 (0.02)
			500	436.8 (7.8)	139 (10.4)	0.32 (0.03)*	385.2 (20.5)	176.9 (6.7)	0.36 (0.06)	638.3 (15.8)	254.6 (1.2)	0.37 (0.07)	399.5 (8.9)	99.3 (3.1)	0.19 (0.03)
	DRW (-)	P supply rate ($mg\ kg^{-1}$)	0	343.2 (3.5)	19 (1)	0.14 (0.004)	256.5 (6.7)	9.3 (1.2)	0.18 (0.01)	439.8 (13.9)	39 (4.2)	0.2 (0.01)	279.5 (11.4)	5 (0.4)	0.06 (0.01)
			250	406.9 (12.7)	94.3 (2.8)	0.17 (0.01)	303.1 (11.6)	128.5 (9.2)	0.2 (0.02)	580 (48.4)	168.2 (3.4)	0.28 (0.05)	350.6 (20.9)	74 (6)	0.13 (0.02)
			500	449.4 (9.9)	132 (7.2)	0.19 (0.04)	377.5 (32.3)	168.9 (3.7)	0.3 (0.02)	697.8 (12.5)	248.4 (3)	0.3 (0.06)	411.7 (29.4)	98.5 (2.2)	0.14 (0.02)

* The mean difference is significant at the 0.05 level.

Note: Values are means of four soil replicates. The row separately arranged in accordance with the soil type variation. Different letters in each column indicated statistical differences (significance reported as $p \leq 0.05$) between different P variables.

4.4.2 Soil biological indicators

This section analysed the biological responces of the soil to the immediate *i*-DRW events. The *i*-DRW effect can induce the enzymatic activities and carboxylates exudation associated with P solubilisation processes.

Soil enzymatic activities

The level of dephosphorylating enzyme activities showed a significant, upto three-fold increases after episodes of *i*-DRW events, as compared with the control soils. The following observations were found:

(1) before experiment, alkaline phosphatase activities were 61.6, 84.5, 116.1 and 154.8 µg PNP h^{-1} g^{-1} in the Shandong, Chongqing, Heilongjiang, and Beijing soils respectively and at the end of the experiment (a) DRW (-) Controlled sample 67 and 250 µg PNP h^{-1} g^{-1} at P500 treatment Shandong and Beijing soils (b) DRW (+) 111 and just below 400 µg PNP h^{-1} g^{-1} at P500 treatment Chongqing and Beijing soils.

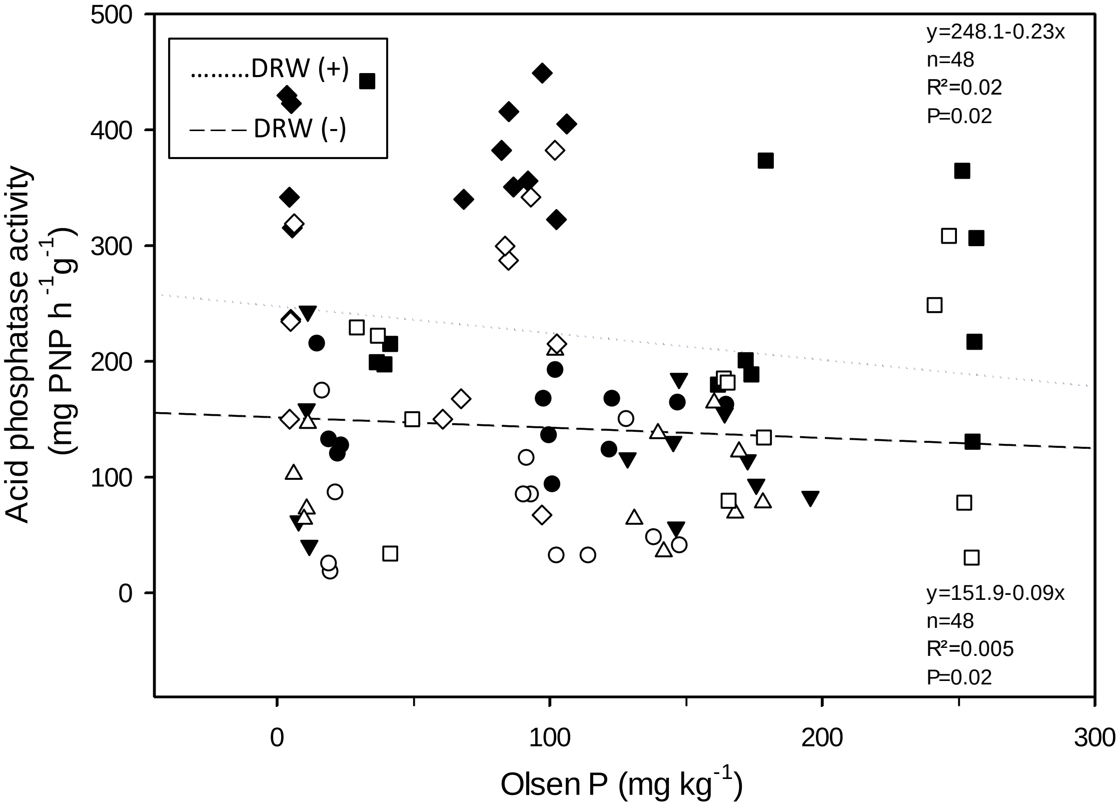
(2) before experiment, acid phosphatase activities were 55.2, 87.5, 141 and 102.8 µg PNP h^{-1} g^{-1} in the Shandong, Chongqing, Heilongjiang, and Beijing soils respectively and at the end of the experiment: (a) DRW (-) Controlled sample 63 and 158 µg PNP h^{-1} g^{-1} in the P0 treatment in the Shandong and Heilongjiang soils (b) DRW (+) 94 and 320 µg PNP h^{-1} g^{-1} in the P0 treatment in the Shandong and Heilongjiang soils.

(3) before experiment, phytase activities were 0.08; 0.07; 0.24 and 0.16 mg P ml^{-1} h^{-1} g^{-1} in the Shandong, Chongqing, Heilongjiang, and Beijing soils and at the end of the experiment (a) DRW (-) 0.07 and 0.2 mg P ml^{-1} h^{-1} g^{-1} in the Shandong soil with P250 and Heilongjiang soils with P0 control and (b) DRW (+) 0.11 and 0.3 mg P ml^{-1}

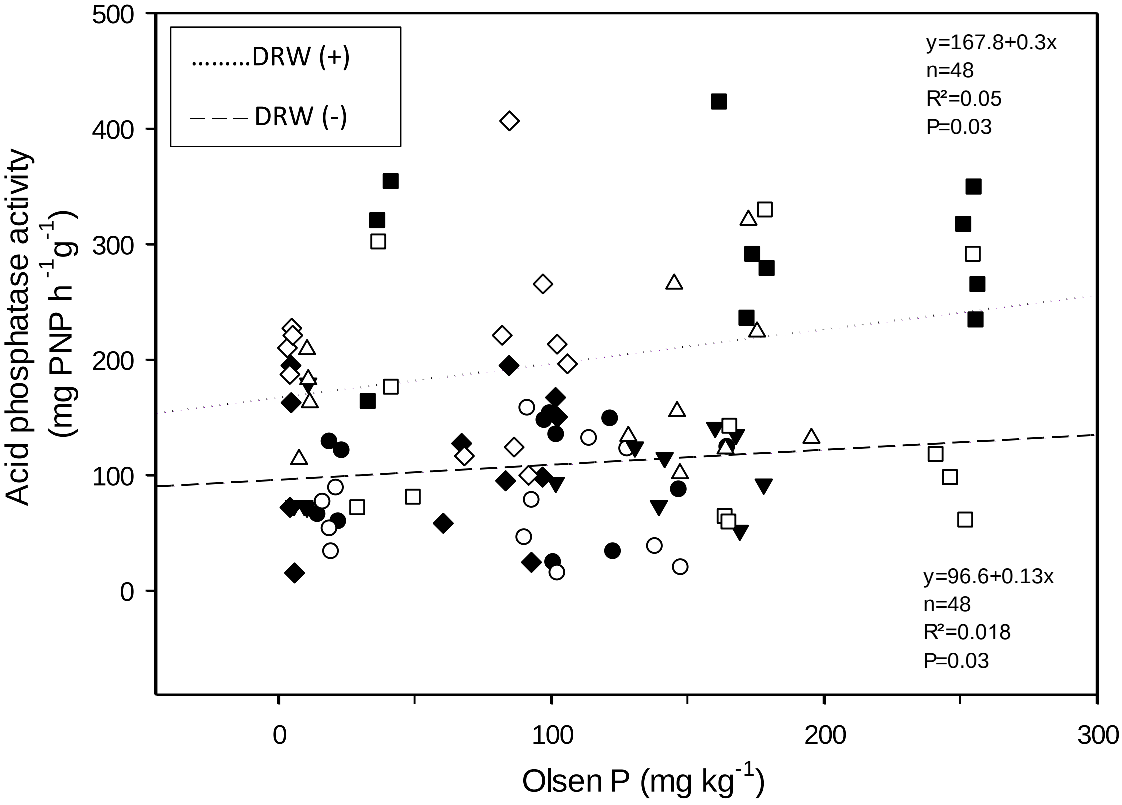
The statistical significance of dephosphorylation enzyme activities noted in DRW soils (a) alkaline phosphatase: all P treatments of all soil types except Chongqing soil; (b) acid phosphatase: all types of soil samples and (c) phytase: all P treatments groups of the Shandong. The level of average enzyme activities was dramatically changed in the soil where it has the most soluble P contents, for example, in the Heilongjiang soil at P500, whereby the average alkaline phosphatase recorded at 254 in DRW (+) and 166 in DRW (-) Controlled soil, acid phosphatase 291 DRW (+) and 142 µg PNP h-$^{-1}$g^{-1} DRW (-) Controlled sample, and phytase 0.29 DRW (+) and 0.15 DRW (-) Controlled sample mg P ml^{-1} h^{-1} g^{-1}. In all DRW treated Heilongjiang soils, a slight increase of phytase activities occurred by 11-18% particularly in increments of 0.03 and 0.05, regardless of the general increase by 30- 50 % other soil types collected from the other three regions under DRW.

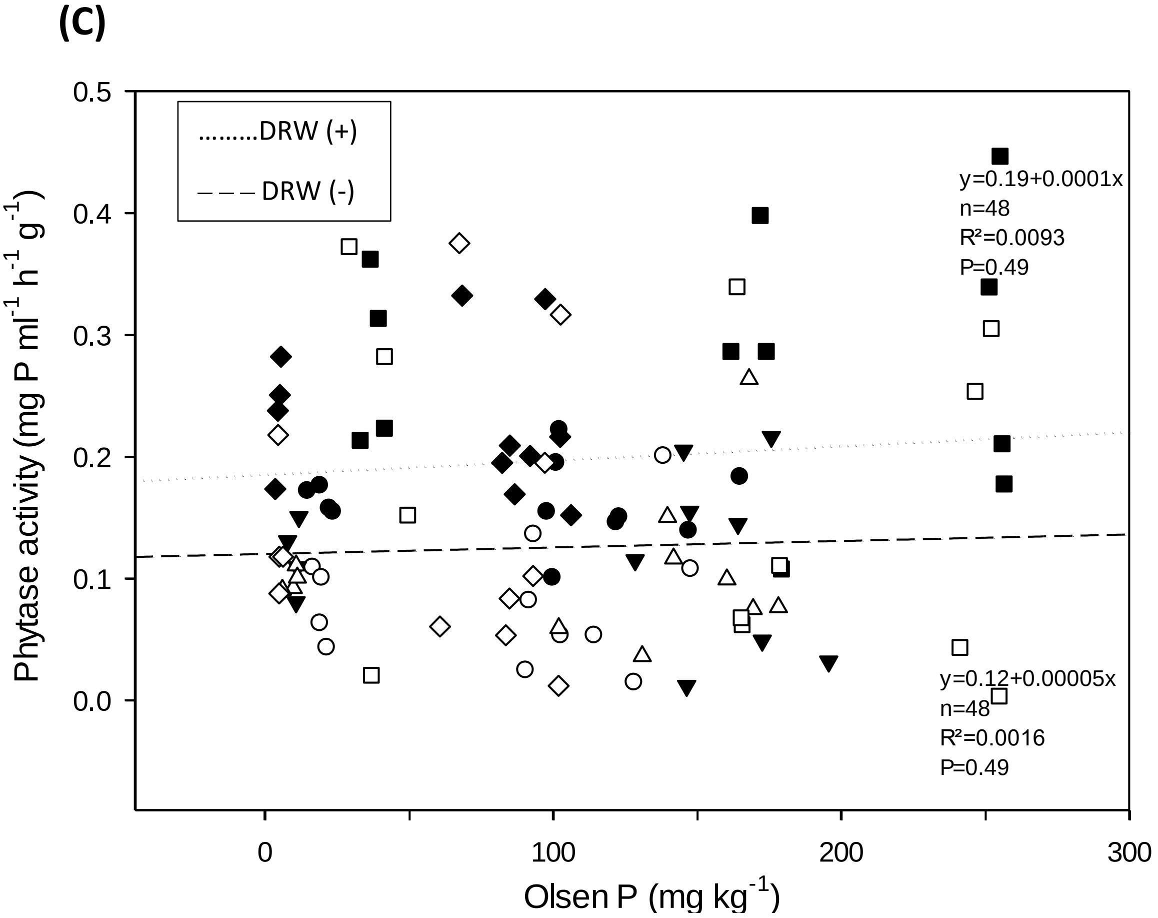
The DRW can induce enzymatic activities of the soil at variable rate, depending on the different soil types. The sandy loam textured Cambisol soils from Shandong with pH 7.3 displayed the highest level of phytase activities, followed by fine and coarse loamy textured Lithosol soils from Chongqing with pH 4.7, as compared with soil types from the other two regions (a) phytase activities of Shandong soil was ranging between 0.16 and 0.17 ml^{-1} h^{-1} g^{-1} and little higher than Chongqing soil at 0.11 and 0.12 ml^{-1} h^{-1} g^{-1}, (b) alkaline phosphatase activities in Shandong soil at 147-154 ml^{-1} h^{-1} g^{-1} and Chongqing soil at 111-126 ml^{-1} h^{-1} g^{-1} and; (c) acid phosphatase activities in Shandong soil at 94 to 115 µg PNP h^{-1} g^{-1} and Chongqing 164-200 ml^{-1} h^{-1} g^{-1} after the 4th DRW cycles. A noticeably higher increase of enzyme activities occurred within all P treatments of silt loam textured calcareous Luvic Kastanozems soils from Beijing with pH 8.9, for example, (a) alkaline phosphatase 372-383 ml^{-1} h^{-1} g^{-1} and (b) acid phosphatase activity just above 200 µg PNP h^{-1} g^{-1}, (c) phytase approximately 0.23 ml^{-1} h^{-1} g^{-1}. The increases of phytase activities were extraordinarily little as compared with the elevation of other two enzyme activities, with the largest increase in the Shandong soil. However, no significant difference of enzyme activities between P supply rate group was evident, except acid phosphatase activities.

(A)


.........DRW (+)
– – – DRW (-)
y=248.1-0.23x
n=48
R²=0.02
P=0.02
y=151.9-0.09x
n=48
R²=0.005
P=0.02
Acid phosphatase activity
(mg PNP h -1g-1)
Olsen P (mg kg-1)
500
400
300
200
100
0
0
100
200
300

(B)


.........DRW (+)
– – – DRW (-)
y=167.8+0.3x
n=48
R²=0.05
P=0.03
y=96.6+0.13x
n=48
R²=0.018
P=0.03
Acid phosphatase activity
(mg PNP h -1g-1)
Olsen P (mg kg-1)
500
400
300
200
100
0
0
100
200
300



●	Shandong	DRW (+)
○		DRW (-)
▼	Chongqing	DRW (+)
△		DRW (-)
■	Heilongjiang	DRW (+)
□		DRW (-)
◆	Beijing	DRW (+)
◇		DRW (-)

Figure 0.3 The relationship between the response of soil enzymatic activities and Olsen P concentration to the immediate drying and rewetting effect across four different soil types including control with stable soil moistening conditions.

The enzymatic activities and Olsen P concentration were measured after a cycle of four drying and rewetting events, demonstrating Olsen P versus (A) acid phosphatase activity (B) alkaline phosphatase activity and (C) phytase activity. Soil moisture treatments highlighted in two different colours: DRW (+) is black, DRW (-) is white. Each soil type is represented by distinct shape within designated box for visual identification.

Soil carboxylate excretion and pH

Generally, the changes of soil pH may be greatly associated with the organic acid exudation that was triggered by the soil moisture stress. This change in soil pH level is presented in figure 4.4.3, associated with emission of carboxylates by using trans-aconitate as an example. The level of pH in all soil types dropped slightly at the end of the experiment from an initial range between 4.7 and 8.9. A maximum decrease of 1 unit was noted in all soil samples with and without moisture treatment of the Shandong and P control Beijing soil. Apart from Heilongjiang soil, average pH was dropped in greater rate in DRW processed soil than moisture control soils, with a statistically significant decrease in DRW treated non-P added soils from Shandong. Conversely, the least pH changes of DRW soils were identified in all soil samples from Chongqing and Heilongjiang, which had the little undergoing difference below 0.3 units, the except stable moistened Heilongjiang soil without P addition.

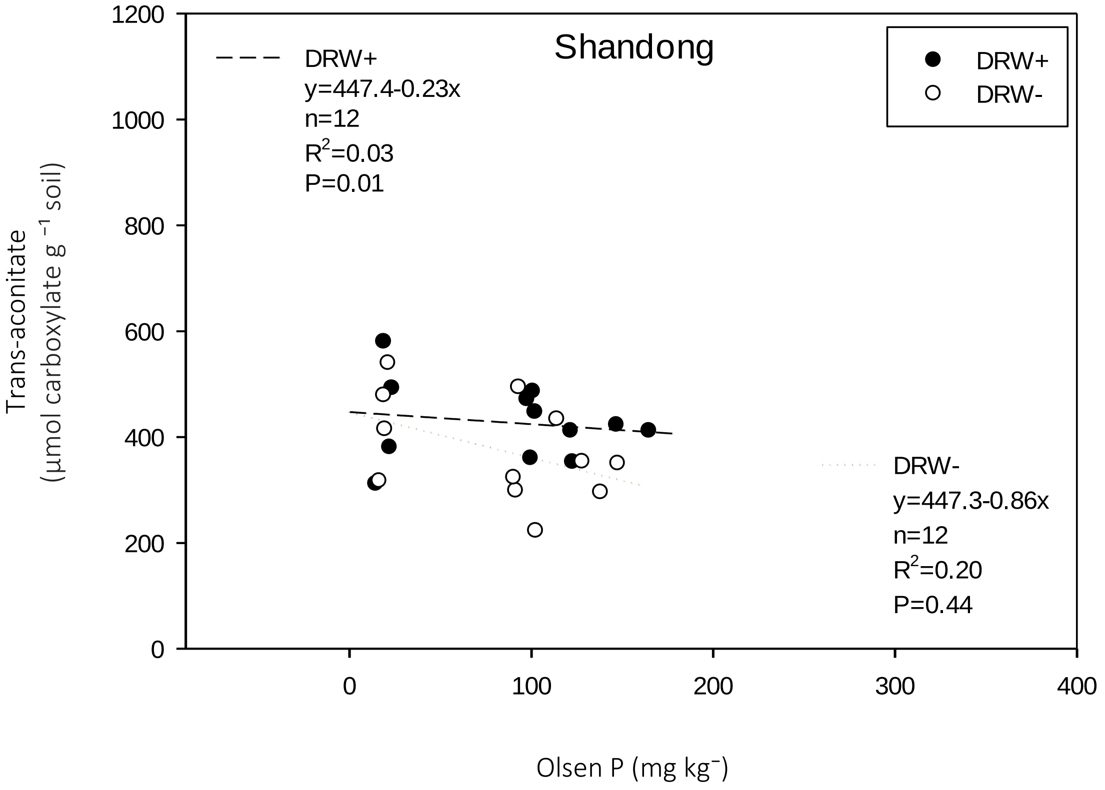
The link between soil moisture treatment and exudation of the commonly detected carboxylate can be laid in patterns for the effect of soil type characteristics and P application rates, for which the greatest amount of organic acid emission was found in the DRW (+) Shandong soils, P added DRW (+) and DRW (-) Chongqing soils, and DRW (-) Heilongjiang. The detection of organic acid exudation amongst all DRW treated Shandong soils was higher than the average level, for example, in (a) P0: 2477, 431, 862 and 441; P250: 1460; 198, 440, and 442; (c) 2879, 476, 616, and 400 µmol carboxylate g $^{-1}$ soil of malate, citrate, succinate and trans-aconitate respectively, where the occurrences of highest amount of P in the leachate water were found with statistically significant drops of pH in the DRW (+) soil as compared with DRW (-) Controlled soil. However, the level of organic acid was the lowest in all treatments of silt loam textured (pH 8.9) Luvic Kastanozems soil from Beijing with Olsen P content at 2.6 mg kg^{-1}, except the P 500 treatment of stably moistened soil. This may suggest that while the rewetting of drying soils may affect the organic acid exudation and the changes in soil pH, however, is not capable being support the idea of an increase in an amount of solubilised P.

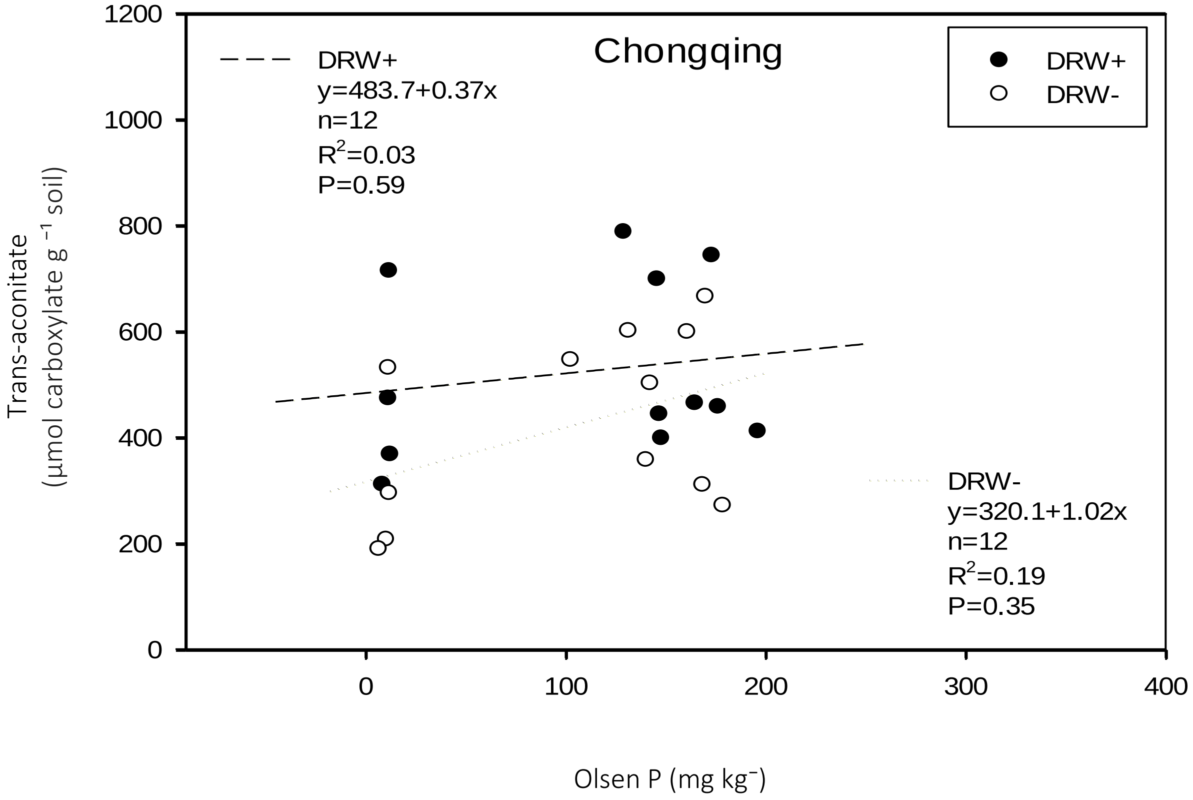
To explore potential physiological factors of the soil affected by moisture stress, I performed the stepwise regression, particularly on the trans-aconitate as a random exemplification that included an emission of organic acids over the P solubilisation of each soil type and pH changes, represented in figure 4.4.3. The P solubilisation after DRW stress varied based on the soil types and characteristics in combination with its pH ranges. The malic acid was the predominant carboxylate throughout all treatments and soil types, resulting in the considerably different quantity in the collection solution sample from 19 µmol carboxylate g $^{-1}$ in DRW (-) Controlled Beijing soil with no P addition to 2879 µmol carboxylate g $^{-1}$ in DRW (+) soils from Shandong with P500 addition. A number of other carboxylates, including tartrate and fumarate, were presented in trace quantities (with some of the recorded data not at a sufficient level to analyse).

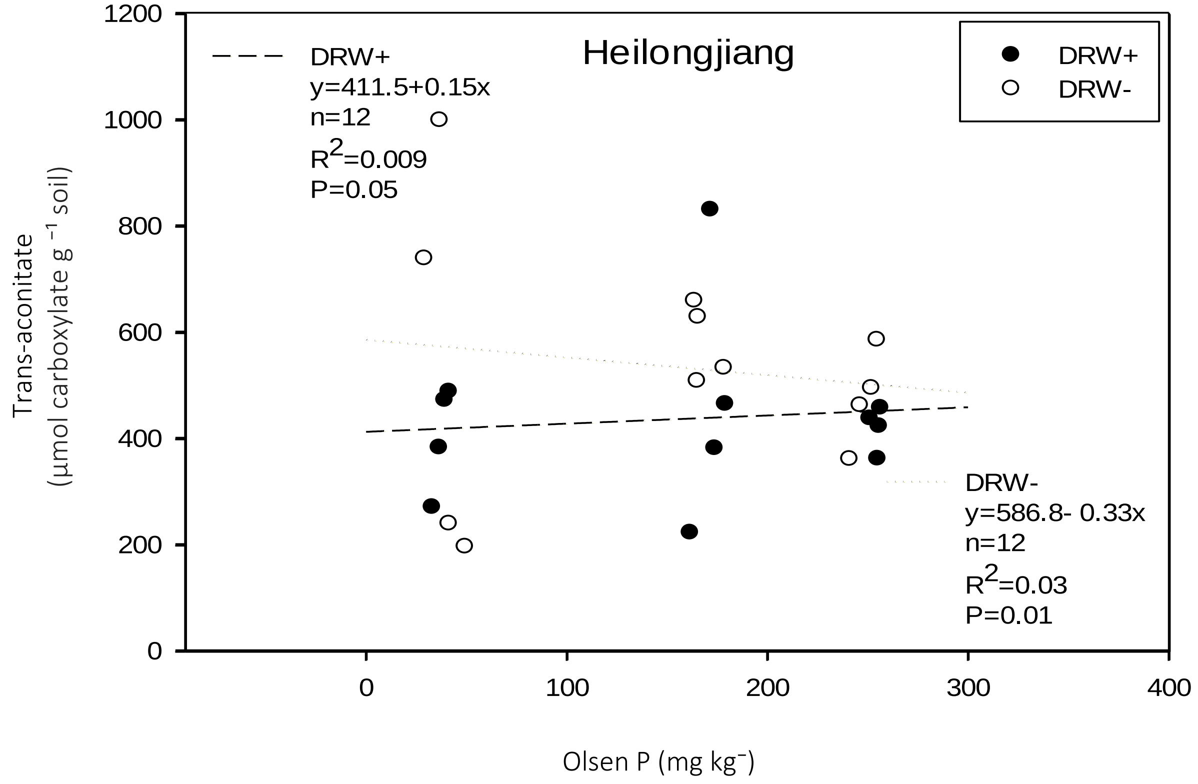
Figure 0.4 The relation between the emission of trans-aconitate and (A) Olsen P concentration and (B) pH changes of soil associated with i-DRW effect of soil including drying and rewetting DRW (+) and stable moistening DRW (-) conditions.

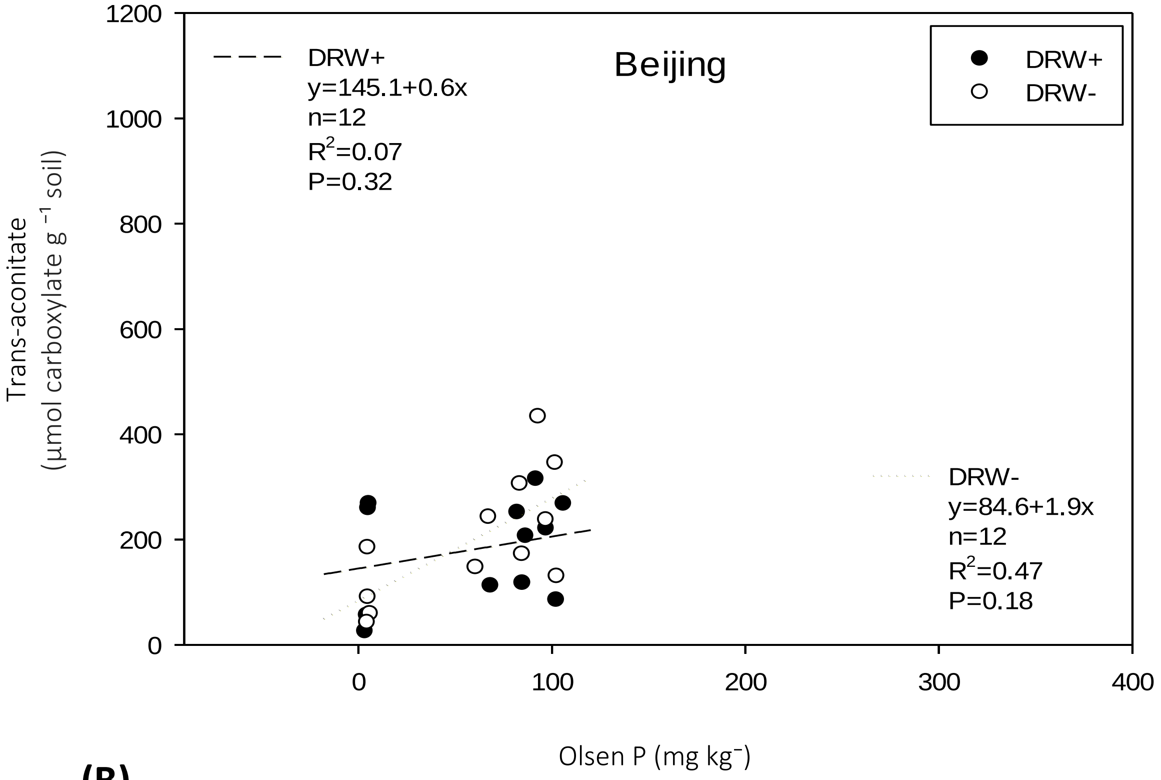
Note: Trans-aconitate was intentionally selected for the calculation below along with equation and report

(A)

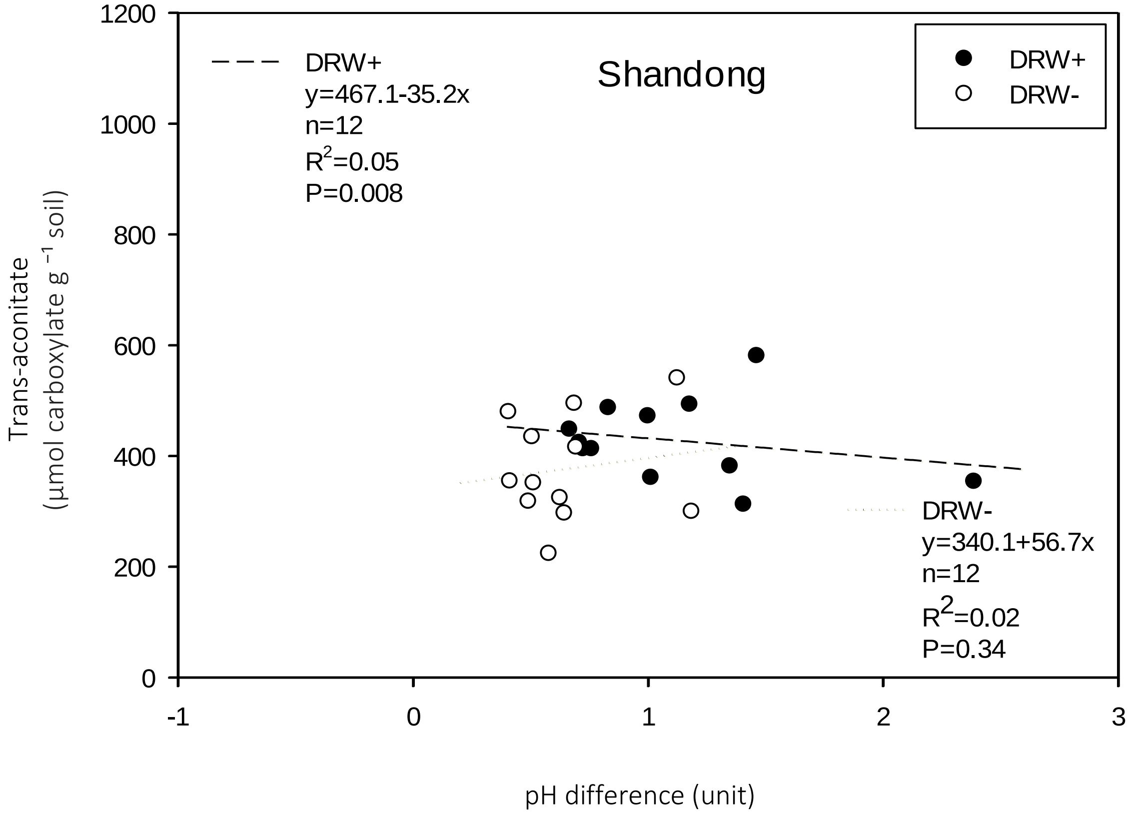


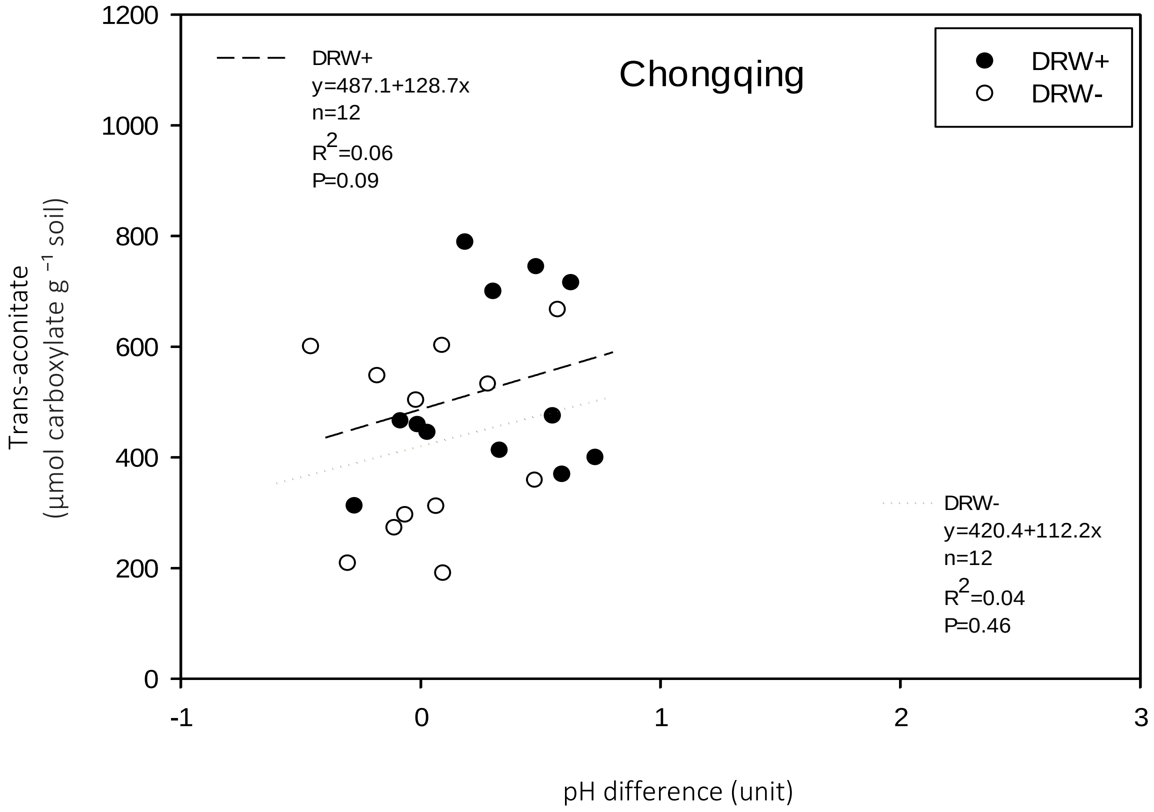

Chongqing
DRW+
y=483.7+0.37x
n=12
R2=0.03
P=0.59
● DRW+
○ DRW-
DRW-
y=320.1+1.02x
n=12
R2=0.19
P=0.35
Trans-aconitate
(µmol carboxylate g⁻¹ soil)
Olsen P (mg kg⁻)
0
200
400
600
800
1000
1200
0
100
200
300
400

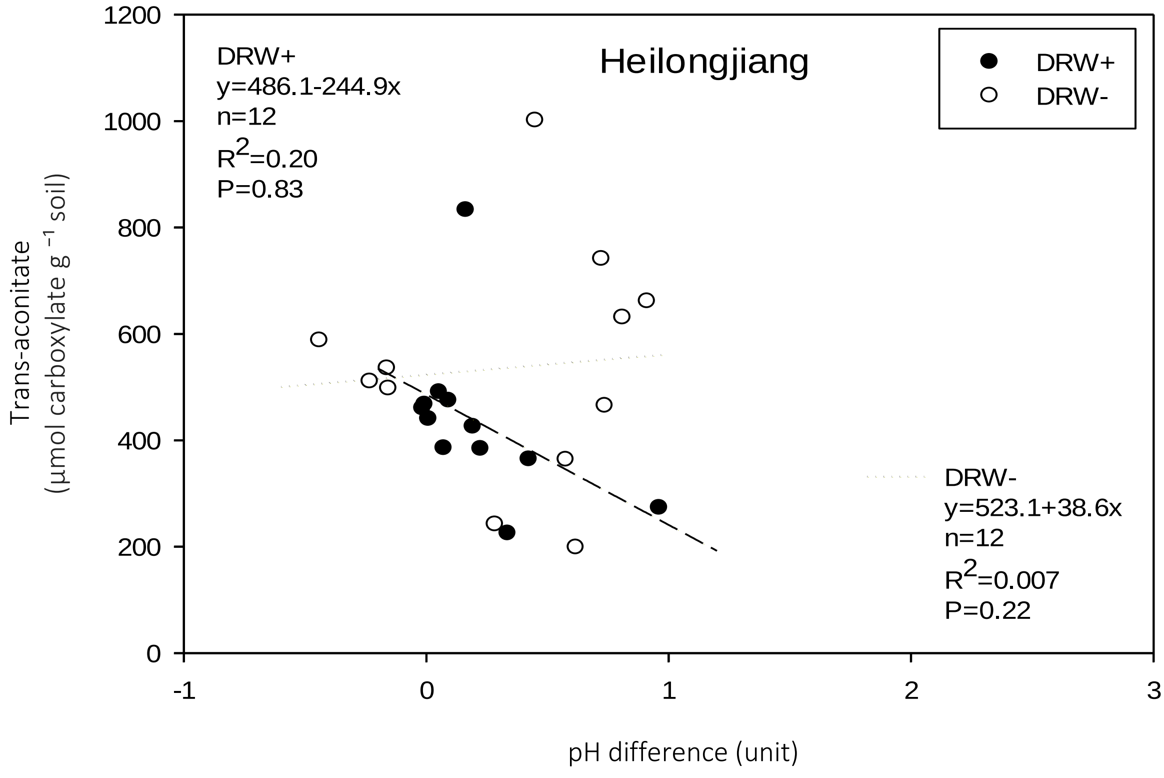

Heilongjiang
DRW+
y=411.5+0.15x
n=12
R2=0.009
P=0.05
● DRW+
○ DRW-
DRW-
y=586.8- 0.33x
n=12
R2=0.03
P=0.01
Trans-aconitate
(µmol carboxylate g⁻¹ soil)
Olsen P (mg kg⁻)
0
200
400
600
800
1000
1200
0
100
200
300
400

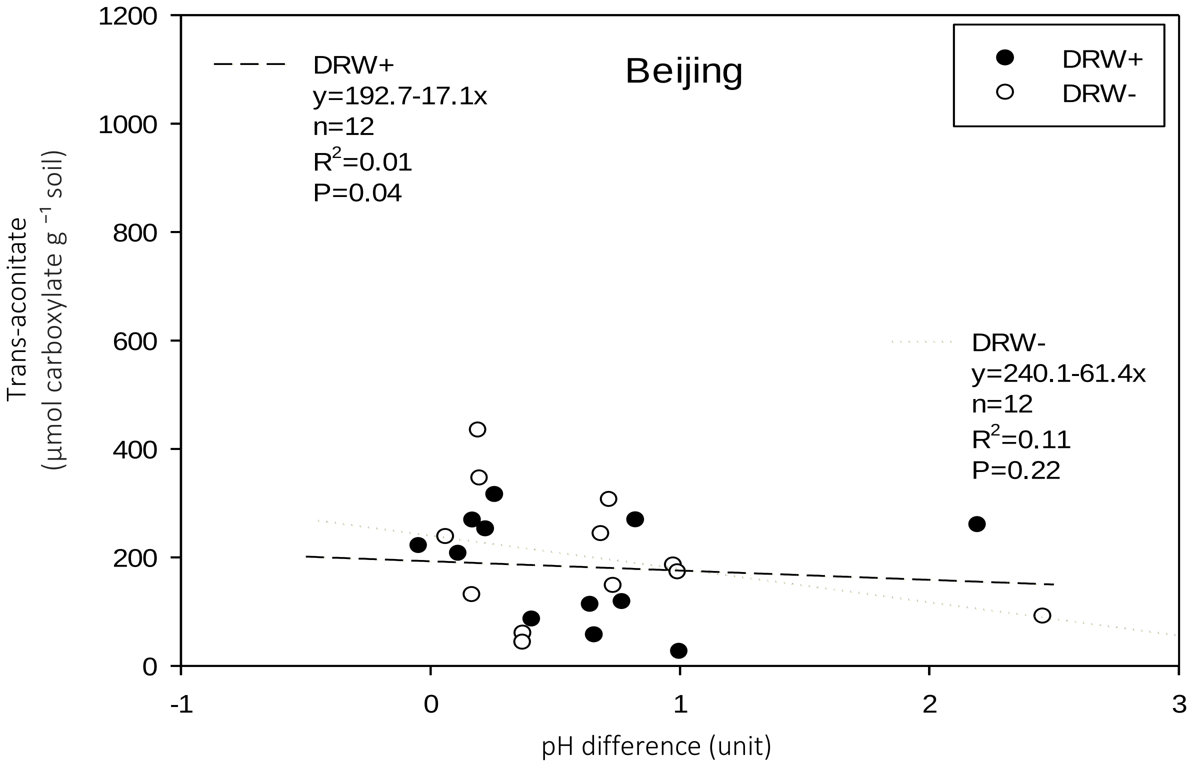

Beijing
DRW+
y=145.1+0.6x
n=12
R^2=0.07
P=0.32
DRW-
y=84.6+1.9x
n=12
R^2=0.47
P=0.18
● DRW+
○ DRW-
Trans-aconitate
(µmol carboxylate g $^{-1}$ soil)
0
200
400
600
800
1000
1200
0
100
200
300
400
Olsen P (mg kg⁻)

(B)


Shandong
DRW+
y=467.1-35.2x
n=12
R^2=0.05
P=0.008
DRW-
y=340.1+56.7x
n=12
R^2=0.02
P=0.34
● DRW+
○ DRW-
Trans-aconitate
(µmol carboxylate g $^{-1}$ soil)
0
200
400
600
800
1000
1200
-1
0
1
2
3
pH difference (unit)


Chongqing
DRW+
y=487.1+128.7x
n=12
R2=0.06
P=0.09
DRW-
y=420.4+112.2x
n=12
R2=0.04
P=0.46
● DRW+
○ DRW-
Trans-aconitate
(µmol carboxylate g −1 soil)
pH difference (unit)
0
200
400
600
800
1000
1200
-1
0
1
2
3


Heilongjiang
DRW+
y=486.1-244.9x
n=12
R2=0.20
P=0.83
DRW-
y=523.1+38.6x
n=12
R2=0.007
P=0.22
● DRW+
○ DRW-
Trans-aconitate
(µmol carboxylate g −1 soil)
pH difference (unit)
0
200
400
600
800
1000
1200
-1
0
1
2
3



This linear regression between the responses of physiological processes to the soil moisture fluctuation and P solubilisation in the soil of four diverse types having bio-physiochemical composition under variable rates of P supply by plotting an emission of trans-aconitic organic acid against the Olsen P concentration (A) and soil pH changes (B) on the basis of *i*-DRW soil drying and rewetting -DRW (+) and stable moistening -DRW (-) conditions.

4.4.3 The characteristics of the soil rewetting and drying performance

The characteristics of drying across different soils were indeed vary, including the duration and drying features of the soil. The duration of soil drying periods to reach 30% of field capacity in each soil differed based on the number of event cycles, ranging between (a) 14 and 19 days in the Shandong soils; (b) 17 and 22 days in the Chongqing soils; (c) 19 and 24 in the Heilongjiang soils, and (d) 17 and 20 days in the Beijing soils in respect of the texture including sandy loam, fine coarse loamy, clay loam and silt loam. To reach the same level of soil moisture at 30 % of field capacity, the length of drying periods resulted in prolonged drying periods from three to five days when the greenhouse temperature ranged from 17 and 28°C during the experiment period between July and September.

The highest temperature was recorded during the experiment occurred in July and August, specifically at 24 and 28°C, leading to change the moisture levels rapidly through an increased evaporation rate. On the other hand, these soils experienced a slower rate of moisture loss towards to end of the repeated cycles, caused by a temperature drop in the greenhouse. This may imply the potentiality of the soils that exhibited reduced water evaporation or transportation rates. The greenhouse without temperature control can create the environment that is more susceptible to natural temperature fluctuations as the experiment progressed, for example, ambient temperature throughout the day and night, as well as seasonal variation. However, the precise temperature of greenhouse was not accurately recorded on a daily basis. This may pose challenges in interpreting experimental results and limit our ability to understand the effects of temperature on the soil responses.

Whilst length of drying period varied (as can be seen from the Figure 4.4.5), drying was not always uniform throughout the soil types, such as patchy drying (Chongqing), sandy drying (Shandong and Beijing) and compact drying (Heilongjiang). These variations were based on the soil types with different physico-chemical contents. For example, the Chongqing and Heilongjiang soils contain more significant proportion of C and N than the Shandong and Beijing soils (Table 4.4.2). Carbon to nitrogen ratio was calculated as 9, 06, 8.98, 9.79, and 7.49 for Shandong, Chongqing, Heilongjiang, and Beijing, respectively, with pH determined for the Shandong, Chongqing, Heilongjiang, and Beijing as 7.3, 4.7, 5.1, and 8.9. With regard to hydrological parameters of soil, the pattern follows the same rewetting layout, with the greatest water holding capacity of soil at 41 and 39 % and the lowest percolation rate at 3.2 and 3.5 cm h^{-1} in the Heilongjiang and Chongqing soils, respectively.

4.4.4 Leachate water analysis

The P solubilisation may be influenced greatly by the *i*-DRW processes of the soil resulting in the leaching with a broad variety of concentrations and forms of P (mg L^{-1}) in the drainage water over the course of DRW cycles. The findings of P leaching from each soil type were included in the part of this study by performing through an exploratory analysis of variable by univariate statistics and distribution plots under three different P supplies. The ability of all forms of P to drain away with water penetrating throughout the soil length associated with the condition of rewetting varied at a certain degree among the different soil types under variable lengths of drying time, shown in the Figure 4.4.4.

4.4.4.1 Phosphorus analysis in the leachate water

The physio-chemical fractionation of P in the leachate from each soil type under the subsequent rewetting is also illustrated in Figure 4.4.5. As can be seen from the detailed assessment of P species in the (Fig 4.4.5), the level of P loss in the each soil type, depends on rate of P loading into the soil, for example, the greatest rate of P leaching was observed in the high P input at 500 mg kg^{-1} and it was followed by 250 mg kg^{-1}, with the lowest level of P in the leachate observed for the non-added control soil.

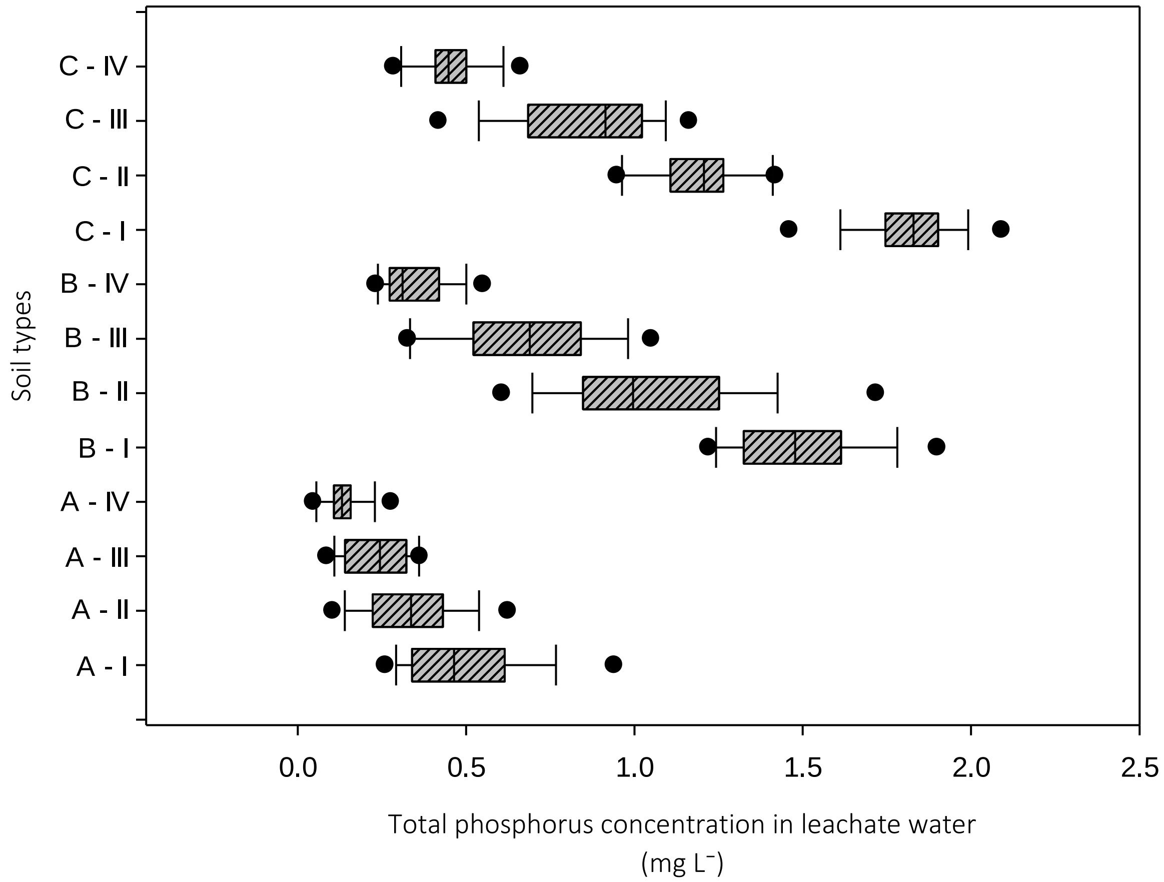


Figure 0.5 Summary of total phosphorus losses in the leachate water derived from four different type of soils, categorised based on phosphorus application rate, in relation to the *i*-DRW effects over a four-cycle of events.

Distribution of P in the drainage water can be compared using four different types of soil, which are denoted using Roman numerals: (IV) Shandong, (III) Chongqing, (II) Heilongjiang and (I) Beijing, on the basis of three different phosphorus supply rates (A) non-P added soil, and P added soil at the rate of (B) 250 mg kg^{-} and (C) 500 mg kg^{-1}.

A consistent observation across of all P species derived from all filtered and unfiltered leachate water samples included a downward trend as frequency of cycle increases, shifting from first to fourth rewetting events. The concentrations of all forms of identified P species in leachate water appeared to decline in the steady state across the four drying and rewetting cycles, with the exception of all forms of P in the Shandong leachate as can be seen from fig. 4.4.6. The detectable P in the leachate from the Shandong soil had no apparent decrease during the experiment, with the significantly highest amount observed throughout the experiment, with 60.6 mg L^{-1} in total. The leaching loss of total P from Shandong soil constitutes a greater proportion of unreactive P than the other three soil sources, which can be associated with its soil hydrological characteristics and soil properties. Sandy textured Beijing soils allowed more leaching losses of dissolved P than others do through their pore spaces by gravitational water movement mechanisms, even the detected TP in the leachate water was the lowest.

The potentiality of all forms of P leaching derived from all types of soil can, of course, be a soil type specific process that may be associated with co-leaching of P complexation compounds that compete with sorption sites as well as level of P availability of soil. The lowest level of TP in the leachate was identified in the leachate collection from Beijing soil, due to the low P availability. Occurrence of significantly higher unreactive P fractions in the leachate samples, for example, accounted as dissolved forms (<0.45 µm) with ranges: 54-60% in the Shandong soil, 27-46% in the Chongqing soil, 35-43% in the Heilongjiang soil and 38-54% in the Beijing soil. On the contrary, the amount of RP in the filtrate water constantly fluctuated under the range between 0.02-1.7, 0.03-0.2, 0.02-0.09, and 0.02-0.1 mg L^{-1} in the Shandong, Chongqing, Heilongjiang, and Beijing leachate, respectively without any marked change throughout all four DRW cycles. The detailed changes of physio-chemically separated P level in the leachate under the four cycles of DRW by each soil type are as follows:

(1) The sandy loam textured brown Cambisol soils with pH 7.3 from Shandong:

The biggest proportion of leached P was measured as unreactive dissolved P species (<0.45 µm), which was consistently high throughout the experimental period, for example, measuring in each treatment between 0.1-0.27 mg L^{-1} in the P0; 0.8-0.9 mg L^{-1} in the P250 and 0.9-1.1 in P500 mg L^{-1}, which doubled the total amounts of particulate unreactive P except in P0 treatment. The proportion of total unreactive dissolved P form in the leachate were accounted 54%, 59%, 57% and 57% in first (I), second (II), third (III) and fourth (IV) DRW cycle, respectively.

(2) The fine and coarse loamy textured red Lithosol soils with pH 4.7 from Chongqing:

The leaching losses of P from the Chongqing soils related with all DRW events were largely consisted of the particulate unreactive P species, except in the first DRW event. Total amounts of unreactive particulate P in the leachate water under the three different P treatments ranged between 0.09-0.22 mg L^{-1} in the P0, 0.5-0.6 mg L^{-1} in the P250 and 0.6-0.7 in P500 mg L^{-1}, as general trend has declined during a cycle of four DRW event. The proportion of total unreactive particulate P form in the leachate were measured to be 45%, 52%, 48% and 44% (in first (I), second (II), third (III) and fourth (IV) DRW cycles, respectively.

(3) The clay loam textured black Haplic Phaeozems soils with pH 5.1 from Heilongjiang:

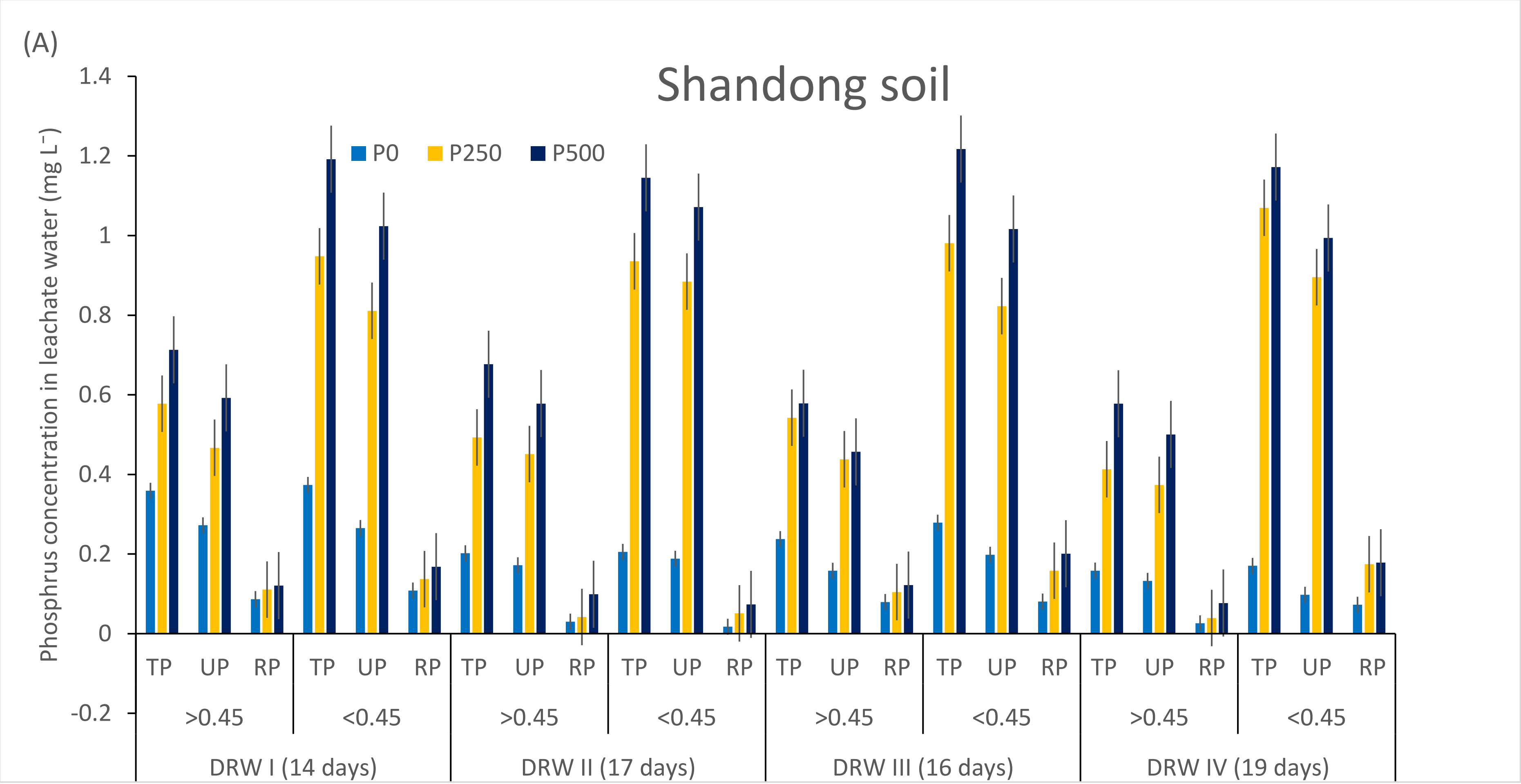
Apart from the second cycle, all forms of dissolved and particulate unreactive P species in the leachate water were almost at the same level, with a general downward trend in over the course of four DRW events. The level of total P in the leachate was gradually decreased throughout the experiment periods, for example, ranging under variable P supply 0.12-0.3 mg L^{-1} in the P0, 0.42-0.86 mg L^{-1} in the P250 and 0.6-1 mg L^{-1} in P500 treatments. As a result of this gradual decline, the proportion of total unreactive particulate P form in the leachate has dramatically changed at 56%, 63%, 73% and 74% in the first (I), second (II), third (III) and fourth (IV) DRW cycles, respectively.

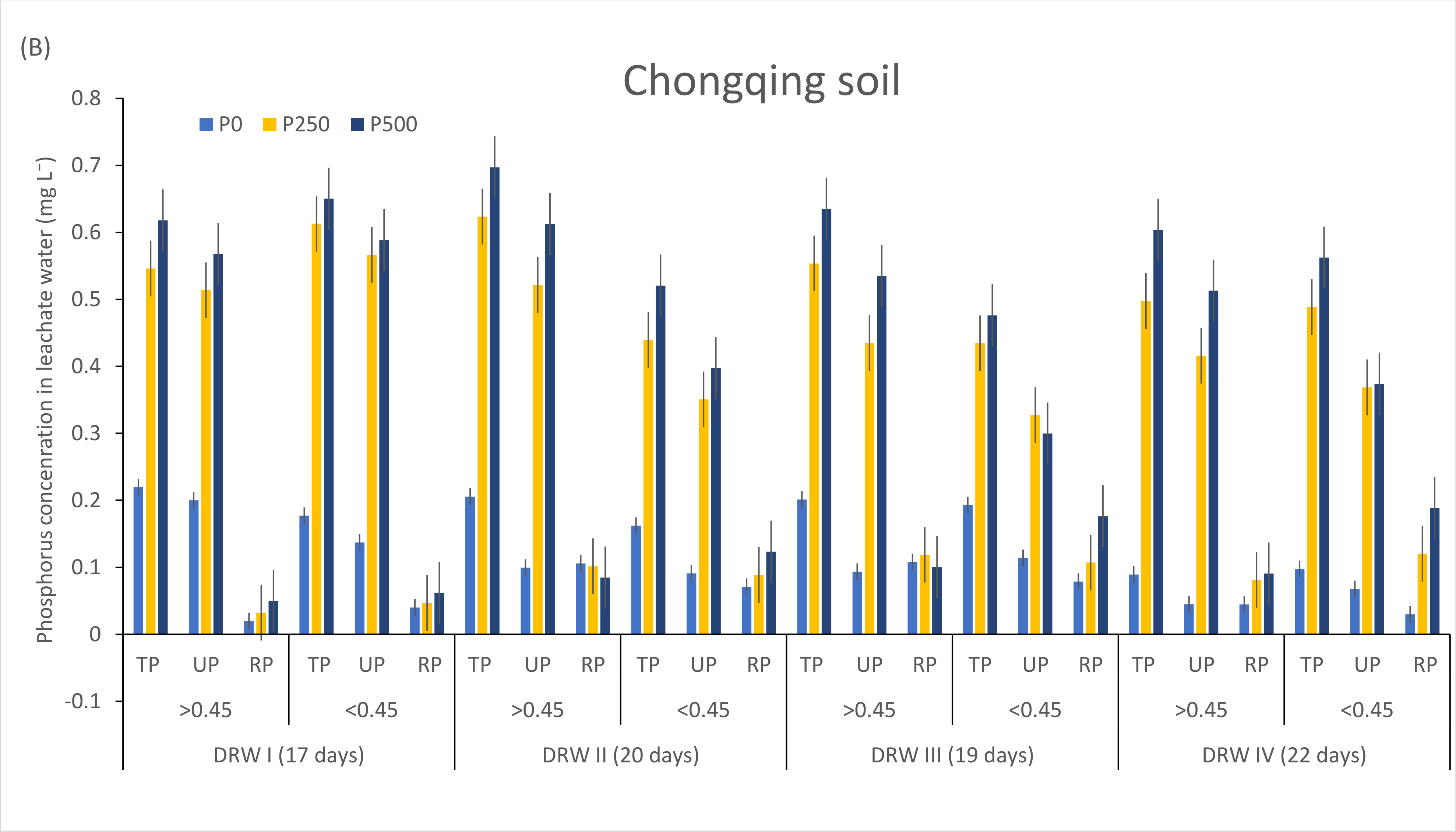
(4) The silt loam textured calcareous Luvic Kastanozems soils with pH 8.9 from Beijing:

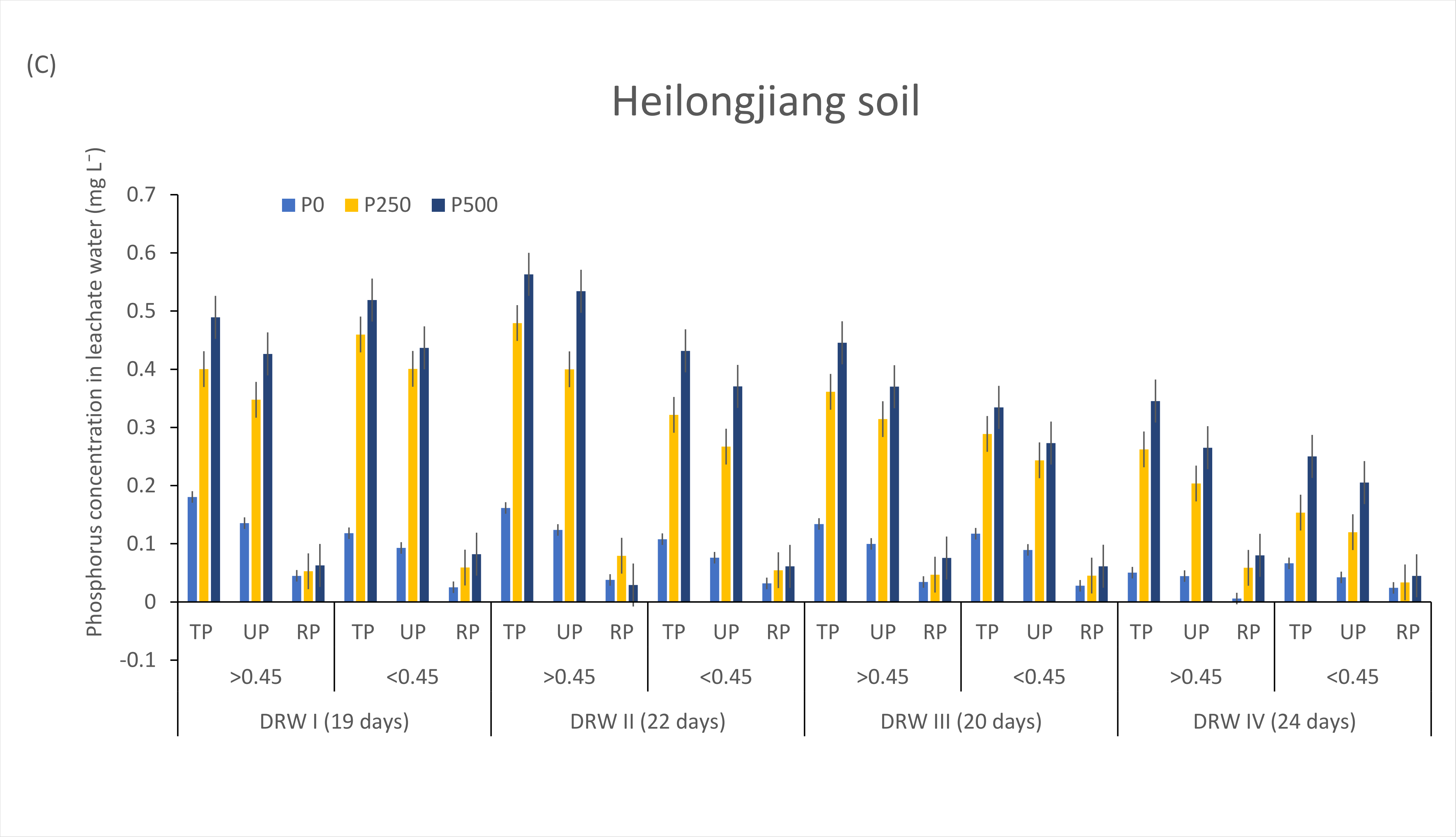
The majority of the P species in the leachate consisted of unreactive dissolved P forms with significant fluctuations over the series of DRW stress. As compared with particulate forms, a considerably higher concentration of dissolved P was detected in the filtrate water, for example, total dissolved unreactive P contents were at around 0.06-0.09 mg L^{-1} in the P0 control; 0.19-0.25 mg L^{-1} in the P250 and 0.24-0.3 mg L^{-1} in P500 treatments, which is far higher than the particulate P in the leachate at around

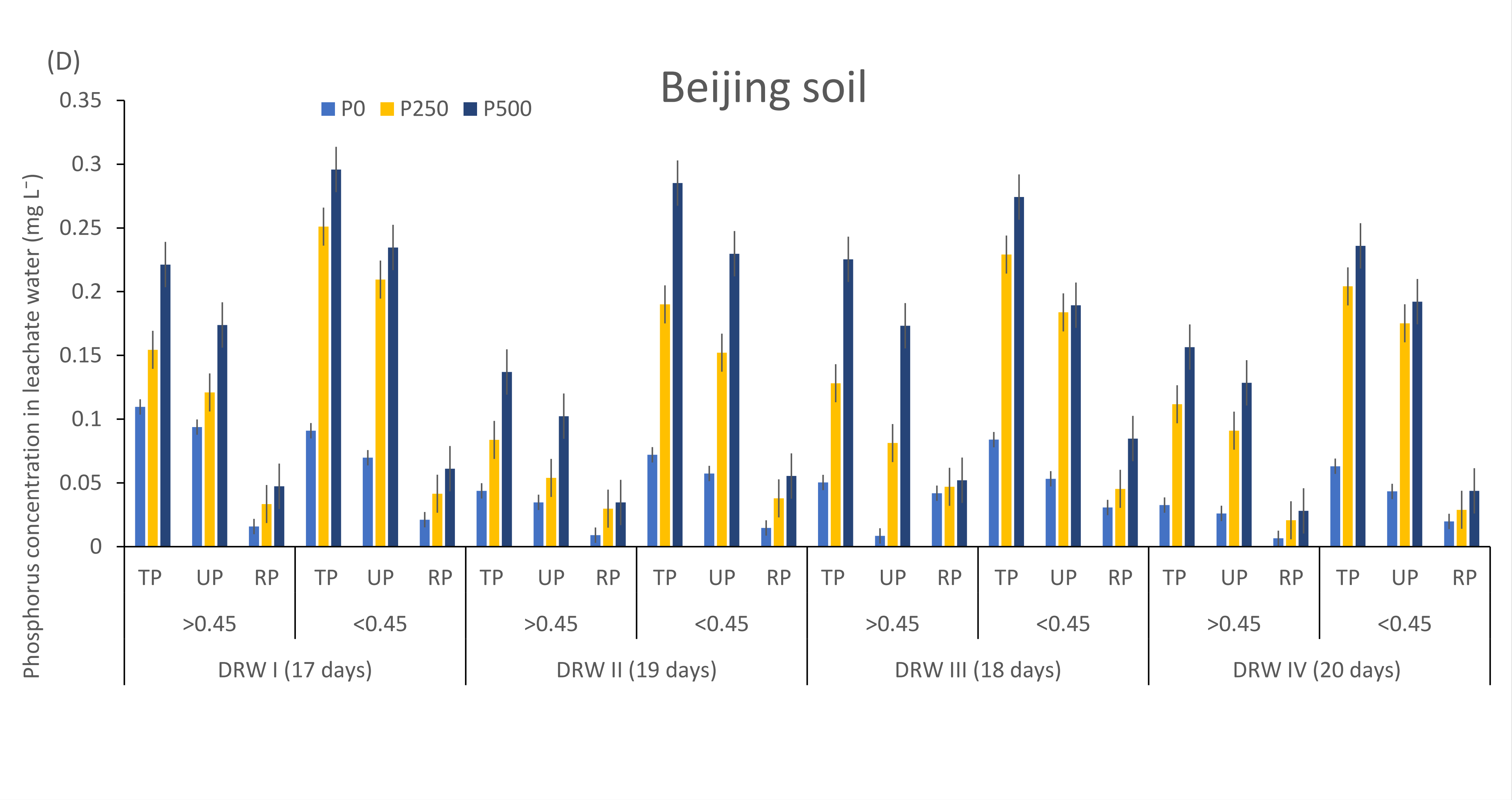
0.03-0.1 mg L^{-1} in the P0; 0.08-0.15 mg L^{-1} in the P250 and 0.14-0.23 in P500 treatments mg L^{-1}. The proportion of total unreactive dissolved P form in the leachate measured 45%, 54%, 37% and 49% in the first (I), second (II), third (III) and fourth (IV) DRW cycles, respectively.

Figure 0.6 The concentration of P species (mg L⁻) in leachate water of four contrasting types of soils subsequent to cycle of rewetting events for each replicate with the incubation of drying periods from (a) sandy loam textured Shandong soils with pH 7.3, (b) fine and coarse loamy textured Chongqing soils with pH 4.7, (c) clay loam textured Heilongjiang soils with pH 5.1, and (d) silt loam textured soils with pH 8.9 from Beijing.




(B)
Chongqing soil
P0
P250
P500
Phosphorus concenration in leachate water (mg L⁻)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
TP
UP
RP
>0.45
<0.45
DRW I (17 days)
DRW II (20 days)
DRW III (19 days)
DRW IV (22 days)


(C)
Heilongjiang soil
Phosphorus concentration in leachate water (mg L⁻)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
P0
P250
P500
TP
UP
RP
>0.45
<0.45
DRW I (19 days)
DRW II (22 days)
DRW III (20 days)
DRW IV (24 days)


(D)
Beijing soil
P0
P250
P500
Phosphorus concentration in leachate water (mg L⁻)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
TP
UP
RP
>0.45
<0.45
DRW I (17 days)
DRW II (19 days)
DRW III (18 days)
DRW IV (20 days)

4.5 Discussion

Data collected from the *i*-DRW experiment did not support hypothesis one that were initially proposed (DRW increases the P solubilisation and thus the potential for leaching). The Olsen-P generated in the soil solution associated with *i*-DRW appears to contradict the initial assumptions made in this chapter. The result may suggest, the *i*-DRW may not mobilise more P than in onset drying and rewetting conditions at greenhouse temperatures. However, it is important to consider that this observation is specifically employed under the characteristics of the soil, variations in soil types, the experimental setup, temperature regimes and other relevant parameters. For example, the non-statistically significant Olsen P level observed may be associated with the methodology of this experiment. The soils were sampled for the analysis, just after the fourth DRW cycle, without disturbing too much, taken from a 3cm from surface. This limited soil sampling approach, which was sampled only a fraction of the soil from a shallow depth, affected the results by not capturing the accumulated P in the bottom layers of the soil, after the leaching of soluble P in the surface. Soil sampling from the top provides a limited perspective of the overall picture of P dynamics in the soil from top to bottom layers. This was possible to avoid by sampling down to the core, where leached P may accumulate in the soil. Sampling down to the core without disturbing soil hydrology allows to assess P content distributed in different soil layer.

Most findings can still offer valuable insights into the broader implications of repeated *i*-DRW events, particularly in relation to the biological makeup of the soils.The labile organic P can be solubilised through the exudation in the rhizosphere and other compounds in responses to DRW, which influence the availability and cycling of organic P in the rhizosphere. Exudation process involves the production, secretion, and reactivation at varying degrees. It indicates, the soil responses associated with (a) non-microbial (Blackwell et al., 2009; Nannipieri et al., 1996) and (b) the microbial contribution (Blackwell et al., 2010; Bünemann et al., 2013; Gordon et al., 2008) may play a role in the observed soil responses. The significant changes of exudation in the rhizosphere derived under DRW stimuli align with previous studies such as Manzoni et al. (2014, 2012), specifically the activities of dephosphorylating enzymes as mentioned in the references by Chepkwony et al. (2001) and Navarro-Garcia et al. (2012). The possible explanations of the increased extracellular enzymes during the soil moisture fluctuation include either the releases of intracellular solutes of the lysed cells due to the cell wall disruption or excretion of the living organism (Meisner et al., 2018). This release may be a mechanism to avoid excessive turgor pressure (Halverson et al., 2000, Fierer et al., 2003). However, the biological responses of the soil to the *i*-DRW depend on the nature of the DRW process including the rate of soil rewetting (Turner and Haygarth, 2001, Blackwell et al., 2009, Blackwell et al., 2009, 2013), intensity of drying, DRW cycle number (Bünemann et al., 2013; Sardans and Peñuelas, 2007; Sun et al., 2017; Zhao et al., 2010) and drying duration (Forber et al., 2017).

Despite these changes observed in the rhizosphere, the first hypothesis of this chapter remained rejected, due to the similarity of Olsen P concentration between the soil groups with and without moisture fluctuation treatments. Even though the statistically significant increase of water-soluble P concentration was observed following the episodes of soil DRW events particularly in the Shandong and Chongqing soils, the hypothesis 1 is still rejected because of the Olsen P level in the soil used as a key indicator of soil P solubilisation performance. Unlike other studies, one limitation may be noted due to the soil preparation procedure for the laboratory analysis, as a continuous decline of soluble P is highly likely to occur under the prolonged drying time for the preparation of soil P analysis. However, the incubation period of soil air-drying is required to take several days prior to the P analysis, which may introduce an opportunity for possible interactions evading clear predicable responses of the soil, as a rapid increase of P bioavailability can only persist for 1 day in the field after the subsequent rewetting of dried soil (Nguyen and Marschner, 2005). Thereby, the observation associated with the recurrent sudden moisture fluctuation of this study could be questioned to fully explain the nature of the P solubilisation processes under effects of *i*-DRW fully.

The possible link between *i*-DRW effects and dephosphorylating enzyme activities formed over a cycle of four moisture fluctuation events may help to answer some important questions. Indeed, the *i*-DRW induced P solubilisation performance has been predicted to be stronger in soils with greater carbon (C) content than low C content, which can explain for example, the dramatic increase of acid phosphatase activities in the Heilongjiang soil, which is consistent with other studies (Navarro-García *et al*. 2012; Meisner *et al*. 2015). Heilongjiang soil has the highest C content than three other soils. The largest part of soil P sources (Brookes et al., 1984) exists as a microbial biomass (MBP). This is an exceedingly small fraction of the soil organic matter, around 1–3% (Blackwell et al, 2010). The immediate moisture change conditions of *i*-DRW with intensive soil drying having higher temperatures for longer duration, resulted in significant reductions in microbial biomass (Brödlin et al., 2019; Chen et al., 2016; Kaiser et al., 2015) coupled with occurrence of the highest rates of microbial responses, up to 70% in the soil (Blackwell et al., 2010). In this experiment, however, the microbial biomass was not measured, therefore no direct evidence is available to clarify the impact on soil P solubilisation related to soil microbial origin.

This study provides insight into the immediate responses of the soil biological component to *i*-DRW effects associated with sudden soil moisture disturbance and data to support the fifth hypothesis that dephosphorylating enzymes and organic acids affect the P solubilisation in the rhizosphere after DRW. As mentioned previously, the soil biological responses are largely contributed by soil microbes. However, the soil P solubilisation processes may be benefiting from additional enzyme activities attributed by soil enzyme fractions under extreme weather condition (a) immobilised form attached with soil colloids of clay and humus that can be released in longer term period under *d*-DRW effects and (b) free form in the soil solution (Dick and Burns, 2011; Nannipieri et al., 1996; Shukla and Varma, 2010) that can be rapidly released in the shorter term period under the effect of *i*-DRW. The immobilised form exists as biochemically active for a prolonged period and less susceptible to denaturation (Burns, 1982) as compared with free enzymes, due to the protective organic matter cover that can be stabilised in soil matrix (Schnecker et al., 2014; Zornoza et al., 2006). Therefore, an increased concentration of dephosphorylating enzyme brings into question whether the soil enzyme fraction is an integrated part of this complex P solubilisation system of *i*-DRW effects in addition to the soil bacterial community involvement. The assessment of which was beyond the scope of this study. This would make insightful future work, which may also raise the question, is an increase in soil enzyme fraction, or are the microbial extracellular enzyme activities.

It has been suggested that the higher the amount of P was applied into the soil, the higher the level of solubilised P measured, demonstrating by the Olsen-P, with 6 and 38-fold increases at rates of P application in 500 mg kg^{-1} as compared with control soil without any supply. The outcome of these results demonstrated, the quantities of both water-soluble and Olsen P in the soil of all treatments varied significantly among the different rates of P fertiliser application with a noticeable difference between non-P added and P added soil samples. It is highly likely that the more P supply applied to the soils, the greater the effects exerted on the P solubilisation and potential leaching (Haygarth et al., 1998; Hedley et al., 2002; Sims, 1993; Tunney et al., 1997). Of most concern, the level of total P leaching in the drainage water ranged from 0.1 to 1.9 mg kg^{-} in the P application at the rates of 250 and 500 mg kg^{-} respectively, where the greatest amount of solubilised P appeared in the soil (Heckrath et al., 1995).

Further, the ability of P to move down throughout the soil length along with draining water varied from one type of soil to the other, which is reversely correlated with water holding capacity. The volume of drained water was almost the same size in all soil types except the Heilongjiang and Shandong soils where small and large volumes of leachate water were released, respectively. Total P lost in the leachate water following a cycle of DRW event may differ for the soil types with natural undisturbed structure (Gerhard et al., 2021). However, the measurement of water volume drained from each soil sample has not been included in this experiment. Therefore, it is arguable that more in-depth investigation of the leachate, particularly total mass P lost was excluded for the analysis of this thesis.

The experiment described the level of total P in the drainage water varied widely across the different soil types and cycling periods. The Shandong soil exhibited higher levels of total P (0.3-1.9 mg L^{-1}) in the leachate, while the Beijing soil showed lower levels of total P (0.1-0.5 mg L^{-1}) throughout the four cycling periods. The highest amount of P penetrated throughout the soil presented in the leachate was observed in the Shandong soils with P application at P250 and P500, and the Chongqing soils with P500. This observation may suggest that the application of higher amounts of P fertiliser resulted in increased P movement through the soil. However, the observed variations in total P levels in the leachate after rewetting can be attributed to the soluble P concentration in the soil solution and physio-chemical properties of the soil, which affect the retention and movement of P in the soil-water system on the basis of the interactions between P and soil particles (McDowell and Condron, 2004; Simmonds, 2016).

As can be seen from figure 4.4.1, the largest increase in Olsen P in the soil was observed between control (P0) and the addition of P at 250 mg kg^{-1}. However, the increase in Olsen P was not dramatic between P250 and P500, when P was added at the dosage of 500 mg kg^{-1}. This observation may be related to the leaching of P, which could be explained by the mass balance of total P in the system and extent of leaching. In this case, the mass balance could not be calculated, due to the lack of measurement of leachate volume, associated with the spillage of liquid from an unsealed container. Some of containers were too little to collect leachate over 40ml. Without accurate measurement of leachate volume, it is difficult to determine the absolute quantity of P in the leachate and complete P balance.

Different soil types have varying characteristics, which affect the movement of P throughout the soil profile at various degrees under the rewetting condition, depending on texture, sorption capacity and pore structure. For example, calcareous sandy soil with low sorption capacity carries a considerable risk of losing the large amounts dissolved P compared to other soil types. This is primarily due to the gravitational water movements, through their large pore spaces, especially under high rates of P supply (Jalali and Jalali, 2016; Schoumans and Groenendijk, 2000; Van der Zee and Van Riemsdijk, 1986). The considerable amount of P transfer has also been recorded in the clayey soil via formation of macropores(cracks) (Yan et al., 2021) under the DRW and freezing and thawing, due to the capabilities of high clay content to shrink and swell (Farewell et al., 2012). Fine-textured soils, which are typically characterised by high clay content are generally not associated with a high-risk of P leaching when soluble P levels in the soil are moderate. This is attributed to the high P sorption capacity of fine-textured soils (Svanback et al., 2014). However, this is clearly an exceptional finding of this experiment by the results of the Chongqing soil with the second highest P leaching (0.2 to 1.3 mg L^{-1}), despite being a fine textured soil, which deviates from general trend observed regarding P leaching.

While focusing on rewetting of soil, the drying incubation can also have significant implications on P solubilisation (potential for leaching). By setting a target WHC and subjecting the soil to severe drying conditions, this allows us to understand the fundamental changes of soil responding to decreased soil moisture levels. Studying the processes of P solubilisation can provide insights into natural soil drying that is increasingly relevant in the context of climate change, especially considering the projected increase in drought condition (Pearce et al., 2007), as it is expected more frequent and intense many regions. However, naturally occurring soil drying under future climate scenarios has not been specifically replicated at a peak WHC of 30%. This extreme water deficiency of soil was not informed by how climate change may affect weather patterns. As there is limited number of existed literatures, conducting a reductionist experiment can conceptually stimulate an interesting result and further discussion on the processes involved in P solubilisation (Turner and Haygarth, 2001, Whalley et al., 2013).

To reach this threshold of drying, the length of drying period varied in each of the soils under the same drying conditions. Different soil types have varying matrix potentials, which exhibit different rates of drying and duration periods under each number of events (please see figure 4.4.1). For example, the timeline of drying periods observed in specific soil types ranged over the four cycle of DRW events were as follows:

(a) Shandong soils: (I)-14; (II)-17; DRW (III)-16 and (IV)-19 days;

(b) Chongqing soils: (I)-17; (II)-20; DRW (III)-19 and (IV)-22 days;

(c) Heilongjiang soils: (I)-19; (II)-22; DRW (III)-20 and (IV)-24 and

(d) Beijing soils: (I)-17; (II)-19; DRW (III)-18 and (IV)-20 days.

The length of drying periods increased from three to five days when the greenhouse temperature ranged between 17 and 28^{0} C throughout the experimental period, from July to September. This suggests that temperature can play a role in the drying process, with higher temperatures (up to 28^{0}C recorded in July and August) leading to faster drying. However, accurate recording of greenhouse temperature was not done in the experiment, which limits our understanding of the precise relationship between temperature fluctuations in the environment and the drying duration of the soil.

The variations in drying periods indicate that each soil type has its own characteristic drying behaviour associated with the particle sizes, organic matter content and soil structure. For example, soils with smaller particle sizes (as observed in the Heilongjiang soil) tend to retain water for longer periods during the drying condition, as a finer matrix of smaller particles offers more resistance of water molecules to move, as compared to sandy soils with larger particles (the Shandong soil) with a coarser matrix. Furthermore, the presence of organic matter in the soil which has a higher WHC also influences drying rate. Therefore, the drying characteristics of soil types can lead to non-uniform drying patterns (Diel et al., 2019) with formation of patches, pores and certain areas that remain moist for longer, caused by the combination of particle sizes, organic matter content and soil structure.

As the length of the drying period varied in each of the soil types, the drying time of soil types were unsynchronised. Therefore, the soil microbes experience the stresses at varying levels (Abdul et al., 2021), associated with the unequally limited water availability under the different soil types. Some of them, dried quicker, causing more microbial death. However, the presence of organic matter in the soil can affect water retention and drying dynamics (Brady and Weil, 1999), as it acts a bridge between large particles and surface charge of mineral aggregates to hold the water (discussed in chapter 2). Therefore, *i*-DRW can notably impact on the biological performance of the soils at varying degree through the processes of P solubilisation in the rhizosphere of diverse soil samples and subsequently cause the leaching loss of P following rewetting. The results showed, the frequency of drying and rewetting exhibit different trends of total P in the leachate under a single DRW event. In this experiment, a substantially higher proportion of unreactive P to TP in all leachates coupled with greater enzyme activities amongst all type of soil samples found under *i*-DRW with repeated moisture fluctuation disturbance may indicate the underlying processes of P movement originating from soil microbial and non-microbial contributions, including soil microbial biomass P (Turner and Haygarth, 2001, Turner et al., 2003), microbial osmoregulatory compounds (Halverson et al., 2000) and soil non-living organic material (Wu and Brookes, 2005).

In this experiment, the direct responses of a single DRW event were not measured, therefore any changes occurred to reflect the achievements of soil P solubilisation shifting toward a cycle of four DRW events. However, the changes of dephosphorylating enzyme activities and excretion of organic acids observed at the end of the experiment in relation to Olsen P level cannot fully answer all questions, as these parameters may not characterize whole P solubilisation (potential leaching) processes. Many chemical characteristics of the soil emphasized the importance of immediate responses of the soil to the frequent *i*-DRW stimuli, including the quantities of soil nutrients, their availability, pH, the redox potential (Pulford and Tabatabai, 1988), available P sorption sites of the soil clay-sized particles (Atalay, 2001), Fe- and Al-oxide minerals (Sharpley and Rekolainen, 1997) and soil organic matter (Schoenau and Bettany, 1987). In addition, other factors aforementioned can also be included in this assumption if the experiment is conducted in a natural environment, such as soil moisture content, cropping system, drainage design and soil management (Heathwaite and Dils, 2000; Mcdowell and Sharpley, 2001; Sims et al., 1998). In terms of the potential leaching, the acidic soils selected in this experiment generated similar amounts of P in the leachate water, including Heilongjiang (0.3-1 mg L^{-}) and Chongqing (0.2-1.3 mg L^{-}), which displayed the same behavioural pattern of leaching throughout all frequencies of DRW events. This was despite these acidic soils from Heilongjiang and Chongqing containing different amount of carbon at the rates of 0.68% and 0.42%; and 0.07% and 0.05% for nitrogen, respectively.

Generally, the level of TP drained into the leachate water appears to decrease throughout the experimental period, with a steady downward pattern as the frequency of DRW event increased over time for all soils, except the Shandong soils. For example, noticeable changes of TP in the leachates were clearly observed in the Heilongjiang soils. This downward pattern may suggest that repeated DRW events (Pailler et al., 2014) are likely to increase the resistance of the bacterial community to sudden soil moisture fluctuations over time (Butterly et al., 2009) through the biofilm formation (Bouskill et al., 2016), inoculation in the intact soil aggregates (Navarro-García et al., 2012) and adaption of dormant status (Schimel et al., 2007), thereby leading to a decline in the P transfer rate in the leachate water. The bacterial adaption mechanism can provide interesting results which warrant consideration and further investigation in more in-depth relation to the P solubilisation (and potential leaching) under the frequency of the DRW events. However, again this was outside the scope of this experiment and as such no specific bacterial testing was conducted following DRW events.

In the field condition, the leaching losses of P can occur at the rate of 0.79 kg ha^{-} $year^{-}$ after the disturbance of the soil via conventional autumn ploughing, which includes particulate P at 87% under the inversion of the soil to the depth of 23 cm (Svanback et al., 2014). `Indeed, the processes involved in the preparation stage are likely to disturb the natural physical structure of the soil found in field settings, through the sieving and air-drying, mentioned earlier in the previous chapter. In addition to this, the increased DRW events can alter soil physical structure itself (Adu and Oades, 1978). Therefore, as a part of this experiment, I have attempted to mimic the soil natural structure by adding base layers such as sand and cotton net plugs to compensate for the potential impact of the soil preparation. A spout was plugged with cotton mesh and additionally 100 g coarse sand was placed in the lower half of the lysimeter tube to simulate layering found natural soil for water retention purposes (Figure 4.3.1). However, despite these steps taken to re-structure the soil artificially, it appears that the physical disturbance of the soil caused by the preparation procedure and DRW processes could not fully mitigated. On the other hand, the composition of sand used in this experiment is unknown. This uncertainty may affect the interpretation of the results, particularly in relation to the losses of soluble P concentration, as the presence of minerals, clay, organic matter and limestone components of sand can affect the adsorption capacity and mobility of soluble P.

4.6 Conclusions

The objectives of this experiment were to gain a better understanding of how *i*-DRW can influence the efficiency of P solubilisation processes in the rhizosphere, the amount of dissolved P in the soil solution and the leachate drained with water under a cycle of repeated events. The P solubilisation (and potential leaching) of the soil under *i*-DRW effect associated to the sudden soil moisture fluctuations were assessed amongst the different soil samples with varying P application rates. Four contrasting soil types with different textures, pH levels and chemical compositions were used in this experiment, sampled from distant geographical locations of China to compare true effects of *i*-DRW on the different soil types. Simultaneously, I was also interested in assessing how the frequency of DRW events maintain the differentiation of P leaching pattern in each soil type over the period of four rewetting episodes.

Overall, four out of five hypotheses of this experiment were accepted under the same *i*-DRW experimental structure through the statistically significant changes observed in the rhizosphere. However, no evidence was found to establish the link between soil moisture fluctuation stimuli and amount of solubilised P in the soil particularly, Olsen-P. This leads to the hypothesis (1) of this chapter being rejected, even though there was a statistically significant difference of water-soluble P concentration observed between two moisture groups. The prevalent pattern of each soil type with upward trends of solubilised and leached P, which corresponds with upscaling rate of P supply, directly support hypothesis (2). This chapter also provided data on the immediate effects of DRW on the P solubilisation through the biological function of the soil on the basis of the soil types with different physiochemical properties, accepting hypotheses (3) and (4). The concentration gradients of P species in the leachate water fluctuated over the frequency of DRW cycles and its dynamics, demonstrated the different patterns in the correlation between level of TP concentration against the number of frequencies of the DRW events. These outcomes suggested to accept the Hypothesis (5) are accepted. Although the different P forms in the leachate still considerably varied depending on the soil type, as the frequency of DRW cycles increases. The highest amount of P penetrated throughout the soil, presented in the leachate, was observed in the Shandong soils with P application at P250 and P500, and the Chongqing soils with P500.

The next Chapter will investigate the delayed effect of DRW (d-DRW) by introducing plant performance under the different soil types that were prior treated with a cycle of four DRW events. It seems increasingly likely that an amount of bioavailable P in the soil generated during the DRW processes would later benefit the plant particularly growing in the arid regions through the efficient rhizosphere processes.

CHAPTER 5

The effects of soil drying and rewetting history on delayed phosphorus solubilisation and growth of maize under contrasting agricultural soils from different regions of China

In the previous chapter, I explored the *immediate* responses of soil to the moisture fluctuation under the greenhouse setting associated with rapid drying and rewetting effects (*i*-DRW) and how a cycle of four DRW events could affect the phosphorus (P) solubilisation (and potential leaching) by using widely divergent types of soil collected from four different agricultural regions of China. This chapter will be building the experimental infrastructure established in the chapter 4 by continuing previous *i*-DRW study with plant performance to understand the potential impacts of delayed DRW (*d*-DRW) effect holistically on P solubilisation processes of the rhizosphere. This chapter will test hypothesis number 7 by growing maize on the four contrasting types of soils, which was previously treated with a cycle of four DRW events. Hypothesis 8 was: agricultural soils with a recent history of drying and rewetting can trigger P solubilisation in the rhizosphere and a subsequent growth response of maize (page 21 from Chapter One). This page also includes objective 1, which seeks to test the working hypothesis of this chapter by comparing the P solubilisation processes in the rhizosphere and growth responses of maize on the soil samples that simultaneously simulated a cycle of four drying and rewetting events: DRW in processed soil (DRW+) and a single maintained soil moisture: controlled soil (DRW -) prior to this experiment.

This work was presented in the Virtual EGU General Assembly, 2021 BG6 Sustainable phosphorus management and recovery: linking phosphorus and other element/material cycles.

5.1 Abstract

The experiment in this chapter tested the hypothesis that agricultural soils with a recent history of drying and rewetting (DRW) can trigger P solubilisation in the rhizosphere and a subsequent growth response of maize (*Zea mays*). Specifically, it aimed at investigating a possible delayed effect of DRW stresses on the soils by studying P solubilisation in the rhizosphere, plant P acquisition and performance, and root growth under different types of agricultural soils with the previous history of a series of DRW events. The soils collected from four different arid agricultural regions of China, Shandong, Chongqing, Heilongjiang and Beijing, intentionally treated with four varying cycles of DRW events prior to the experiment to raise levels of soil biotic and abiotic activities. A controlled pot experiment was conducted in order to establish the Olsen P in the soil, maize shoot P concentrations, root morphology and other rhizosphere parameters, for a duration of 43 days after planting. The results show a positive relationship between plant biomass, plant P concentration and Olsen P. The effect was most clearly demonstrated by level of plant growth and their biological performance in the rhizosphere, as the plants responded better in the soil with a DRW background than to a soil that did not have a history of DRW in the past. Notably, the most positive results were obtained from the Haplic Phaeozems soil of Heilongjiang, leading to an acceptance of Hypothesis 8. However, the soluble P concentration and plant growth response varied depending on P application rates and soil types. For example, the silt loam textured Luvic Kastanozems soil sample from Beijing did not support the hypothesis.

5.2 Introduction

Phosphorus is a major plant nutrient, cycling rapidly throughout the soil-rhizosphere-plant system. The organic acids and CO_2 released from the biochemical respiration of plants initiate the cycling process by increasing the acidity around the root hairs and degrading organic matter (OM). In order to acquire P, the plants convert it to accessible forms in their rhizosphere. The required concentration of phosphate ion in the soil solution for optimal crop production can be defined as more than $2x10^{-1}$mg L^{-1} in the field (Kruse et al., 2015). However, in the natural environment, many P sources exist as insoluble forms found in mineral compounds. Indeed, about 98% of the P in the soil, including most forms of biomolecules, are not immediately accessible for plants, due to the stable and insoluble nature of natural occurring inorganic and organic P sources. The level of labile P forms in soil matrices can be maintained through a variety of chemical, biological, and physical processes in the rhizosphere that equilibrates the orthophosphate ions rapidly into the soil solutions, particularly within the P applied soil.

The dissolution processes are achieved through dissolution-precipitation with mineral equilibria sorption-desorption, mineralisation–immobilisation and oxidation-reduction. The poorly crystalline P-bearing minerals (Schlesinger, 1997) and weakly sorbed phosphate monoesters such as D-Glucose 6-phosphate (Turner, 2010) can be dissolved within the root pore spaces under the acidic condition. Upon dissolution, the orthophosphate releases into the soil solutions from the complexed inositol phosphates (IP) and biomaterial decays through the oxidation reactions by enzymatic catalysis of soil microorganisms (Menezes-Blackburn et al., 2016b). As plants absorb the soluble P in soil solutions, it is incorporated with plant tissue. Then P converts into an organic form through their photosynthesis and metabolism. The various different environmental factors are considered to be materially impacted on the P solubilisation processes e.g., the presence of a sufficient amount of water in the soil, the P fixing and P buffering capacities of soil types (Syers et al., 2008), pH, concentrations of Al and Fe, and weather patterns such as dry spell and intense rewetting .

With subject to soil drying and rewetting (DRW), the decreased level of soil water potential leads to the accumulation of highly concentrated solutes in the cells over the longer period (Harris, 1981) with the breakdown of protecting coating of the soil aggregates (Denef et al., 2001). These processes may attribute the enhanced level of solubilised P associated with non-microbial organic P. At the same time, this can immediately contribute the soluble P leached out from the soils under *i*-DRW (Blackwell et al., 2009) discussed in chapter 4 and later indirectly renders an improvement of nutrient availability and crop production through by creating “enabling soil condition” for the subsequent plant growth under *d*-DRW effect (Sawada et al., 2019) via slow mineralisation process of organic P (Blackwell et al., 2013). Under the soil moisture fluctuation, the osmotic shock releases the intracellular solute (Fierer and Schimel, 2003; Halverson et al., 2000) and disposes the osmolytes (Schimel et al., 2007), then increases mineralisation of exposed organic matter (Miller et al., 2005) for an extended length of time during the d-DRW. The intensity of these processes of the soil depend on soil types (Czyż and Dexter, 2013), tolerance of microbial species to drought (Swift et al., 1979) and nature of DRW with regard to the degree of intensity, rate and frequency. It was further discussed in detail in Chapter 2 with visual illustration of Figure 2.3. However, the underlying mechanisms of subsequent plant responses with delayed P solubilisation to soil with previous history of DRW are still uncertain.

The hotter and drier summer following with intense rainfall events predicted in climate change scenario are likely to impact the soil respiration via activating CO_2 efflux toward living roots (Vargas and Allen, 2008) and increasing levels of CO_2 belowground. Consequently, the soil respiration may accelerate the nutrient turnover rates and its availability (Hasegawa et al., 2016) coupled with metabolic activities of mycorrhizae and the root growth, specifically fine roots (Liu et al., 2002). However, it remains somewhat unclear how the enhanced soil nutrients obtained via the *i*-DRW process will ultimately improve subsequent crop yields and decrease the leaching of bioavailable P into water sources from agricultural sites in arid and semi-arid regions, achieved through plant P uptake. A more extensive examination is necessary to precisely identify appropriate management measures aimed at enhancing P use efficiency within a wider agricultural context.

Whilst the previous chapter focussed on the i-DRW effects, this chapter aims to reflect on the longer-term issues building on the experiment in the previous chapter. The rationale of this study is to gain a better understanding how soil subsequently responds to the d-DRW effect of temporal dynamic moisture change events through the efficient processes of the rhizosphere to solubilise P. The experiment discussed in this chapter tests the Hypothesis 8 that agricultural soils with a recent history of drying and rewetting (DRW) can trigger P solubilisation in the rhizosphere and a subsequent growth response of maize (*Zea mays*). The objectives of this study are to observe a possible d-DRW stresses by studying plant performance on the soil with previous history of series of DRW events including P solubilisation in the rhizosphere, plant P acquisition, and root growth parameter. The prior drying and rewetted, highly variable types of agricultural soils with contrasting generic makeup used in this experiment can support to compare the subsequent plant growth.

5.3 Materials and methods

The experimental methodology of this chapter was trying to design a rhizosphere model exercise associated with the *d*-DRW effect by establishing a platform for the previous experiment outlined in Chapter 4. In this experiment, the selection of soils tried to represent a high variability of soil types with distinctive physio-biochemical characteristics by choosing sampling location of four different agricultural sites the within position from southwest to northeast regions of China, where these regions are the most susceptible areas to the natural occurrence of DRW during the cropping period. Detailed information about four different soil types is now available to view at Materials and Methods outlined in Chapter 4. Two separate groups of prior moisture treated soils, which was used in the chapter 4, intentionally continued throughout this experiment, acts as second stage of DRW experiment: (i) DRW group (DRW+) was subject to a cycle of four DRW events; and (ii) controlled group (DRW-) was maintained the soil moisture stably throughout the experimental period of chapter 4.

In this experiment, *Zea mays* was used as a focus crop. Maize seeds (*Zea mays* L. cv. ZD958) with weight between 0.45 and 0.50g, coated by antibiotics, were obtained from Plant and Nutrition Lab, CAU. The greenhouse temperature was ranged from 15-25^{o} C during the experiment. To support this experiment, I have applied different rates of P fertiliser applications at the rate of zero (0), 250 and 500 mg per kg soil. The P supply was used as monopotassium phosphate KH_2PO_4 and applied into both soil groups before introducing the first DRW cycle in chapter 4. The amount of P was calculated based on molar mass of KH_2PO_4. Thus, a sample layout of a completely randomised design with three P treatments under four types of soils within the two moisture groups, each replicated four times. Four soil samples (n = 4) were used for the analysis under standard procedures and quality controls.

5.3.1 Experimental procedure

The pot experiment was conducted between 10th October and 24th November 2018 in a naturally lit greenhouse at the China Agricultural University (the photo of this greenhouse was taken in July, 2018 and shown in the Figure 5.3.1). Immediately after the fourth rewetting, the soils were then used for the plant seedlings. The preparation of experiment and collection of soils were outlined in detail in Chapter 4 together with the analysis of: (i) soil physical (texture, permeability, and water holding capacity); (ii) chemical (pH, total P, water-soluble P, Olsen P, total nitrogen (N), total carbon (C) and the C:N ratio); (iii) and biological (acid and alkaline phosphatase and phytase activities and carboxylates) characteristics, which is also summarised below. In all cases, these tests were performed shortly before the pot experiment (see Table 4.4.1 and 4.4.2 and Figure 4.4.1 in Chapter 4).

Figure 0.1 Naturally lit greenhouse in the China Agricultural University, photo taken by the author during the summer (2018)

The preparation of the experiment

Closed-ended conical shaped 15 cm long tubes with one 0.5 cm hole in the spout at the bottom, which was designed for the earlier experiment in chapter 4 (Figure 5.3.2) continued to use in this experiment. This tube has a gradual sliding side with diameter 6 cm at the top and 2.5 cm at the bottom. The cotton mesh at the size of 10 x 10 cm was plugged the spout. The tube was filled with 300 g of soil with 100 g coarse sand underneath in height of 10 cm, without the basal micronutrient addition.

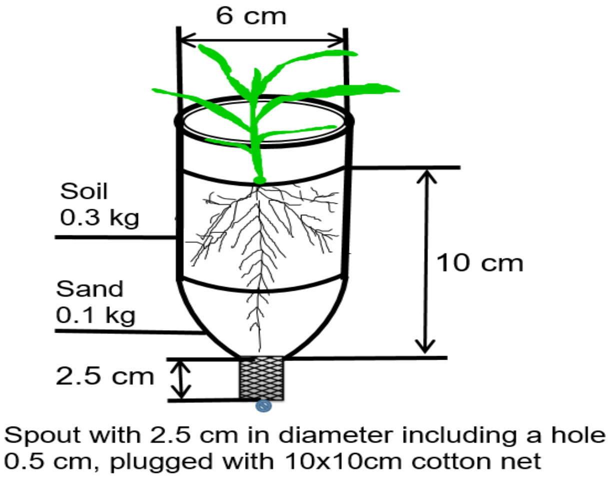


Figure 0.2 Conical shaped pot design

The soil analysis carried out prior to the first part of DRW experiment (as set out in Chapter 4) are as follows:

1. Sandy loam textured brown Cambisol soils collected from the fallow land in Qingdao, Shandong, China (Latitude: 29^{o}03’N, Longitude: 106^{o}11’E 170m) by Prof, Ding of Qingdao University experiment station. It contains Olsen P 22.3 mg kg^{-1}, TP 389 mg kg^{-1}, organic carbon 11.5 g kg^{-1}, and soil pH 7.3.
2. Fine and coarse loamy texture red Lithosol soils were collected from Huangzhuang village, Yong Xing town, Jiang Jin, Chongqing, China (Latitude: 29^{o}03’N, Longitude: 106^{o}11’E, 266 m). The land is used for the crop rotation of winter wheat with summer maize or wheat with rice. The soil contains Olsen-P 7.85 mg kg^{-1}, TP 270 mg kg^{-1}, organic carbon 15.5 g kg^{-1}, and pH 4.7.
3. Clay loam textured black Haplic Phaeozems soils were collected from Heilongjiang Province (Latitude: 45^{o}40’N, Longitude: 126^{o} 35’E, 151 m). The land is utilised for a single crop rotation of wheat- soybean-maize. Soil contains Olsen-P 34 mg kg^{-1}, TP 448 mg kg^{-1}, organic carbon 24.5 g kg^{-1}, and pH 5.1.
4. Silt loam textured calcareous Luvic Kastanozems soils with low P availability were collected from the fallow Shangzhuang field, long-term fertiliser CAU experiment station, Beijing, China (Latitude: 40^{o}01’N, Longitude: 116^{o}16’E, 130 m). Soil contains Olsen P 2.6 mg kg^{-1}, TP 288 mg k^{g-1}, organic carbon 12 g kg^{-1}, and pH 8.9.

The seed germination and planting of seedlings

Seeds were surface sterilised by 10% H_2O_2 for 30 minutes, then rinsed by distilled water several times, and after that these imbibed in well-aerated saturated $CaSO_4$ solution in dark room for 8 hours. All seeds were germinated for 3 days in paper towels, which are placed on the tray soaked with distilled water, in the dark and humid environment at 22^{o}C (Mollier and Pellerin, 1999). The tray was covered with a loose-fitting lid in order to allow some gas exchange. One uniformly germinated seedling was then transplanted into each conical pot within the depth of 2 cm from surface. Pots were watered via deionised water every other day to maintain the weight at 75% field capacity whilst controlling infection. I kept observations in aerial parts of maize periodically in 12 days for the plant growth performance while taking photos. Plants were harvested in the middle of 4th observation periods of this experiment at day 43 after sowing, before sudden drop of greenhouse temperature during the night expected at the end of November.

5.3.2 The data collection and laboratory analyses

As mentioned, plants were harvested 5 days earlier as planned. Shoots were cut just above the soil surface. It was oven dried for 30 minutes at 105°C and then 3 days at 70°C for the measurement of biomass weight. Roots were carefully separated from the soil by shaking into 50 ml 0.2 mM $CaCl_2$ solution and then collection was used for the rhizosphere extraction solution modified from the published methods (Pearse et al., 2007; Veneklaas et al., 2003). A subsample of rhizosphere extract: (1) one stored at -20°C is used for the carboxylates HPLC analysis with adding microbial inhibitor Micropur (Sicheres Trinkwasser, Germany) at the dose of $0.01gL^{-1}$ including 2 drops of concentrated phosphoric acids and (2) another stored at 4°C for determination of acid phosphatase activity and rhizosphere pH measurement. The rhizosphere extraction solution remaining in the tube was dried in open air in order to measure rhizosphere soil weight and further rhizosphere P testing.

The soil phosphorus analysis

Three different P analysis was performed by using air-dried soil of this experiment. Firstly, the soil bicarbonate extractable phosphorus was determined by the Olsen methods (1954). 5 g of the air-dried rhizosphere and bulk soil were extracted in 100 ml of 0.5 M $NaHCO_3$ solution, adjusted to pH 8.5 along with a shaker for 1 h. Extracts were filtered by Whatman No. 42 and then used for the determination of P availability on the basis of the colorimetric method by the molybdenum-blue reaction of Murphy and Riley (1962). Secondly, TP in the soil was analysed by using the digestion method. The finely ground soil was digested with perchloric acid ($HClO_4$) and sulphuric acid on a hot plate for about 40 min at 203°C until the dark colour (organic matter) disappeared. After the digestion, the extract was subsequently diluted by suspending in deionised water and filtered via Whatman 541 paper. Then the colorimetric method was performed on the basis of the reaction with acidic molybdate in the presence of phosphomolybdate complex and measured spectrophotometrically at 880 nm. Finally, to determine water soluble P, malachite green oxalate salt with polyvinyl alcohol is used after the molybdate acidifying reaction in soil solution, with absorption of wavelength spectrophotometric measurement at 610 nm, method is modified from Feng et al., (2011).

The soil enzymatic activities, carboxylates and pH analysis

Determination of the acid and alkaline phosphatase activity was performed by the colorimetric analysis (Alvey et al., 2001; Neumann, 2006) of the *p*-nitrophenol. 0.4 mL of 0.04M sodium acetate buffer at pH 5.2 is used for acid phosphatase activity and Trizma buffer at pH 7.4 for alkaline phosphatase activity. 0.1 mL 0.15 mol L^{-1} *p*-nitrophenol phosphate (PNP) were diluted in sodium acetate buffer at pH 5.2 for acid phosphatase, and in Trizma buffer at pH 7.4 for alkaline phosphatase activity and then added to 0.5mL rhizosphere suspension. After incubation of soil at $28^{o}C$ for 30 minutes, the reactions were terminated off by 0.5 M NaOH. The absorbance substance released by phosphatase activities was measured spectrophotometrically at 405nm. One unit of acid and alkaline phosphatase activity was defined as the activity per gram soil that produced 1 µmol p-nitrophenol per hour.

Phytase activity was assessed by using methods modified from Richardson et al., (Richardson et al., 2000, George et al., 2010). The soil suspension was prepared at 2 g soil in 8 ml deionised water. 0.5 mL of soil solution was mixed with 0.4 ml InsP6 MES buffer at pH 5.5 including 30mM MES, 2mM EDTA, and 4mM InsP6. After incubation of soil at $37^{o}C$ for 1 hour, the reactions were terminated by 1ml 10% trichloroacetic acid (TCA). The sample was subsequently centrifuged at 12000 r.p.m for 10 min to remove soil particles. The orthophosphate concentration in the supernatant was determined by colorimetric assay at wavelength 882 nm using the molybdenum-blue reaction of Murphy and Riley

(1962). One unit of phytase activity was defined as the activity per gram soil in dissolved is 1ml $CaCl_2$ that liberated 1 mg P per hour.

Carboxylates were analysed by a reversed-phase high-performance liquid chromatography (HPLC) system, in prior filtration of soil suspension through a 0.22-µm filter to avoid blocking the machine. The chromatographic separation was conducted on a 250 × 4.6 mm reversed-phase column (Alltima C18, 5 µm; Alltech Associates, Inc., Deerfield, IL, USA). The mobile phase was 25 mmol L^{-1} KH_2PO_4 (pH 2.3) with a flow rate of 1 mL min^{-1} at 31 °C with detection of carboxylates at 214 nm.

Rhizosphere pH was measured on the day of harvesting by using Seven-Compact S210 pH meter, whilst measurement of bulk soil was suspended in deionised water at the ratio 1:2.5 in soil to water after the air-drying of 7 days (www.globe.gov).

Plant analysis

Root scanning: Thoroughly washed frozen roots were used for the root image analysis by the EPSON scanner at 400 dpi (Epson Expression 1600 pro, Model EU-35, Japan) and followed by the software calculation via Win-Rhizo (Regent Instruments Inc., Quebec, Canada). In addition to the total root length, the specific root length (SRL) was measured by calculating the root length per 1g dry weight (mg^{-1}) modified from Jing et al. (2010). This can be used as an indicator of environmental changes (Ostonen et al., 2007). After root scanning, the roots were oven dried for 30 minutes at 105^oC and then 3 days at 70^oC to measure the dry weight and R/S ratio.

Plant phosphorus analysis: Oven dried roots and shoots were used for the plant P analysis. The plant P concentration was determined by vanadomolybdate method by spectrophotometry at 440 nm (Johnson and Ulrich, 1959) either after wet or heat digestion via a mixture of concentrated H_2SO_4 and H_2O_2 or electric furnace at 550^oC respectively.

Data analysis

The statistical analyses were performed by using the software SPSS version 23 (IBM, SPSS 2014). The data are reported as mean ± standard deviation with a confidence threshold of 0.05 applied in all cases. A two-way ANOVA test was used to determine the interaction effects of delayed DRW on soil types in terms of rate of P supply through univariate REGWQ *post-hoc* range analysis for multiple comparison (significance reported as $p < 0.05$) by using SPSS statistical software. A one-way analysis of variance was also performed on all datasets between two moisture treatments using the least significant difference (LSD) and Duncan's multiple range analysis at the $P \leq 0.05$ probability through the mean differences of treatments among soil types. The data are reported as mean ± standard deviation. The data between DRW and stable moistening soils were tested by the *t*-test. The exponential equation analysis was performed using SPSS to explore the relationship between root/shoot ratio, soil pH and shoot P concentration or soil Olsen-P (Li et al., 2008). Empirical polynomial (inverse third order) equations were used by SigmaPlot (SigmaPlot 10.0, USA) to analyse the relationship between root morphological traits (i.e., total root length, specific root length or proportion of fine roots) and shoot P concentration or soil Olsen-P based on the published method (Deng et al., 2014).

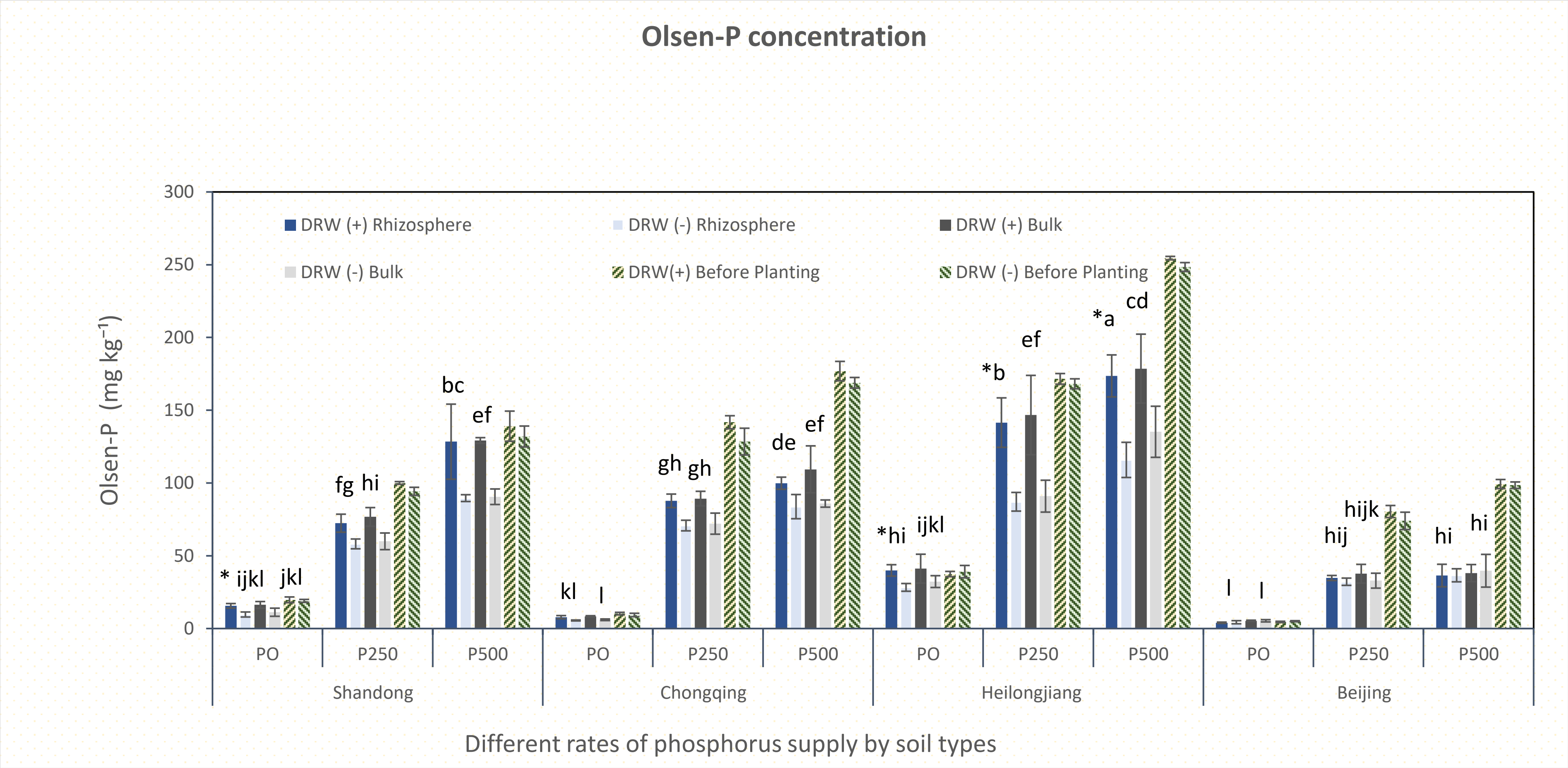
5.4 Results

Findings of the experiment determined delayed effects of DRW can be grouped into three categories: (a) soil P dissolution; (b) plant indicator parameters and P uptake; and (c) rhizosphere excretions and soil pH changes. The significant differences of plant growth were observed under the different moisture and P treatment at the 4th observation period. The plant parameters are positively correlated with amount of dissolved P in the soil, with representing simultaneous upward trends in both soluble P in the soils and P concentration of plants.

5.4.1 Soil phosphorus dissolution

The level of P solubility varied noticeably at the harvest stage, depending on the soil types with variable bio-physiochemical compositions, delayed effect of soil moisture changes and rate of P fertiliser inputs. The results obtained from the test to determine the level of both bicarbonate extractable and water-soluble P are shown below at Figure 5.4.1 and 5.4.2, respectively, with the considerable fluctuation of the P concentration under the soil types. The result set out in the figure 5.4.1 demonstrated the Olsen P concentration of the bulk soil (shaded in deep colour) and their changes in the rhizosphere of maize (in lighter colour), and also includes Olsen P concentration of the soil prior to the planting (highlighted in yellow dots). The representation of Olsen P components of this experiment is denoted in (a) blue: the soil moisture treatments (DRW+) and (b) grey: the moisture control (DRW).

Figure 0.1 Olsen P concentrations of the soil and their changes in the rhizosphere of maize that are grown in different types of soils with variable rates of phosphorus supply.



*The mean difference is significant at the 0.05 level. The data represented are the means of four replicates and error bars shown on standard error.

In this figure, the statistically significant changes of Olsen P between two moisture treatment groups such as DRW and Controlled group was noted by analysing *t*-test. Different letters indicate significant differences by one-way ANOVA in a Duncan post hoc test.

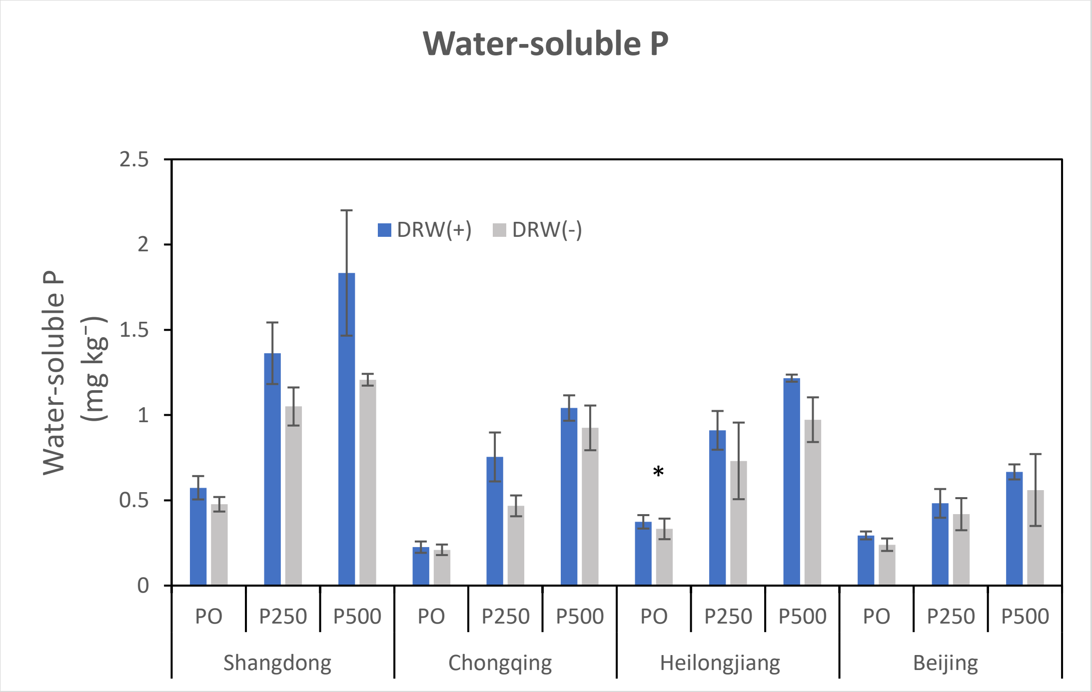
The significant changes of Olsen P between pre and post experimental results were always reported with presence of lowering Olsen P at the end of experiment. For example, the most statistically significant changes of 2.6-fold decline was identified among the all moisture treated Beijing soils within the highest rate of P supply, whilst a slight decrease occurred in the DRW Heilongjiang soil without P addition. Furthermore, when concentration of Olsen P in the rhizosphere soils was totalling between moisture treatment groups, Olsen P in DRW (+) group soil dropped higher than the soils in Controlled DRW (-) group, except in the soils from Beijing, for example, DRW (+) and DRW (-) at 42 and 88; 134 and 147; 108 and 224; and 109 and 104 mg kg^{-1} in the Shandong, Chongqing, Heilongjiang, and Beijing soil, respectively among the all P treatments. The general conclusion that can be drawn from this result is, the plant grown in the prior DRW processed soils may efficiently utilise more soluble P during the growth period. In addition to this general decline, I also observed the decline of Olsen P between the bulk and rhizosphere soils, due to the immediate response for the plant P uptake. However, this change between bulk and rhizosphere soil was not apparent in statistical significance under the delayed effect of DRW.

Olsen P level of each soil category was showing a general upward pattern in accordance with the rates of P supply, regardless of the delayed effect of moisture changes. The level of soluble P was being lower with the non-P addition and high with the P addition, for instance, there was the highest concentration of Olsen-P in the P application at a rate of 500 mg kg^{-1}. A dramatic elevation of Olsen P with statistical significance was observed by 3 to 13-fold increase as soluble P added to soil at a rate of 250 mg kg^{-1}, in comparison with nil rate of P application of the soil. It is followed by a more moderate increase of Olsen P when the soluble P supply is increased from 250 to 500 mg kg^{-1} P treatments by

56, 12, 32 and 2 mg kg^{-1} in the prior DRW processed soils of Shandong, Chongqing, Heilongjiang and Beijing, respectively.

Whilst the foregoing general observation made against the entire soil types subject to this experiment, a further different reaction to soluble P can also be observed when different soils from different regions are compared. For example, one can be seen from Figure 5.4.1, an increase of Olsen P is significantly higher in Chongqing and Beijing soil as soluble P was added at the rate of 250 mg kg^{-1} of soil with following same pattern of plant growth. Conversely, when comparing soils from Heilongjiang, this rise sees the most modest, increasing by lowest with 4-fold from 40 to 142 mg kg^{-1} at the rate of 0 and 250 mg kg^{-1} respectively.

It is also interesting to see the water-soluble P concentration that ranged considerably different between the DRW (+) and DRW (-) Controlled groups. However, overall results still indicate that the general pattern of water-soluble P was showing a similar pattern of Olsen-P, except in Heilongjiang soils. That being, the highest concentration was detected in all DRW processed soils with P500 treatments, followed by the P250. In particularly when a direct comparison is made between soil types, the concentration of water-soluble P given DRW processed Heilongjiang soil was considerably lower than that of the Shandong soil and the almost the same as Chongqing soils around 0.4, 0.9 and 1.2 mg kg^{-1} in PO, P250 and P500 respectively.



*The mean difference is significant at the 0.05 level.

Figure 0.2 The water–soluble P concentrations in various types of soil, measured at the harvest under the different phosphorus (P) supply rates. Data presented to represent the means of four replicates and error bars indicate the standard error. To assess the statistical significance of differences between two moisture treatment groups, a *t*-test was conducted.

5.4.2 Plant indicator parameter and phosphorus uptake

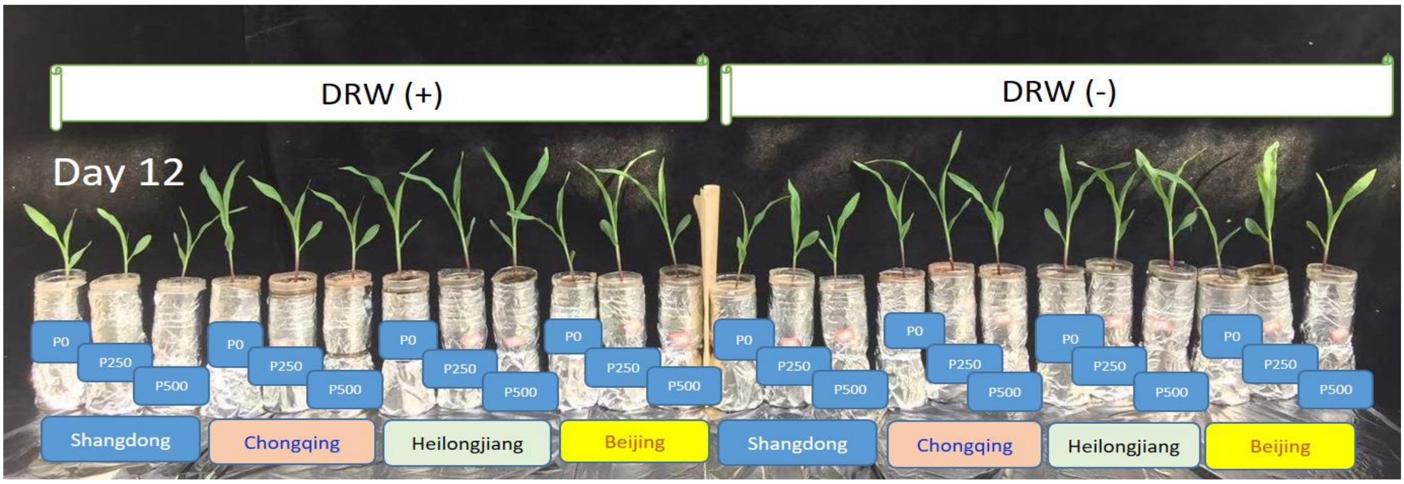
As part of this experiment, I have also tracked the growth process of plants and their responses against the level of soluble P generated from the DRW group during the delayed effects of soil moisture temporal dynamic changes, as well as measuring the same against the Controlled group. For the purpose of this experiment, I have categorised the plant growth performances into two subcategories: (a) parameters related to the aerial part of the plant parameters and (b) distribution of the root morphological parameters. The entire plant indicator parameters performed under the four different types of soils are set out in Table 5.4.1. I will deal with the high-level findings for each subcategory below.

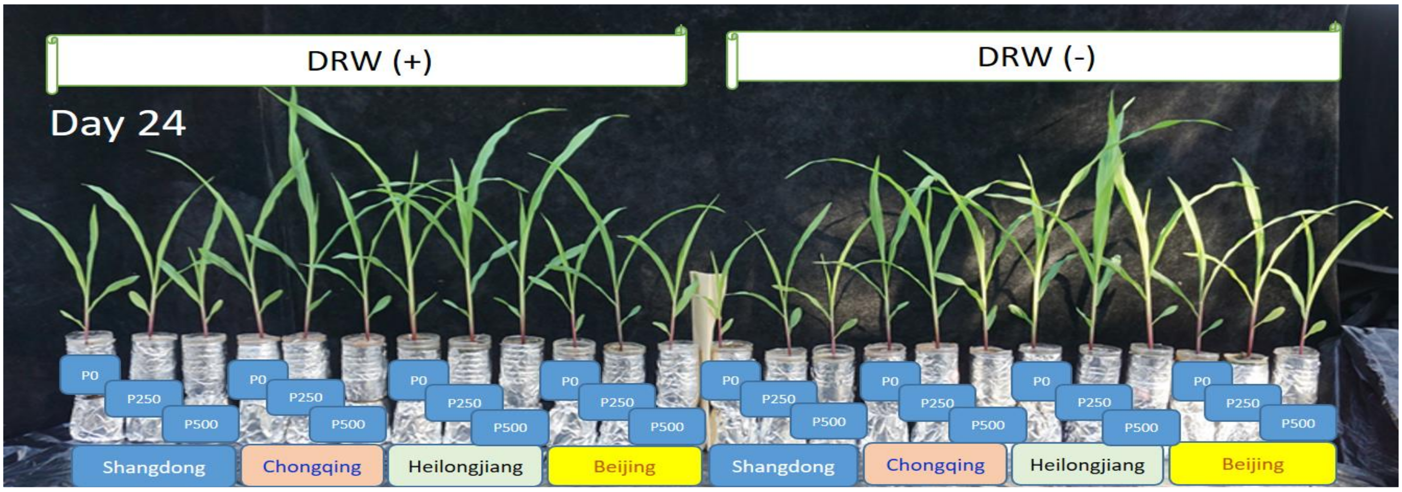
Plant aerial part parameters

This subcategory includes plant P parameters and shoot growth performance. Periodic observation of plant aerial growth was shown in Figure 5.4.3. The plant P uptake was consistent with soil P availability. Furthermore, the improved plant P uptake from soil appears to be achieved by the soil type specificity, soil moisture treatment and P application rate. For example, the occurrences of remarkable elevation in the plant P happened in the prior DRW processed Heilongjiang soil with P addition, where an increased level of soil Olsen P was observed at P250 and P500 treatments at 142 and 174 mg kg^{-1}, respectively. At the same time, the shoot P concentration equally increased in the DRW (+) group of Heilongjiang soil within the supply at the rate of 250 and 500 mg kg^{-} at 12 and 13 mg g^{-1}, respectively. On the other hand, the presence of lower Olsen P was detected in the Controlled group of Beijing and Shandong soils at a supply rate of zero P around 1 mg P taken up by gram biomass.

Shoot P concentration range constituted 1-14 mg g^{-1}, from non-P added (PO) Controlled group of soil from Beijing to P added (P500) DRW group from Heilongjiang, with a mean concentration of 6.4 and 4.7 mg g^{-1} in the DRW (+) and DRW (-) Controlled groups, respectively. Shoot P concentration of all treatments was listed in the ascending order in accordance with increasing P supply rates (P0; P250; P500) for each soil type as follows (i) the Shandong soil 3; 7; and 8 mg g^{-1} in the DRW (+) and 2; 4; and 6 mg g^{-1} in the DRW (-) Controlled group (ii) the Chongqing soil- 4; 6; and 5 mg g^{-1} in the DRW (+) and 3; 5; and 5 mg g^{-1} in the DRW (-), (iii) in the Heilongjiang soil- 7; 12; and 13 mg g^{-1} in the DRW (+) and 7; 10; and 10 mg g^{-1} in the DRW (-), and (iv) the Beijing soil- 3; 4; and 5 mg g^{-1} in the DRW (+) and 1; 3; and 3 mg g^{-1} in the DRW (-) Controlled groups. The detection of the most noticeable changes of dry weight were proportional to the natural pattern of soluble P concentration of the soil types, ranging from 0.3 g all soils in DRW (-) Controlled soil group from the Shandong to 1.1g DRW (+) group with P addition soils from Heilongjiang with 86% of increase. Whilst the mean dry weight of the maize was 0.7 g and 0.6 g in DRW (+) and DRW (-) Controlled group, respectively shown in Table 5.4.1. The shoot biomass can generally be divided into two parts: (i) below 6.5 g: all Shandong and Beijing soils and the Chongqing soils except DRW (+) group with P addition; and (ii) above 6.5 g: all Heilongjiang soils and Chongqing DRW (+) group with P addition.

An interesting observation was noted with regards to the root shoot ratio (R/S) of the sampled groups. The mean R/S ratio of all the plants subject to the experiment was in the DRW (+) group at 0.4 and the DRW (-) Controlled group at 0.4. However, when looking at this at granular level, the R/S ratio of each plant appears to differ widely ranging from 0.83 to 0.24, the general trend of R/S ratio depending on the soil type and the rates of P supply. For example, the soil types from the Shandong and Beijing had a significantly high ratio (0.83 and 0.28, respectively) than the R/S ratio in the soil types from Chongqing and Heilongjiang regions (0.24 and 0.38 respectively). In each case, the R/S ratios gradually decrease in direct correlation to the increase of supply rate of P.





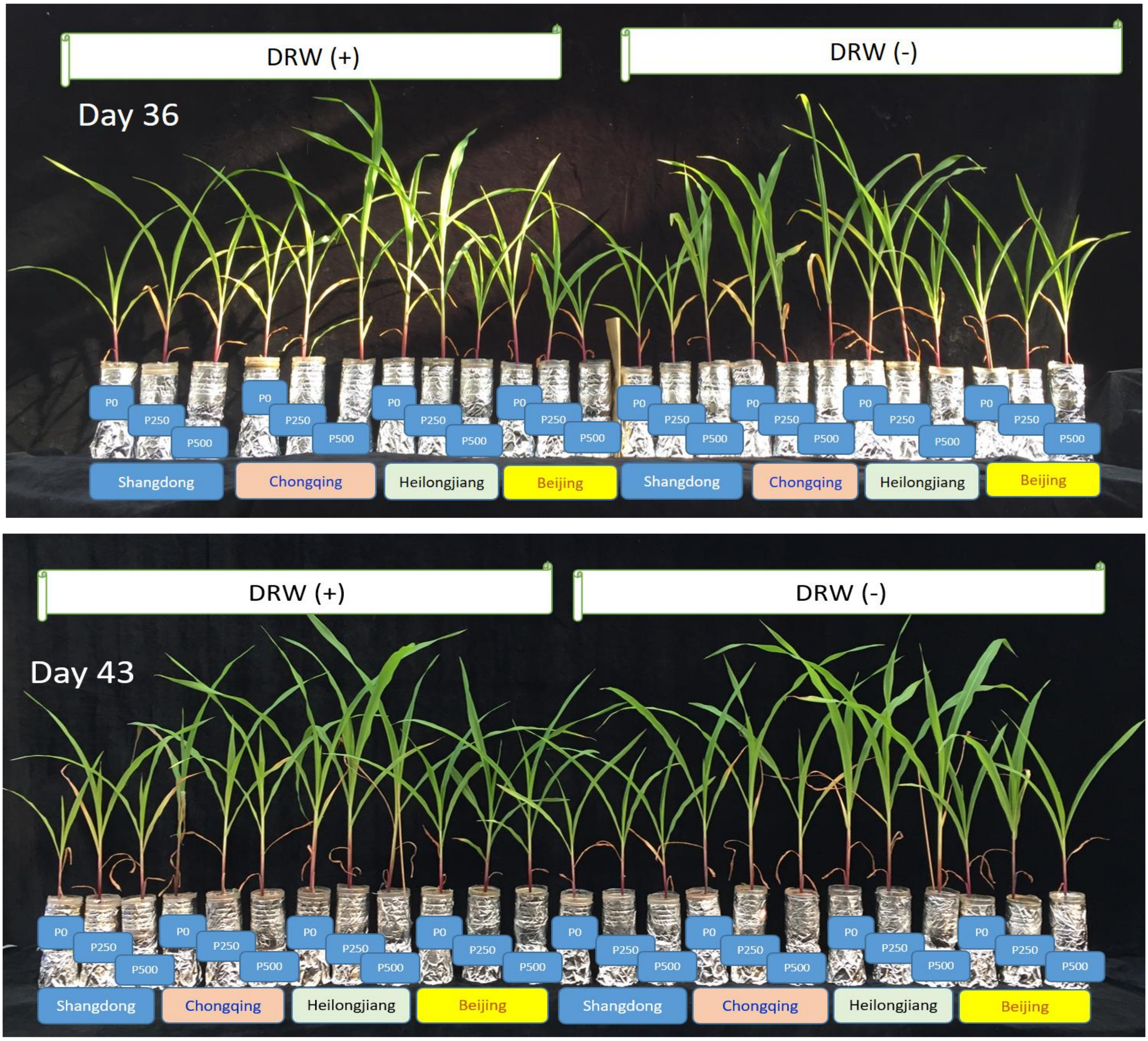


Figure 0.3 Periodic observation of maize growth under the length of 12 days including 43th day before harvesting.

Root morphological distribution

The total length of the root varied from 6.8 to 23 m plant $^{-1}$ with a mean of 13 m plant $^{-1}$. The greatest root length increase was observed at 22.9 m and 21.6 m in maize planted in both DRW (+) and DRW (-) Controlled groups of soils from Shandong region respectively. At the same time, the specific root length of these plants appears to be notably high with a significantly greater proportion of the fine root volume than expected at 61% (DRW (+) group) and at 58% (DRW (-) Controlled group. However, their root length was not in correlation with their shoot biomass. For example, shoot biomass of these maize grown on Shandong soil was slightly less than average plant biomass grown in the Beijing soil where the shortest length of the roots was detected.

The plant grown in the DRW (-) moisture-controlled Beijing soils particularly without P supply had the lowest total length of root at 6.8m. When compared the fine root proportion, it was the highest at 83% (as can be seen from the Table 5.4.1). Despite the comparable average root length of plants in soils from both Heilongjiang and Chongqing, there was a significantly different in the dry weights of the plants (shoot and root) grown on these two soil types, exhibiting a disparity of nearly 35%. The specific root length in the DRW (+) of Chongqing soil consequently, appears to be higher than that of the Heilongjiang soil regardless of the rate of P supplied, having a slightly lower proportion of the fine root volume, ranging from 50 to 70 %.

The plant shoot and root morphological parameters such as dry biomass weight, specific root length and proportion of fine root with the diameter below 0.2 cm were shown. Plants were sampled at the 43 days of planting. The column is arranged in order of soil types. Values are means of four biological replicates. Different letters in each column indicated statistical differences ($P \leq 0.05$) between different P variables.

Table 5.4.1 Summary of the experiment of the delayed effects of DRW on maize including the soil phosphorus availability and plant performance of four different types of soil under the different levels of phosphorus supply.

		P supply rate per treatment (mg kg^{-1})		*Rhizosphere Olsen P (mg kg^{-1})*	*Total P Content of Plant (mg g^{-1})*	*Shoot P concentration (mg g^{-1})*	*Plant P uptake (mg plant^{-1})*	*Dry weight (g plant^{-1})*		*Root length (m plant^{-1})*	*R/S ratio*	*Specific root length (m g^{-1})*	*Fine root volume (%)*
								Shoot	*Root*				
Shandong	DRW (+)	**P0**	**0**	16 (1.6) *ijkl	5.04 (0.47)	3.3 (0.54) de	1.39 (0.2) e*	0.4 (0.03)	0.2 (0.04)	22.9 (2.3) a	0.57 (0.09)	101.6 (15.3)	61 (0.04)
		P250	**250**	72.4 (6.2) fg	8.7 (1.65)	6.6 (1.74) bcde	3.2 (0.88) de	0.5 (0.06)	0.2 (0.03)	14.6 (1.8) abcd	0.39 (0.07)	81.4 (5.1)	57 (0.03)
		P500	**500**	128.4 (25.8) bc	8.8 (0.97)	7.5 (1.02) abcd	3.1 (0.79) de	0.4 (0.06)	0.2 (0.004)	16.4 (0.62) bcde	0.43 (0.07)	102.2 (3.9)	61 (0.03)
	DRW (-)	**P0**	**0**	9.6 (1.7) jkl	3.3(0.45)	1.85 (0.57) de	0.56 (0.18) e	0.3 (0.02)	0.2 (0.004)	21.6 (2.4) ab	0.67 (0.04)	108 (10)	58 (0.02)
		P250	**250**	58.2 (3.4) hi	6.3 (0.38)	4.43 (0.3) cde	1.5 (0.13) e	0.3 (0.02)	0.2(0.04)	11.2 (2.4) cde	0.44 (0.09)	77.6 (8.8)	56 (0.02)
		P500	**500**	89.6 (2.4) ef	8(2.8) b	6.16 (1.63) cde	2.05 (0.6) de	0.3 (0.2)	0.2 (0.02)	16.1 (3.2) abc	0.48 (0.08)	105.9 (18.1)	63 (0.03)
Chongqing	DRW (+)	**P0**	**0**	7.8 (1.1) kl	5 (0.66)	4.3 (0.65) cde*	2.6 (0.29) de*	0.63 (0.03)	0.2 (0.01)	12.3 (2.2) cde	0.3 (0.03)	69.9 (17)	71 (0.1)
		P250	**250**	87.7 (4.6) * ef	8 (1.08)	6.3 (1.04) bcde	4.4 (0.72) cde	0.7 (0.02)	0.2 (0.02)	11.7 (3.2) cde	0.27 (0.02)	59 (13.3)	41 (0.05)
		P500	**500**	99.9 (4.2) de	6.6 (0.47)	5.2 (0.56) cde	3.5 (0.23) cde	0.7 (0.4)	0.2 (0.02)	14.3 (3) bcde	0.24 (0.01)	90.7 (22.4)	46 (0.06)
	DRW (-)	**P0**	**0**	5.6 (0.3) l	4.17 (0.39)	2.5 (0.2) de	1.4 (0.14) e	0.6 (0.05)	0.2 (0.03)	12.5 (2.02) cde	0.38 (0.02)	58.1 (3.9)	53 (0.02)
		P250	**250**	70.8 (3.7) gh	6.6 (1.4)	4.7 (1.1) cde	2.9 (0.65) de	0.6 (0.02)	0.2 (0.01)	11.4 (1.1) cde	0.35 (0.03)	50.8 (2.3)	51 (0.02)
		P500	**500**	83.8 (8.3) ef	7.3 (0.65)	4.9 (0.51) cde	2.9 (0.27) de	0.6 (0.04)	0.2 (0.01)	9.1 (1.07) cde	0.31 (0.02)	49.4 ((5.6)	59 (0.14)
Heilongjiang	DRW (+)	**P0**	**0**	39.9 (3.9) *hi	8.2 (0.94)	7.27 (1.01) abcd	7.6 (1.32) bc	1 (0.04)	0.3 (0.01)	11.5 (1.2) cde	0.27 (0.01)	42.3 (3.9)	53 (0.03)
		P250	**250**	141.5 (17) *b	13.4 (0.77)	12.05 (0.87) ab	13.4(1.1) a*	1.1 (0.05)	0.3 (0.02)	14.8 (3.5) bdd	0.26 (0.03)	51.8 (10)	58 (0.05)
		P500	**500**	173.6 (14.4) *a	14.3 (0.58)	12.7 (0.57) a*	13.7 (0.55) a*	1.1 (0.03)	0.3 (0.03)	14.9 (3.05) bcd	0.24 (0.02)	57.8 (7)	56 (0.04)
	DRW (-)	**P0**	**0**	28.3 (2.7) ijkl	8.8 (1.95)	6.9 (1.9) abcd	5.9 (1.5) bcd	0.9 (0.03)	0.3 (0.02)	12.7 (1.4) cde	0.37 (0.02)	40.4 (3.6)	49 (0.05)
		P250	**250**	87.1 (6.4) ef	11.6 (1.6)	9.5 (1.21) abc	8.7 (1.4) b	0.9 (0.04)	0.4 (0.02)	15.2 (1.6) bcd	0.38 (0.02)	44 (3.5)	53 (0.02)
		P500	**500**	115.8 (12.1) cd	11.5 (1.3)	9.9 (1.05) abc	8.9 (1.45) b	0.9 (0.08)	0.3 (0.03)	10.2 (2.2) cde	0.33 (0.03)	37.7 (9.7)	56 (0.14)
Beijing	DRW (+)	**P0**	**0**	4 (0.4) l	3.3 (0.88)	2.6 (0.93) de	1.1 (0.28) e*	0.5 (0.05)	0.3 (0.04)	11.3 (3.7) cde	0.58 (0.12)	53.6 (23.4)	63 (0.13)
		P250	**250**	34.7 (1.7) hij	5.7 (3.13)	4.3 (2.76) cde	1.9 (1.06) de	0.5 (0.03)	0.2 (0.03)	10 (3.1) cde	0.5 (0.08)	47.6 (2o.3)	66 (0.15)
		P500	**500**	36.4 (7.7) hi	6.2 (1.01)	4.57 (1.26) cde	2.4 (0.59) de	0.5 (0.04)	0.2 (0.04)	11 (1.7) cde	0.36 (0.1)	78.7 (31.3)	50 (0.07)
	DRW (-)	**P0**	**0**	4.3 (1.1) l	2.3 (0.27)	1.13 (0.16) e	0.4 (0.09) e	0.4 (0.05)	0.2 (0.02)	6.8 (1.8) e	0.61 (0.07)	33 (10.3)	83 (0.1)
		P250	**250**	32.2 (2.5) hijk	3.8 (0.46)	2.5 (0.39) de	1.2 (0.33) e	0.5 (0.05)	0.2 (0.03)	8 (1.1) de	0.42 (0.02)	41.8 (7)	57 (0.14)
		P500	**500**	36.6 (4.5) hi	4.8 (0.26)	2.7 (0.1) de	1.3 (0.16) e	0.5 (0.06)	0.1 (0.02)	10.1 (1.2) cde	0.28 (0.02)	76.2 (13.7)	61 (0.13)

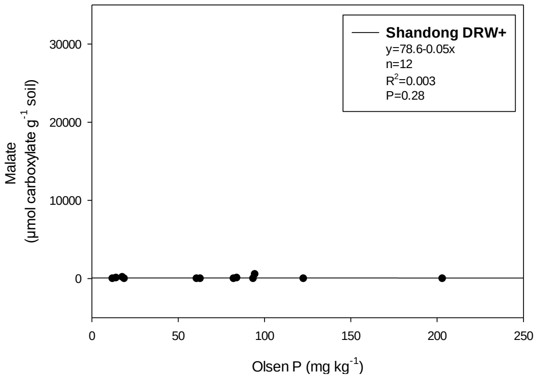
5.4.3 Rhizosphere excretions and soil pH changes

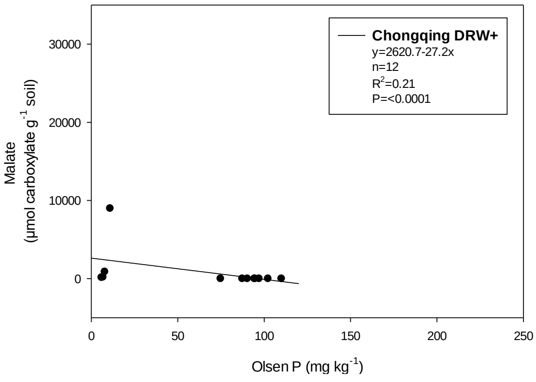
In the given experiment, the arithmetical measure of pH value ranged between 4.3 and 8.3 amongst the different soil types with no significant changes at the harvest stage. This suggests, acidity of soil remained relatively stable throughout the experiment. Figure 5.4.4 represents the physiological responses of maize to different levels of Olsen P in the soil, based on the emission of root exudates. This figure presumably illustrates the correlation between Olsen P levels in the soil and the production of organic acids, as well as the activity of dephosphorylation enzymes. In general, the malate and citrate were predominantly detectable organic acids among all the treatments. The behavioural patterns of organic acid excretions were notably similar under the prior moisture treated and P applied soils. The highest amount of organic acids was observed in the unfertilised DRW proceeded soil, as compared with a detectable organic acid rate for several other treatments at low concentrations. For example, the malic acid was detected at 8349 and 3738 µmol carboxylate g $^{-1}$ soil in the DRW (+) Heilongjiang soil with 0 and 250 rate P supply, respectively. It was followed by the DRW (+) Chongqing soils with P0 treatment supply at 2548 µmol carboxylate g $^{-1}$ soil. At the same time, again the highest level of citric acid was detected under the DRW (+) Heilongjiang soil without P addition at 1036 µmol carboxylate g $^{-1}$ soil, followed by the DRW (+) treated soils with P0 from Beijing at 540 µmol carboxylate g $^{-1}$ soil and Chongqing soil at 286 µmol carboxylate g $^{-1}$ soil.

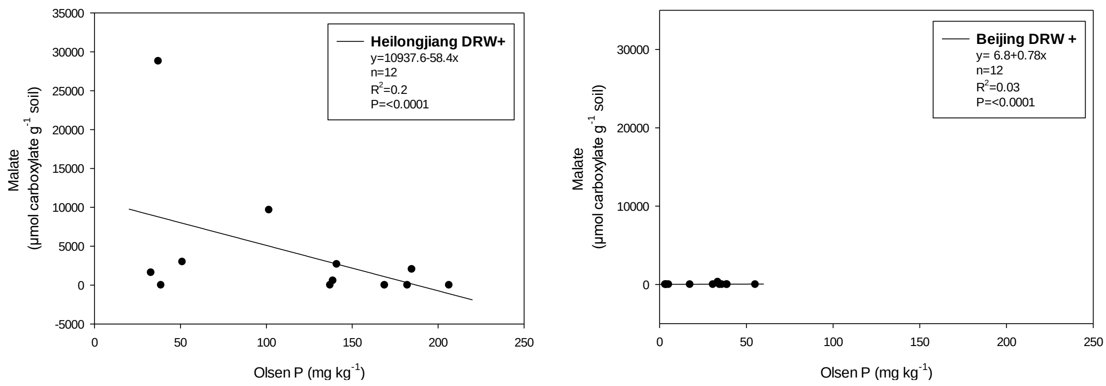
As regards to the excretion of observed dephosphorylation enzymes, the pattern of acid and alkaline phosphatase and phytase was generally similar with negligible differences. The overall increase of all enzyme activities in the rhizosphere were detected in DRW (+) group soils, with illustrative examples of Heilongjiang regions. Acid phosphatase activities were varied in the Heilongjiang soils in the DRW (+) group at 2084 µg PNP h^{-1} g^{-1} (P0), 1809 µg PNP h^{-1} g^{-1} (P250) and 1466 µg PNP h^{-1} g^{-1} (P500); and the DRW (-) group at 1308 µg PNP h^{-1} g^{-1} (P0), 1258 µg PNP h^{-1} g^{-1} (P250) and 836 µg PNP h^{-1} g^{-1} (P500). It seems, the occurrence of gradual increase was observed in the acid phosphatase activities as the P supply rate decreased from 500 mg kg^{-1} to P0 rate, but no material difference of the phosphatase activity observed in the Beijing soils.

Despite the pattern being identical with acid phosphatase, the twice as low alkaline phosphatase activities were identified, except the DRW (+) Heilongjiang soil. For example, regardless of the rate of P application, the level of enzyme activities of the DRW (+) soil group detected under two different pH levels was higher in the acid phosphatase activity of the rhizosphere between (i) 555 and 719 µg PNP h^{-1} g^{-1} in the Shandong, (ii) 1150 and 1406 µg PNP h^{-1} g^{-1} in the Chongqing and (iii) 786 and 836 µg PNP h^{-1} g^{-1} in the Beijing and considerably lower in the alkaline phosphatase at (i) 343 and 513 µg PNP h^{-1} g^{-1} in the Shandong, (ii) 785 and 1003 µg PNP h^{-1} g^{-1} in the Chongqing and (iii) 422 and 630 µg PNP h^{-1} g^{-1} in the Beijing. Whilst the phytase activity of the soil varied without any statistical difference between two moisture treated group, between 17 and 4 µg P ml^{-1}h^{-1} g^{-1} within the maize root medium of DRW (+) group and 4 and 14 µg P ml^{-1}h^{-1} g^{-1} in the DRW (-) Controlled group of all types of soil with and without P addition. The average was 8 and 7 µg P ml^{-1}h^{-1} g^{-1} over DRW (+) and DRW (-) groups, respectively.

DRW (+)







DRW (-)

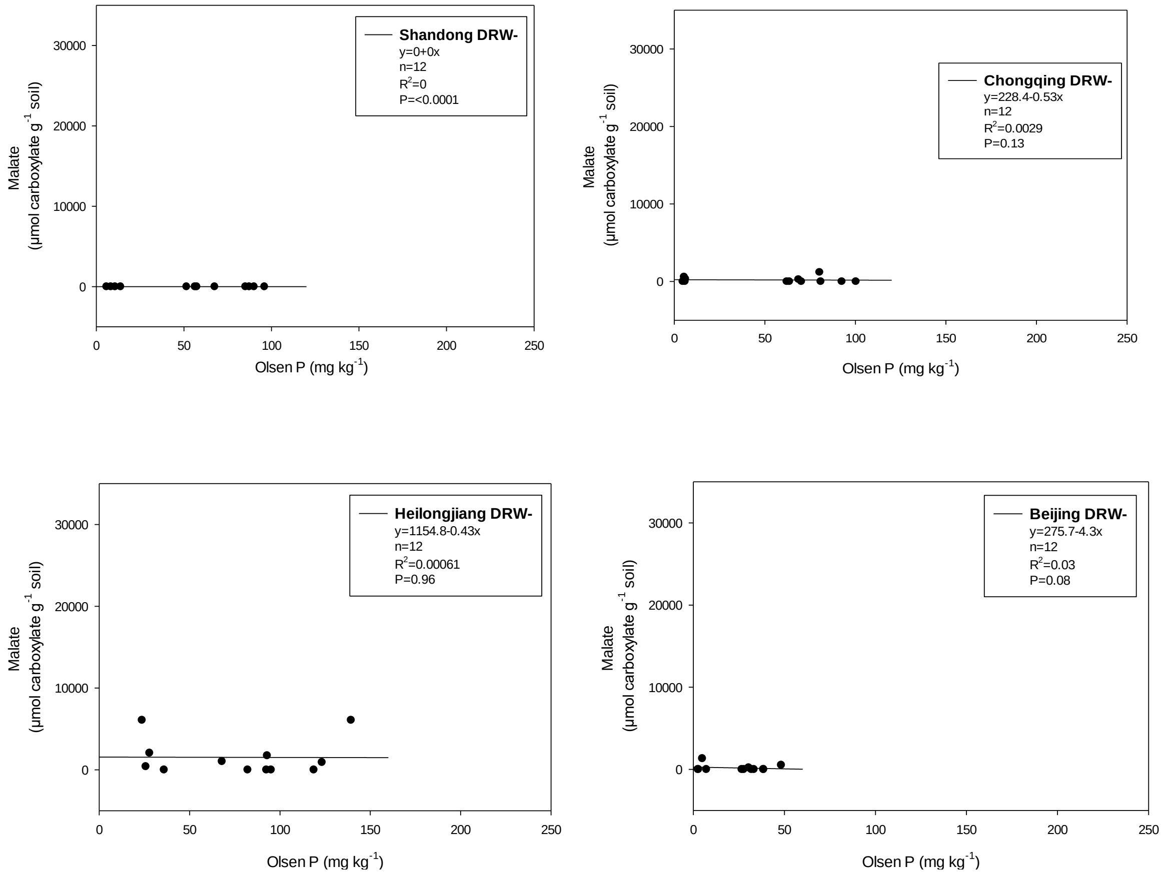


Figure 0.4 The soil physiological responses to the different level of P supply under delayed effect of soil moisture changes, relationship between an emission of malic acid and Olsen P concentration in the drying and rewetting DRW (+) and stable moistened Controlled soils DRW (-).

5.5 Discussion

The results of this experiment clearly demonstrated a relatively large amount of bioavailable P generated from the soil with recent history of DRW. The *d*-DRW effect enables to increase the efficient use of P in the soil through rhizosphere processes and leads with the benefit of the plant performance. The most findings support the hypothesis 8, as stated agricultural soils with a recent history of drying and rewetting (DRW) can trigger P solubilisation in the rhizosphere and a subsequent growth response of maize (*Zea mays*). The evidence provided by the *i*-DRW experiment in the Chapter 4 has been suggested, the DRW events exert a greater stress on the soil biotic and abiotic properties (Frossard et al., 2000), with primary alteration of soil biological activity (Bünemann et al., 2013; Oberson, 2005; Oberson and Joner, 2005; Richardson and Simpson, 2011). Therefore, this impact may not be immediately observable, prior DRW processed soils are later increasingly likely to render an improvement of nutrient availability and crop production in indirect way (De Datta, 1981). As can be seen from the Figure 5.4.1, a comparable difference of the solubilised P level in the rhizosphere between the DRW (+) and DRW (-) Controlled soils allows me to accept the hypothesis 8, for example, Heilongjiang soil under all P treatments and Shandong soil without P addition. However, the *d*-DRW effect rely on the behaviour of different type of soils, which leads to a rejection of the hypothesis in terms of some soil types. This interaction relationship between soil types and recent history of moisture fluctuation is determined by the univariate REGWQ *post-hoc* range analysis under different rates of P loadings for multiple comparison via two-way ANOVA tests (r^2=0.914, $p<0.05$). Further discussion with greater details follows the same flow, used in the order of result section, including soil P dissolution, plant P uptake and rhizosphere enzymatic activities.

5.5.1 Soil phosphorus dissolution

Generally, the P solubilisation of the soil was better in the soil with a DRW background than a soil that did not have a history of DRW in the past when comparing the results of bicarbonate extractable P between DRW (+) and DRW (-) Controlled soil of both experiments in Chapter 4 and 5. Olsen P concentration of four diverse soils from different regions was very variable in the pre-experimental settings, ranged from 2.6 mg kg^{-1} in Beijing, 7.8 mg kg^{-1} in the Chongqing, 22 mg kg^{-1} in the Shandong and 34 mg kg^{-1} in Heilongjiang soil. As plants were introduced in each soil type during the *d*-DRW, the presence of rhizosphere Olsen P category dropped in comparison with bulk soil and soil shortly before maize planting, due to the plant P acquisition. For example, at the harvesting stage, Olsen P in the non-P added conditions between the DRW (+) and DRW (-) Controlled soils fell in the rhizosphere within the range at 4 and 4 mg kg^{-1}; 8 and 6 mg kg^{-1}; 16 and 10 mg kg^{-1}; and 40 and 28 mg kg^{-1} from the regions of Beijing, Chongqing, Shandong and Heilongjiang, respectively. This decline was again apparent with soil types, observed from the smallest (between bulk and rhizosphere soil) to the statistically significant decrease in (I) DRW (+) groups: Shandong P250, Chongqing P250 and P500, Heilongjiang P500 and Beijing P250 and P500; and (II) all soil samples in DRW (-) Controlled group except Beijing without P addition. Likewise, Olsen P in the rhizosphere varied 4-174 mg kg^{-} and 4-116 mg kg^{-1} in same moisture treatment groups, including the level of elevation in each soil type being proportional to the amount of P applied, as already supported by earlier chapter of *i*-DRW effect.

A course of DRW exposures can induce the releases of organic (directly) and inorganic (indirectly) P via subsequent pulse in P availability (Butterly et al., 2009; Styles and Coxon, 2006; Turner et al., 2003) and the following carbon sequestration of soil (Fierer and Schimel, 2003) through the oxidation of soil organic C and Fe and Al associations (Bartlett and James, 1980; Haynes and Swift, 1985; Schlichting and Leinweber, 2002). The soil having the high carbon content, like Heilongjiang soil is a great source of mineralizable organic matter (Boyer and Groffman, 1996) and releases a significant amount of bioavailable P under the repeated DRW events (Chen et al., 2016; Nguyen and Marschner, 2005). However, it has been proposed that increased bioavailable P can be critical to P losses under *i*-DRW (Blackwell et al., 2010; Butterly et al., 2011) via solubilisation and detachment processes described by Haygarth et al, 2005 and potential to plant uptake later under the d-DRW effects (Bünemann et al., 2013) which can help to reduce the relative amount of bioavailable P being leached into water indirectly.

In addition to the rate of P loadings and history of soil DRW, the characteristics of each soil type were likely to reflect a P solubilisation of this experiment. Indeed, the increased soluble P of each soil type is profoundly supported by the P fixing and P buffering capacities of the soils on the basis of soil pH (Lindsay, 1979; Turner and Blackwell, 2013) and concentration of metal cations such as Ca, Fe, Al and organic and inorganic ligands (Hinsinger, 2001). Particularly, Haplic Phaeozems soil with high clay content from Heilongjiang seems vulnerable to *d*-DRW effects (Degens and Sparling, 1995; Goebel et al., 2005), which has a greater potential to release more P than the remaining soil types collected from different agricultural regions. However, the strong positive relation of pulse in soil respiration is not only correlated with clay content of soils (Butterly et al., 2010), but also associated with abundance of P forms in the soils (Stutter et al., 2015), amount of released organic matter (Adu and Oades, 1978), history of soil management, particularly P loading (Evans and Wallenstein, 2012), water stability of soil aggregates, microbial community structure (Chen et al., 2016; Kaiser et al., 2015), and composition (Utomo and Dexter, 1982).

The pattern of Olsen P in the rhizosphere associated with *d*-DRW stimulation (Bünemann et al., 2013a; Lado-Monserrat et al., 2014b) was similar to previous experiment and implied the P solubilisation processes that is achieved through *i*-DRW, discussed in Chapter 4. Hence, a large increase in water-soluble P concentration in this experiment was not the distinctive findings, agreed earlier by Turner and Haygarth, (2001) under soil moisture fluctuation. It has been suggested the relation between the microbial cell lysis (Turner and Haygarth, 2001) and preferential flow of actively growing microbes in P-rich soil (Aponte et al., 2010; Buenemann et al., 2013; Butterly et al., 2009). However, such increased level of solubilised P forms can be an important P source for plant uptake from soil with particularly low P concentration (Styles and Coxon, 2006) and substantial plant growth later under the *d*-DRW effects (Heuck et al., 2015) for example, the fine and coarse loamy textured red Lithosol soils from Chongqing and silt loam textured calcareous Luvic Kastanozems soils from Beijing.

Providing that the recurrent DRW caused by the dynamic precipitation leads to a likelihood of losses of solubilized P into water and reductions SOM, soil microbiota and fertility under the frequent episodes of soil DRW (Fierer and Schimel, 2002; Mikha et al., 2005; Pezzolla et al., 2019). Due to the adaptive strategies of soil microbes under the multiple soil moisture fluctuation (Schimel et al., 1999; Van Gestel et al., 1991) the observable reduction of pulse size has often been found without changing soil microbial biomass (Bagheri-Novair et al., 2020; Chow et al., 2006; Fierer et al., 2003) that may exaggerate the importance of P from non-microbial origin. This can be explained by the physical breakdown of soil aggregates (Denef et al., 2001) that can release protected mineralizable organic matter under the structural integrity of soil (Adu and Oades, 1978) and further increase in mineralisation rate (Fischer, 2009; Kim et al., 2010; Lado-Monserrat et al., 2014a) for prolonged period of time with recent history of DRW. In particular instance, the soil sieving (2 mm) under the preparation stage may cause a sufficient level of physical disruption to release of organic matter that ready to mineralise. However, it is arguable *d*-DRW can enhance the aggregate turnover from cycle to cycle (Denef et al., 2001).

At the same time, the reduction of soluble P may be further associated with an increase in P sorption under the delayed DRW effect that has been found in the earlier studies via resin extraction (Barrow and Shaw, 1980; Olsen and Court, 1982). In the drying period, higher P sorption capacities can buffer against P loss as P applied (Styles and Coxon, 2007). Under the persistence of natural occurring DRW cycles, the adsorption and precipitation reactions can form P compounds with either highly insoluble (Stevenson and Cole, 1999) or less stable non-crystalline (Kodama and Schnitzer, 1980). This is achieved through interaction between P and Fe, Al hydroxides (Walbridge and Struthers, 1993). In the acid soils, P sorption capacity was strongly correlated with concentrations of oxalate-extractable Al and Fe (Richardson, 1985). The red Lithosol soils from Chongqing with pH level at 4.7 could be a case in point, if accurate laboratory analysis of detailed chemo-physical properties were given as examples.

As frequency of events increases, effects of *d*-DRW can be laid in patterns for stable aggregates (Denef et al., 2001) and microbial resilience to disturbance (Griffiths and Philippot, 2013) along with shift in microbial community from bacterial to fungal dominance (Zhang et al., 2007) and long-term biomass accumulation (Fierer and Schimel, 2002). Interestingly, the concentration of water-soluble P in the prior DRW processed Shandong soil with sandy loam characteristic (pH at 7.3) was the considerably highest in this experiment. Whereas, Olsen P sees the lowest reduction rate at the harvest, despite the reasonably high rate in the leachate noted prior to this experiment. It has been suggested that variation of biochemical composition and the physical properties of soils have a great influence in this observation during the experiment. Indeed, sandy soils have been widely recognised as larger air-filled pores than clay soils (Mullins et al., 1990; Mullins et al., 1992). Sandy soils also possess few binding sites for SOM, which can facilitate a faster decomposition (Tahir and Marschner, 2016). Again, laboratory processes for accurate analysis of soil variation including detailed chemo-physical properties per each soil type were a major challenge within the scope for this DRW experiment. A further analysis based on the strength of pH adjustment at the same level will be required to draw a more concrete conclusion of delayed DRW effect on plant performance.

5.5.2 Plant phosphorus uptake

The results showed a difference of observed plant parameters appears to be achieved by the soil type in this experiment. The plant shoot and root morphological parameters varied within the different soil soluble P range driven by *d*-DRW effects under the range of P supply rate. The amount of Olsen P in the soil tended to support the P concentration of plants, which correlated positively with plant growth. Plant growth can be constrained by drought under the i-DRW and low soil P availability (Lynch and Deikman, 1999), for example, affecting crop yield over 80% of bean production in many developing countries (CIAT 2001). Therefore, the direct drying did not introduce on the plant growing in this i-DRW experiment. The discussion will only focus subsequent plant growth. However, plant can optimise their resources allocation under the multiple external constraints, by acquiring through the root morphological and physiological mechanisms (Fernandez and Rubio, 2015; Lambers et al., 2006b; Shen et al., 2011; Vance et al., 2003). These mechanisms are controlled by the plant internal P status, particularly shoot P concentration as well as P availability in the root medium (George et al., 2011; Giehl et al., 2014; Shane et al., 2003a; Shen et al., 2005; Yuan et al., 2016). For example, the shoot P concentration 1-13 mg g^{-1} under the range of rhizosphere Olsen P concentration from 4 to 174 mg kg^{-1} from non-P added (PO) Controlled group of Beijing soil to P added (P500) DRW Heilongjiang soil. Particularly, in low shoot P concentration below 6 mg g^{-1}, there was an increased proportion of fine root observed associated with the low soil P availability of soil.

The fluctuation of biomass from 0.3 to 1.1 g was also proportional to the soil soluble P and shoot P concentration except all Control Group of Shandong soils. To maintain biomass, it is important to enhance P acquisition (Miguel et al., 2015; Postma et al., 2014; Zhang et al., 2012; Zhu et al., 2005; 2010) through the adaptive strategies to improve P use efficiency (PUE), if the resources are limited. A good example of the statistically significant P uptake was presented in all non-P added DRW soils from different regions, except Heilongjiang. As shoot biomass per unit P in shoot can define PUE (Rose and Wissuwa, 2012) plants with higher PUE operate under lower shoot P concentration as opposed to root (Rose et al., 2013). The root P concentration constituted from 0.7 to 2.4 mg g^{-1} in DRW (+) Beijing soil without P addition and DRW (-) Control Chongqing soil with P addition at 500 mg kg^{-1} can estimate the root responses. However, the plant adaptive strategies evolving to cope with demands include the root elongation and more branched root systems with finer roots and lateral roots coupled with increased SRL and R/S ratio. The strategies can increase the root surface area available for soil exploration and acquisition of soil P at a minimal energy cost (Bates and Lynch, 2001; Gaume et al., 2001; Hajabbasi and Schumacher, 1994; Lambers et al., 2013; Liu et al., 2004; Lynch, 2015; Ryan et al., 1996).

The total root length and SRL are a universal feature of root responses to nutrient availability in the soils of variable climatic conditionings (Hasegawa et al., 2016; Li et al., 2016; Zhang et al., 2012). The formation of new roots was observed under the soil respiration derived from soil microbial communities (Hanson et al., 2000), through the increased forage capacity and carbon investment belowground (Mollier and Pellerin, 1999, Fernandez and Rubio, 2015). Whereas, the shortest roots were observed under the DRW (-) Controlled Beijing soils at the P0 treatment having lowest SRL with highest proportion of fine root. This may be associated with inhibition of root growth caused by decreased leaf growth and subsequent photosynthesis (Mollier and Pellerin, 1999; Plénet et al., 2000). Yet, when compared with shoot growth, the root growth is less inhibited by lower level of soil soluble P (Marschner, 2011). For example, the greatest increase in root length was identified in the soil from both groups of Shandong without any P supply with appearance of significantly higher SRL and fine root proportion, however, the longest root was disproportionate notably to the shoot biomass that was almost the same as the Beijing soils.

The root shoot ratio depends upon the partitioning of photosynthesis, which may be predominantly influenced by the environmental stimuli. Root biomass accounting for 12-22% of the total biomass (Qi et al., 2019) usually declines in the late flowering (Husáková et al., 2016). However, the R/S ratio is considerably higher in dryland areas than temperate climates (Lynch et al., 2012). In this experiment, the R/S ratio of both DRW (+) and DRW (-) Controlled soils varied considerably, as a result of the wide alteration in shoot and root biomass. We can still see some general patterns, as the R/S ratio of the DRW (-) Controlled Group plants with a supply of P at zero rate being higher than the plant in the DRW (+) soil group, except the silt loam textured calcareous Luvic Kastanozems soils from Beijing soil with pH at 8.9. In the Beijing soil, the R/S reached at 0.8 in the DRW (+) group at P0 treatments supply, being one of the highest ratios seen at low P bioavailability. This is followed by the R/S ratio seen in non-P added DRW (-) Controlled soil from Shandong region, which was considered exceptionally high in corresponding soil soluble P presence before this experiment. That being said, otherwise opposite direction of such premise of R/S indicated in comparatively higher the soil P of Olsen P at 22.3 mg kg^{-1} sandy loam textured brown Cambisol soils from fallow land in Shandong with pH 7.3. In the Shandong soil, the increased specific root length occurred upon low shoot P concentration, which was associated

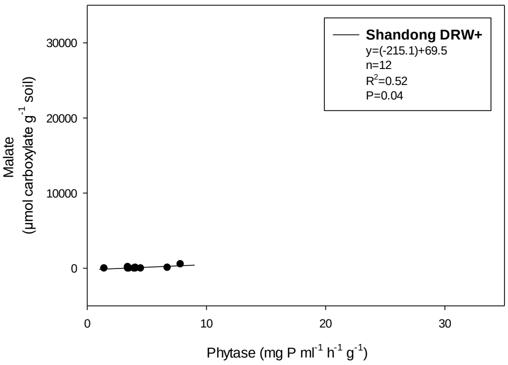
(Table 5.4.1) with a decreased metabolic demand per unit of root length (Eissenstat, 1992; Zobel et al., 2007).

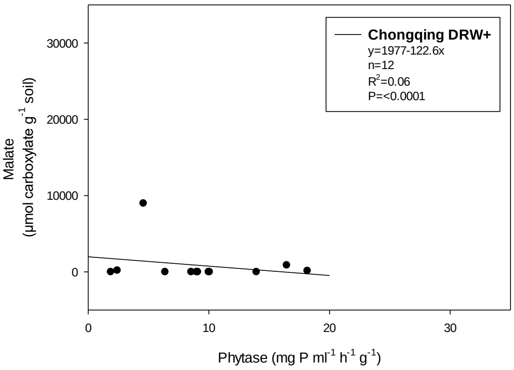
5.5.3 Rhizosphere excretions and soil pH changes

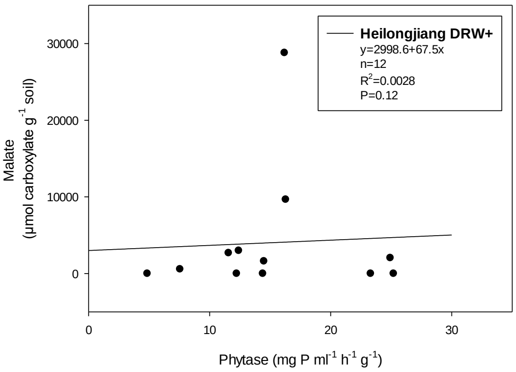
Carboxylate exudation and dephosphorylation enzyme activities are the main representative of adaptive physiological responses that can effectively increase P uptake of maize via modifying pH and solubilising P under varying P inputs (Hinsinger et al., 2005; 2003; Rengel and Marschner, 2005) often accompanied with root morphological changes (Lambers et al., 2006; Shen et al., 2011; Vance et al., 2003). The improved level of efficient P uptake of plants under *d*-DRW exposure appeared to be achieved by soil types and their underlying biochemical features around the root. As compared with root morphological changes, the physiological responses of maize have not been fully clarified to the different rates of P application. It may be related to the P acquisition of maize that heavily rely on root morphology than physiology. Therefore, the observed decline in rhizosphere pH of all soil samples used in variable treatments of this experiment was not statistically significant, with the biggest decline of 2 units seen in the non-P added DRW (+) soil from Beijing with initial Olsen's P at 2.6 mg kg^{-1} (pH 8.9). This result was already agreed by some researchers (George et al., 2002a; Liu et al., 2016) that variable P conditionings do not often induce statistically significant rhizosphere acidification, even in the P deficient condition. However, these results may be influenced by increased proton via exerting an uptake of cation (Braschkat and Randall, 2004) or being different from other research works in terms of experimental design including level of solubilised P in soil, stage of planting (Wouterlood et al., 2004) and properties of growth medium (Carvalhais et al., 2011).

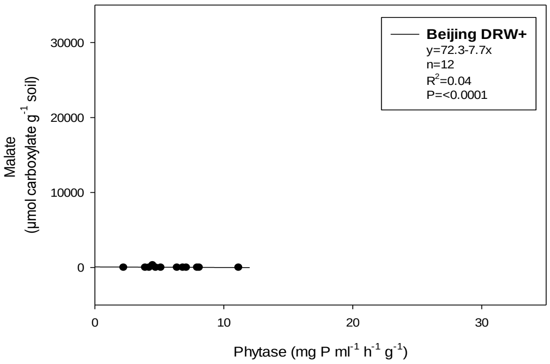
The results generally demonstrated that changes of pH were slightly higher in the prior DRW (+) treated soils than DRW (-) Controlled soils, where the greatest amount of detectable organic acid exudation was observed in the rhizosphere. At the same time, pH level decline in the soils with P0 treatment was also higher than remaining P added soils combined with the greatest number of detectable carboxylates except Shandong soil. Similar findings were reported previously under P deficiency (George et al., 2002; Hinsinger et al., 2003; Rengel and Marschner, 2005) that estimated the value of root exudate in the rhizosphere acidification. Under the P deficiency, increased level of carboxylates can chelate metals to bind organic P in humic substances, thus to dissolve organic P sources (Braum and Helmke, 1995) available for root phosphatase activity (Jungk et al., 1993) and to increase dephosphorylation enzyme activities indirectly (Johnson et al., 1996). The figure below showed how the level of carboxylate exudation under DRW processed affected dephosphorylation enzyme activities by using a malic acid and phytase as an example.

DRW (+)









DRW (-)

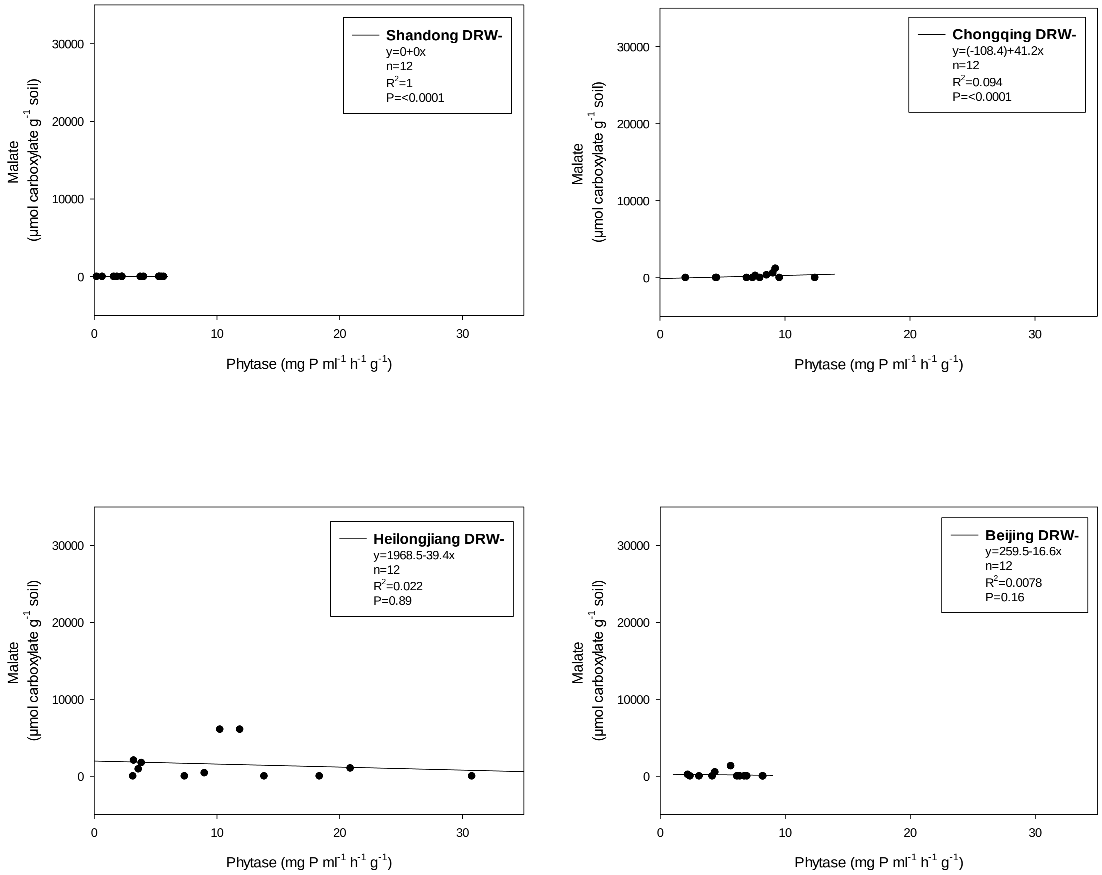


Figure 0.1 The relation between malate and phytase in the drying and rewetting DRW (DRW +) and non- drying and rewetting stable moistened Controlled soils (DRW -).

In the rhizosphere, the production of phosphatases has a potential to enhance P availability of soil, if a large proportion of soil P is presented as organic forms with great composition of phytate (Richardson, 2005). An increase in acid phosphatase activities was observed within the root medium of non-P added soils under the *d*-DRW effect along with phytase activities to enhance P solubilisation processes and plants P acquisition, which may be associated with low P status of plants (Gilbert et al., 1999). However, speculation of the higher enzymatic activities in Chongqing and Heilongjiang soils give an importance to biological behaviour of soil types, which may be related to the respiration of soil among the metabolic sources derived either from autotroph by roots and mycorrhizae and heterotroph by decomposers associated with microbial community composition, decomposition rates and soil organic matter (Bais et al., 2006; Jones et al., 2009; PR Ryan et al., 2001).

Organic acids and enzymes are also excreted from the soil microbes, which is hard to distinguish unless with using non-sterile soil as a control. The fundamental methodology in measuring soil microbiological characteristics, apart from the dephosphorylation enzymes and organic acid per each soil type, in relation with *d*-DRW effects was a major challenge and outside the scope of this experiment. The observed increase in enzymatic activities of different types of soils with recent history of DRW explains, at least in part, the increase in plant growth; as such further research work would be useful to gain better understanding of soil type biological specificity under the parallel-sterilised condition in order to compare the role of adaptation mechanism for the improvement of plant performance under the prior DRW soils.

5.6 Conclusion

This experiment studied the *d*-DRW by testing the hypothesis that agricultural soils with a recent history of drying and rewetting (DRW) can trigger phosphorus (P) solubilisation in the rhizosphere, with a subsequent growth response of maize (*Zea mays*). It followed on from the same experimental structure used in Chapter 4 that focussed on immediate effects of DRW. This study implies, there a time lag between changes of soil moisture and the resulting responses of the soil. The most positive results were obtained from the Haplic Phaeozems soil of Heilongjiang, leading to an acceptance of the hypothesis. However, silt loam textured Luvic Kastanozems soil sample from Beijing showed different results, leading to a rejection of the hypothesis. It seems therefore increasingly likely that time-dependent nature of soil responses generated during the DRW may have a potential to reduce the leaching of a relative amount of bioavailable P in the soil by introducing plants through support from efficient rhizosphere processes. The soil responses can benefit later the plant performance. This moisture fluctuation experimental approach to study the post DRW should be used in both situation in the lab or field, before the soluble P has decreased via the sorption, microbial immobilisation and leaching, on a benefit of plant growth in wider range of soils to further elucidate the processes of enhance plant uptake of P released after DRW

CHAPTER 6

Discussion and conclusion

6.1 Introduction

This final chapter provides a review, synthesis, and conclusion of the work in the previous five chapters. The PhD has focussed on understanding phosphorus (P) solubilisation processes in the rhizosphere, with respect to drying and rewetting (DRW), in an immediate and delayed way. Here, the objectives and hypotheses will be revisited with a summary of the key findings from each of the experimental chapter, revisited from a final wider and more integrated perspective, and future work is proposed.

In this thesis, I have tested the eight hypotheses, each framed under the original overarching hypothesis that P solubilisation in the rhizosphere can be affected by climate driven changes of soil associated with immediate and delayed effect of DRW events and root-zone acidification under variable P amendments, with a particular consideration of the effects of low-grade phosphate rock. The summary of the work, including seven hypotheses, approaches and matching key results are shown as flow chart in Figure 6.1.

6.2 The key findings and conclusions

In this section I revisit the specific conclusions from each chapter and present a general conclusion from the composite results of P solubilisation processes in the rhizosphere reached from the works of this thesis. Data from these smaller scaled controlled experiments demonstrated the different levels of P solubilisation under variable P exposures (e.g. limitation and enrichment). More specifically, the DRW stimulation of soil appears to increase the P solubilisation processes in the rhizosphere. In a state of rewetting, the solubilised P in the soil solution has an increased potential for being leached out, with drainage water, which would reduce crop benefits and contribute water pollution. The onward effect of the climate changes can significantly amplify these phenomena of P losses and potentially threaten global food security (Cordell et al., 2009; Wang et al., 2016) and the stability of aquatic systems. Therefore, optimal P concentration in soil solution is critical, not only for the maintenance of water quality, but also for the improvement of crop production in the agriculture (Pierzynski and McDowell, 2005). The P solubilisation in the rhizosphere as a part of soil P cycling is now more vulnerable under climate change.

The thesis studied: (a) potential rhizosphere management opportunities to increase the efficiency of different P supplies (Chapter 3), via a rhizosphere engineering approach to manipulate root-zone acidification, and; (b) P solubilisation and potential transfer (Chapter 4) under the immediate effects of DRW events (*i*-DRW). The latter was followed by the subsequent plant growth (Chapter 5), under delayed effect of DRW events (*d*-DRW), in order to link two levels of P solubilisation processes, one was established in previous study (Chapter 4). This chapter considers to discuss an inclusion of rhizosphere into the P transfer continuum in order to expand the conceptual four-tiered framework model (Haygarth et al., 2005a) under the extreme climate events (Forber et al., 2018). The rhizosphere is the key zone controlling P solubilisation, as a subcomponent mobilisation in the wider transfer continuum.

Overall, chapters 3 and 5 provided data that showed P source and concentration, rhizosphere manipulations, and accumulative delayed effect of recurrent DRW events, which all had significant impacts on P solubility of soil and plant growth. Specifically, the evidence obtained from the experiments in chapter 3 emphasize the direct application of low-grade phosphate rock (PR) that may benefit the plant growth under neutral soil pH near root zone acidification. In the experiment in chapter 5, the P solubilisation in the rhizosphere of associated the post DRW soil condition has practical implications in the crop productivity over arid agricultural regions, particularly soils with low P availability used in this experiment for example, Beijing and Chongqing soils. Whereas the level of

P solubilisation in the parallel study (chapter 4) using non-planted soil has been noted, without any statistical significance itself when soils were immediately treated with a cycle of four DRW events. However, a direct association between behaviours of P solubilisation processes in the rhizosphere and *i*-DRW stimuli, suggests the needs of more data that can identify the effects of excretion processes in particular.

Here is a summary of the specific conclusions:

Objective 1: To assess the link between behaviour of P dynamics and P solubilisation processes in the rhizosphere, with corresponding plant performance under various levels of different P exposure, using different types of PR, calcium perphosphate monohydrate and urea phosphate. I hypothesised that different types of P fertilisers have differing consequences on the P solubilisation in the rhizosphere.

Conclusion (a).

The hypothesis that different types of P fertilisers have differing resulting effects on the P solubilisation in the rhizosphere was accepted. Different types of P supply at varying rates in the rhizosphere and the efficiency of rhizosphere processes resulted in a substantially different level of P solubility in the soil and corresponding plant performance (growth and biomass production). The efficiency of P solubilisation processes in the rhizosphere varied, increased rate of solubilised P in the rhizosphere corresponds with improved plant performance.

Objective 2: To study the effects of rhizosphere P solubilisation processes, in combination with different types of root zone acidification in the soils treated with low-grade PR. I hypothesised that the emission of organic acids in the rhizosphere at near neutral soil pH increases the P solubilisation of soils with different levels of P exposure.

Conclusion (a). The hypothesis that emission of organic acids in the rhizosphere at near neutral soil pH increases the P solubilisation of soils with different levels of P exposure was accepted. The excretion of organic acids along with dephosphorylating enzymes in the rhizosphere can increase the P solubilisation processes of soil under the root induced changes of soils, via chemical acidifiers and intercropping attempts.

Conclusion (b). The application of low-grade PR onto the soil could help to build a viable case for sustainable use of P in the future, as the P solubilisation of the soil treated with direct application of low-grade PR increased under the different rhizosphere conditionings associated with various forms of chemical acidifiers and intercropping practice. However, it seemed that direct application of low-grade PR alone cannot fundamentally meet the demand of large maize yield, unless it can be accompanied with the efficient rhizosphere approaches that alters the soil acidity.

Objective 3: To study the immediate effects of DRW on the dissolved P forms in both soil solution and leachate water, using a detailed assessment of P solubilisation processes in four contrasting soil types, sampled from different agricultural regions of China, and with different P fertiliser application rates. It tested the following five hypotheses: (1) DRW increases the P solubilisation (and thus the potential for leaching); (2) the amount of solubilised P has a potential for leaching, being proportionate to the rate of P fertiliser applied; (3) dephosphorylating enzymes and organic acids affect the P solubilisation in the rhizosphere after DRW; (4) the different soils respond differently to DRW, and (5) the forms of P in leachate water will vary depending on DRW event cycles and dynamics.

Conclusion (a). The DRW did not increase the level of P solubilisation (and thus the potential for leaching), for which hypothesis (1) DRW increases the P solubilisation (and thus the potential for leaching), was not accepted. This study revealed uncertainty and ultimately highlighted the need for more research in the future, with a greater range of experimental designs and constraints.

Conclusion (b). The positive correlation between level of soil soluble P concentration and the rate of P supply applied into the soil has a great reflection on the quantity of P being penetrated through the soil into the leachate. Therefore hypothesis (2) The amount of solubilised P has a potential for leaching, being proportionate to the rate of P fertiliser applied was accepted.

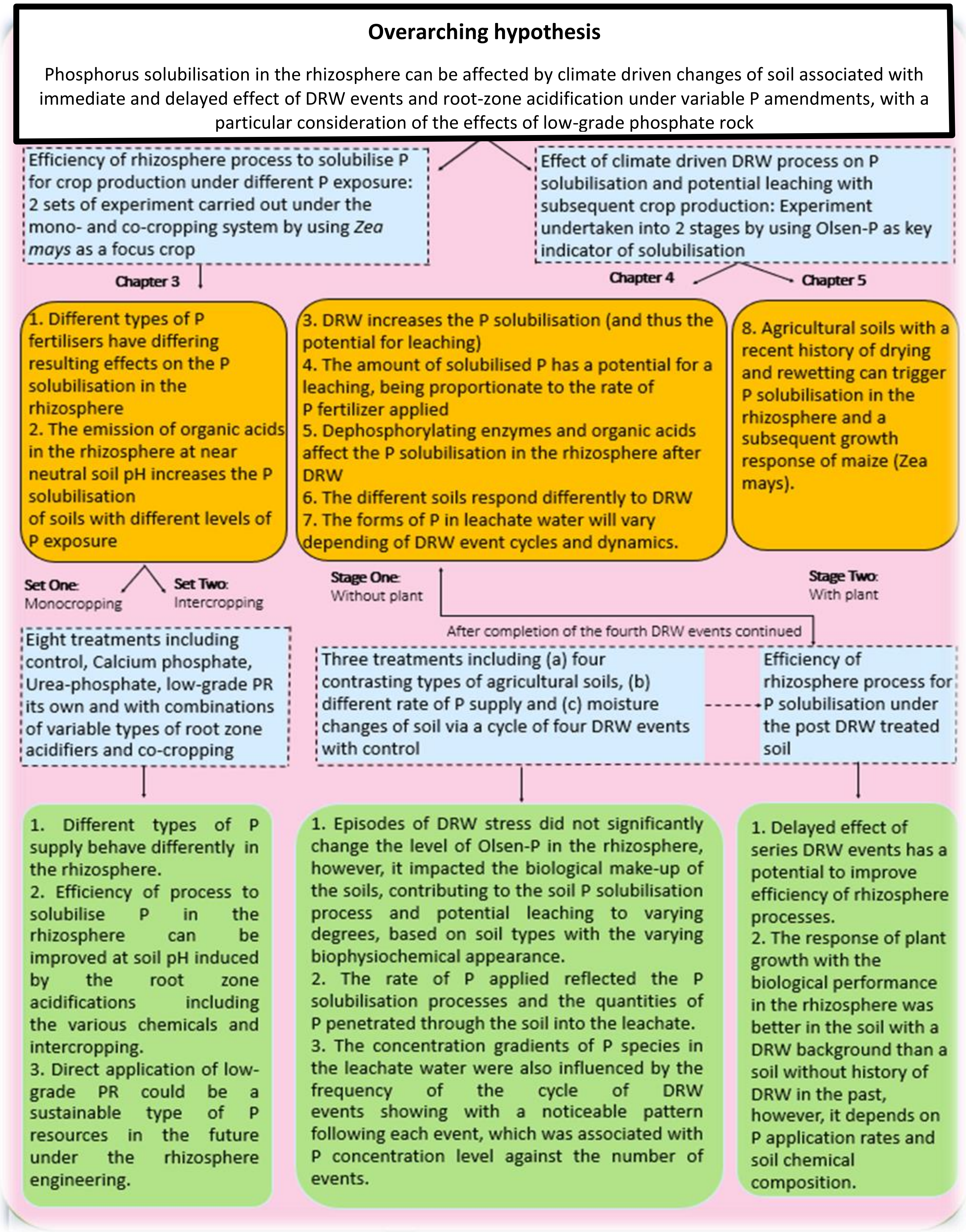
Conclusion (c). The frequent i-DRW events have had a significant impact on the biological makeup of soil. The significance of biological processes may contribute the P solubilisation processes and potential leaching at different rates, depending on the level of excreted dephosphorylating enzymes and organic acids in the rhizosphere. However, the soil responses to the DRW stimulation *varies considerably between soil types. Consequently, both hypotheses (3) Dephosphorylating enzymes and organic acids affect the P solubilisation in the rhizosphere after DRW; and (4) the different soils respond differently to DRW, were accepted.*

Conclusion (d). The concentration gradients of P species in the leachate water under the frequent soil moisture pressures fluctuated over DRW event cycles, showing a noticeable response to the DRW events. I can therefore accept the hypothesis (5) The forms of P in leachate water will vary depending on DRW event cycles and dynamics.

Objective 4: To observe the possible delayed effect of soil DRW stresses by studying P solubilisation in the rhizosphere, plant P acquisition and performance, and root growth after the previous history of series of DRW events, combined with different types of agricultural soils of varied bio-physiochemical characteristics. I hypothesised that agricultural soils with a recent history of drying and rewetting can trigger P solubilisation in the rhizosphere and a subsequent growth response of maize (*Zea mays*).

Conclusion (a). These results led to the acceptance of the hypothesis that 'Agricultural soils with a recent history of drying and rewetting can trigger P solubilisation in the rhizosphere and a subsequent growth response of maize (Zea mays)'. That a post DRW effect on the agricultural soils can be beneficial and has wider implications for regulation of agricultural practice in arid agricultural regions.

Conclusion (b). The post DRW effect on the agricultural soils can be beneficial and has wider implications for regulation of agricultural practice in arid regions.



Overarching hypothesis

Phosphorus solubilisation in the rhizosphere can be affected by climate driven changes of soil associated with immediate and delayed effect of DRW events and root-zone acidification under variable P amendments, with a particular consideration of the effects of low-grade phosphate rock

Efficiency of rhizosphere process to solubilise P for crop production under different P exposure: 2 sets of experiment carried out under the mono- and co-cropping system by using *Zea mays* as a focus crop

Effect of climate driven DRW process on P solubilisation and potential leaching with subsequent crop production: Experiment undertaken into 2 stages by using Olsen-P as key indicator of solubilisation

1. Different types of P fertilisers have differing resulting effects on the P solubilisation in the rhizosphere
2. The emission of organic acids in the rhizosphere at near neutral soil pH increases the P solubilisation of soils with different levels of P exposure

3. DRW increases the P solubilisation (and thus the potential for leaching)
4. The amount of solubilised P has a potential for a leaching, being proportionate to the rate of P fertilizer applied
5. Dephosphorylating enzymes and organic acids affect the P solubilisation in the rhizosphere after DRW
6. The different soils respond differently to DRW
7. The forms of P in leachate water will vary depending of DRW event cycles and dynamics.

8. Agricultural soils with a recent history of drying and rewetting can trigger P solubilisation in the rhizosphere and a subsequent growth response of maize (Zea mays).

Eight treatments including control, Calcium phosphate, Urea-phosphate, low-grade PR its own and with combinations of variable types of root zone acidifiers and co-cropping

Three treatments including (a) four contrasting types of agricultural soils, (b) different rate of P supply and (c) moisture changes of soil via a cycle of four DRW events with control

Efficiency of rhizosphere process for P solubilisation under the post DRW treated soil

1. Different types of P supply behave differently in the rhizosphere.
2. Efficiency of process to solubilise P in the rhizosphere can be improved at soil pH induced by the root zone acidifications including the various chemicals and intercropping.
3. Direct application of low-grade PR could be a sustainable type of P resources in the future under the rhizosphere engineering.

1. Episodes of DRW stress did not significantly change the level of Olsen-P in the rhizosphere, however, it impacted the biological make-up of the soils, contributing to the soil P solubilisation process and potential leaching to varying degrees, based on soil types with the varying biophysiochemical appearance.
2. The rate of P applied reflected the P solubilisation processes and the quantities of P penetrated through the soil into the leachate.
3. The concentration gradients of P species in the leachate water were also influenced by the frequency of the cycle of DRW events showing with a noticeable pattern following each event, which was associated with P concentration level against the number of events.

1. Delayed effect of series DRW events has a potential to improve efficiency of rhizosphere processes.
2. The response of plant growth with the biological performance in the rhizosphere was better in the soil with a DRW background than a soil without history of DRW in the past, however, it depends on P application rates and soil chemical composition.

Figure 0.1 Summary of thesis including the conclusions and relevant to the research hypothesis

Summary of thesis highlighted in colour: box for overarching hypothesis is white, sub-hypotheses are orange, for research approaches and processes are blue, and for key findings are green.

6.3 Integrated summary of key findings

Consideration of P mobilisation via solubilisation process in the rhizosphere under the changing climate patterns, especially seasonal precipitation, is critical not only for water quality, but also for the sustainability of agriculture (George et al., 2011; Ockenden et al., 2016). Understanding the soil DRW effects on rhizosphere microbial activities as a driver of the P solubilisation process, adds further complexity to this. This thesis therefore aimed to understand and consider ways to improve P use efficiency (PUE) of different types of P application in crop yield, particularly low-grade PR supply, whilst reducing the impact of P transfer, based on the new understanding of the effect of DRW on P solubilisation in the rhizosphere. A summary of introduction with inclusion of the general concepts are shown in the figure 6.3.1.

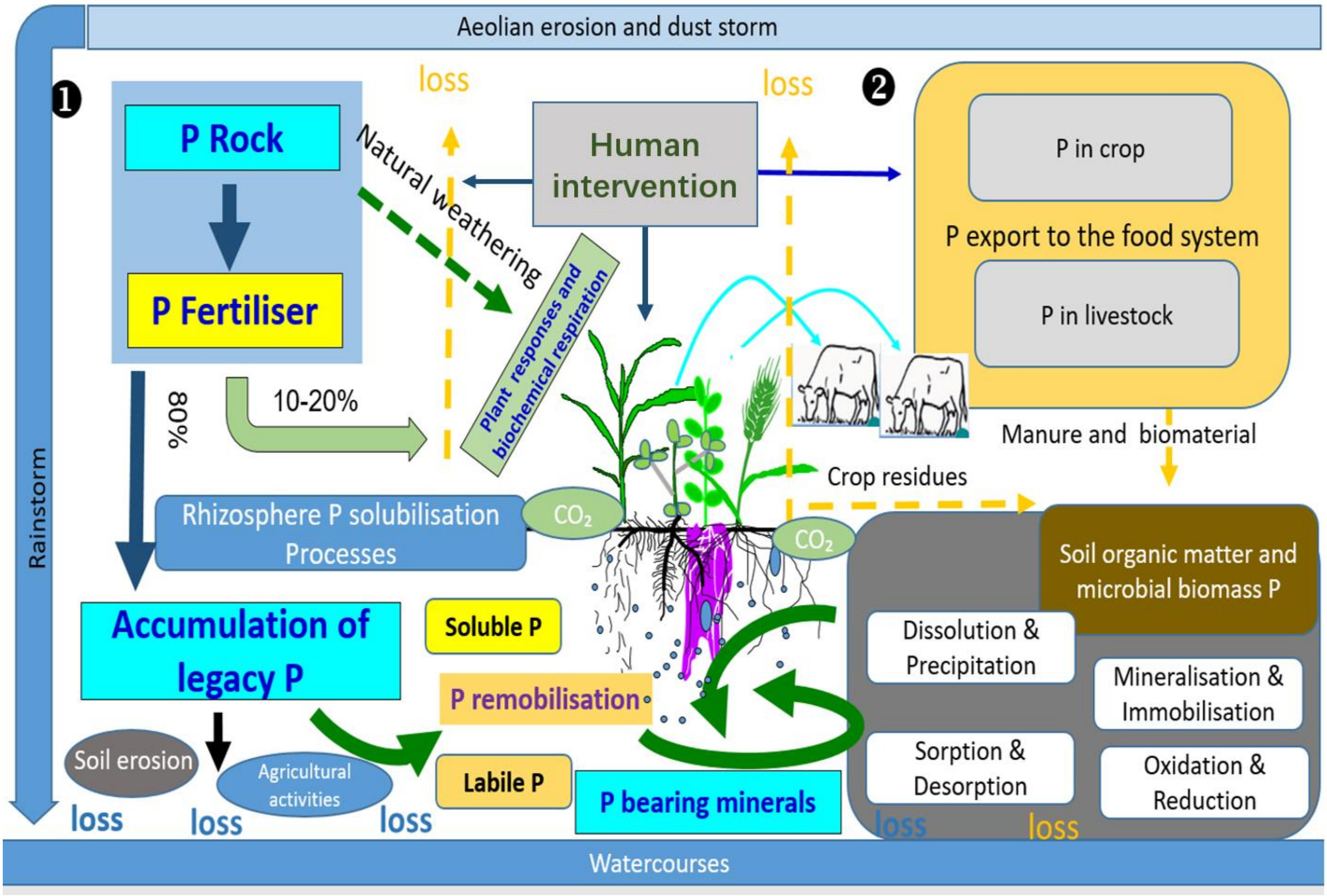


Figure 0.1 Schematic representation of soil phosphorus cycling

The plant response to the soil soluble P concentration demonstrated in the Chapter 3 was achieved by different types and rates of P fertiliser application, particularly low-grade PR included with addition of root zone induced pH amendments of the rhizosphere. A root mycorrhizal inoculation was also considered in this experiment. Given the results (Chapter 4 and 5), there are two levels of dynamics occurring here: First is the rapid (immediate) response of soils to DRW: *i*-DRW immediately induces onward leaching losses of P through the soil depth; and Second is a longer-term response of soils to the cumulative effect of frequent DRW events: *d*-DRW has a potential to improve P solubilisation processes and the subsequent plant, which was discussed in detail in Chapter 4 and 5 respectively. The impacts of soil *i*-DRW and *d*-DRW on P source and concentration and subsequent plant responses (positive or negative), which were identified in the chapter 4 and 5, were also reported by Sawada et al (2019).

Generally, the works in the thesis attempted to address the considerable global concerns related with P supply (Cordell and White, 2011) and the sustainability of P sources in the long-term (Withers et al., 2015). The existing literature reports, 82% of total P manufacturing has been used for fertiliser supply in food production (Lun et al., 2018; Ringeval et al., 2017; Schroder et al., 2010), whilst scarcity of high-grade P sources may threaten global food production in the long term (Cordell et al., 2011). Despite concerns over P supply and potential security, a significant amount of low-grade PR still exists which could address such concerns and meet agricultural demands into the future. However, there is little evidence to support the suitability of low-grade PR in the agricultural context to address such knowledge gap. In this thesis, the different types of P fertiliser application, particularly widely available sustainable types of low-grade PR were employed to demonstrate their behaviour in the rhizosphere for the favour of plant P benefits (plant P content and biomass accumulation). Specifically, in chapter 3 the application of low-grade PR was comprehensively assessed under the impacts of rhizosphere engineering approaches, by adapting the root induced changes of soil in combination with chemical acidifiers and intercropping system.

In my studies, I have used small pot experiments (2 kg in chapter 3 and 0.3 kg in chapters 4 and 5) with addition of four different rates of P application (0, 75, 250 and 500 mg kg^{-1}). I intentionally chose a high P dose value of 500 mg kg^{-1} to the soil by a single application. This range of P applications allowed me to observe (a) the subtle changes of solubilisation process in the rhizosphere with corresponding plant growth over a short practical period (reported in the chapter 3 and 5) and (b) the rate of P solubilisation in the soil and leaching loss of P with drainage water during the *i*-DRW (in the chapter 4). Results reveal that increased levels of P supply applied to the soil are likely to solubilise the larger amounts of P under all experimental designs (a) rhizosphere engineering approach in chapter 3 and (b) frequent DRW stimuli in chapter 4 and 5, resulting in improved plant growth and a greater amount of P leaching in the drainage water. The P application rate affect will of course have wider scale affects and is commensurate with the observations of others, particularly related with a risk of P loss from soils to the waters (Foster et al., 2003; Sharpley et al., 2013). However, of data produced from small scale experiments do yield uncertainties, thus need to be further verified with model predictions and studies in the field.

The rate of P supply reviewed in the chapter 2, was principally demonstrated by the evidence in chapter 4, where the amount of P leaching following DRW was proportionate to the rate of P application history. At the global scale, anthropogenic P input to agricultural land is the dominant driving factor for P transfer to water (Campbell et al., 2017; Collins et al., 2016; MacDonald et al., 2016; Withers et al., 2020). For example, Bouwman et al., 2013 estimated, 4 Tg. P y^{-1} transferred into aquatic systems in 2000, because of fertiliser application and manure recycling (Turner and Haygarth, 2001, Blackwell et al., 2009, Blackwell et al., 2013, Bunemann et al 2013). The movement of this added P is likely to increase dramatically under the climate change induced variable weather events, as far as volume and intensity of rainfall concerned (Ockenden et al., 2016 and Forber et al., 2017).

Future choices about the uses and amounts of fertiliser in the field and the management planning for right quantities and the right form of preferential P sources (Dijkstra et al., 2013; 2012) are a great concern around the world. New P fertiliser applications must match crop P demand of soils. In the

UK this must be based on the soil test to determine a solubilised P level in the soil (Defra, 2018), for vegetable crops this is no greater than 45 mg P L^{-1} and for forage crops this is no greater than 25 mg L^{-1}, (Nutrient Management Guide RB 209). However, it is important to recognise that different soils behave differently (Sharpley et al., 2000), which makes the fertiliser planning more challenging.

Whilst it has been shown that with the installation of mitigation measures in catchments can result in reduction in total P loading into waterways (Withers et al., 2000) of up to 15% (Collins et al., 2016), P losses are still predicted to increase up to 30% by the 2080s under climate change (Ockenden, 2017). Therefore, as commensurate with the aims of this thesis, it remains essential to assess the potential impacts of *i*-DRW and *d*-DRW on P solubilisation processes of the rhizosphere help us to understand any soil primed effects of recurrent short-term changes of moisture, dealt with chapter 4 and 5. This enabled me to observe the decoupling impact of rhizosphere processes in the non-planted (chapter 4) and planted soils (chapter 5) under the DRW treatment, by (a) the level of solubilised P in both in the leachate water and the soil solution after each rewetting event and final episode of series DRW events, and (b) the subsequent plant growth on the post DRW soil.

The rhizosphere processes are highly complex and dynamic in nature, in space and time and thus vary considerably with the degree of intensity, rate and frequency of DRW and length of drying time (Khan et al., 2019, Forber et al., 2017, Ockenden et al., 2016). The role of rhizosphere in these systems was supported by the data captured from both chapters, where dephosphorylating enzyme increased significantly associated with moisture fluctuation, even there was non-significant increase of Olsen-P in the soil under the *i*-DRW (chapter 4). Under the improved exudative processes in rhizosphere, DRW stimulation seems to be a key factor to shift the modes of mobilisation between solubilisation and physical detachment (Haygarth et al., 2005, Forber et al., 2018).

The degree of P mobilisation depends on not only rate of P application, but also soil type and the nature of DRW processes. Across all treatments of *i*-DRW, the total P in the leachate has been changed over the cycle number, with a great deal of fluctuations throughout experiment, which may be associated with soil priming effect due to changing soil moisture. In most field cases, if solubilised P is not captured by the plant and surrounding symbiotic or non-symbiotic microorganisms (Chen et al., 2016; Chiu and Paszkowski, 2019; Schachtman et al., 1998; Vorholt, 2012) or fixed with soil minerals (Arai and Livi, 2013; Bünemann et al., 2013; Dean, 1949) during the incubation period (Chen et al., 2021; Chen et al., 2016; Scalenghe et al., 2012), the vertical P transfer is likely to occur via leaching throughout soil depth (Haygarth et al., 1999; McDowell et al., 2021; Turner and Haygarth, 2000) under rewetting. Beyond the findings of an increase in rhizospheric activities associated with soil *i*-DRW (chapter 4), the changes of P solubilisation process under the extreme climate events could probably suggest both the leaching loss of the freshly applied P and accumulation in the soil over decades (Power et al 2016). However, the extended impacts of repeated DRW (chapter 5) reveals potential exchange of P between pools within the soils, particularly from the legacy stores (Bünemann et al., 2013; George et al., 2002; George et al., 2005; Giles et al., 2017; Power et al.,2016).

The equilibrium P balance between absorption and desorption is an important consideration for P flows around at the root medium (Rose et al., 2013), with implications for crop production. In addition, the wider plant responses to soil with history of DRW is potentially very important for many hectares of land used for agricultural production (Lacerda-Júnior et al., 2019; Reynard et al., 2001).

The chapter 5 was a follow up experiment of the previous chapter that was responsible to continue carrying on *i*-DRW experiment with plant growth. The example of maize was used here to demonstrate how plants may manipulate the underlying process of P solubilisation beyond -DRW effects for in these systems. Four different types of soils were used in this experiment. The improved level of plant performance on the post DRW soils suggested behaviour of P solubilisation processes in cropped land responds differently than that of uncropped land. A potential cause of increased level of subsequent plant growth observed may be related with efficiency of the processes in the rhizosphere, under delayed DRW effects (Bünemann et al., 2013; Chepkwony et al., 2001; Dodd et al., 2015; Van Gestel et al., 1993). The findings of chapter 5 may potentially have a major agronomic benefit through the increased levels of P solubilisation processes at the rhizosphere, with corresponding biomass production and plant P value. However, a potential impact on plant growth, under delayed DRW effects, remain unclear.

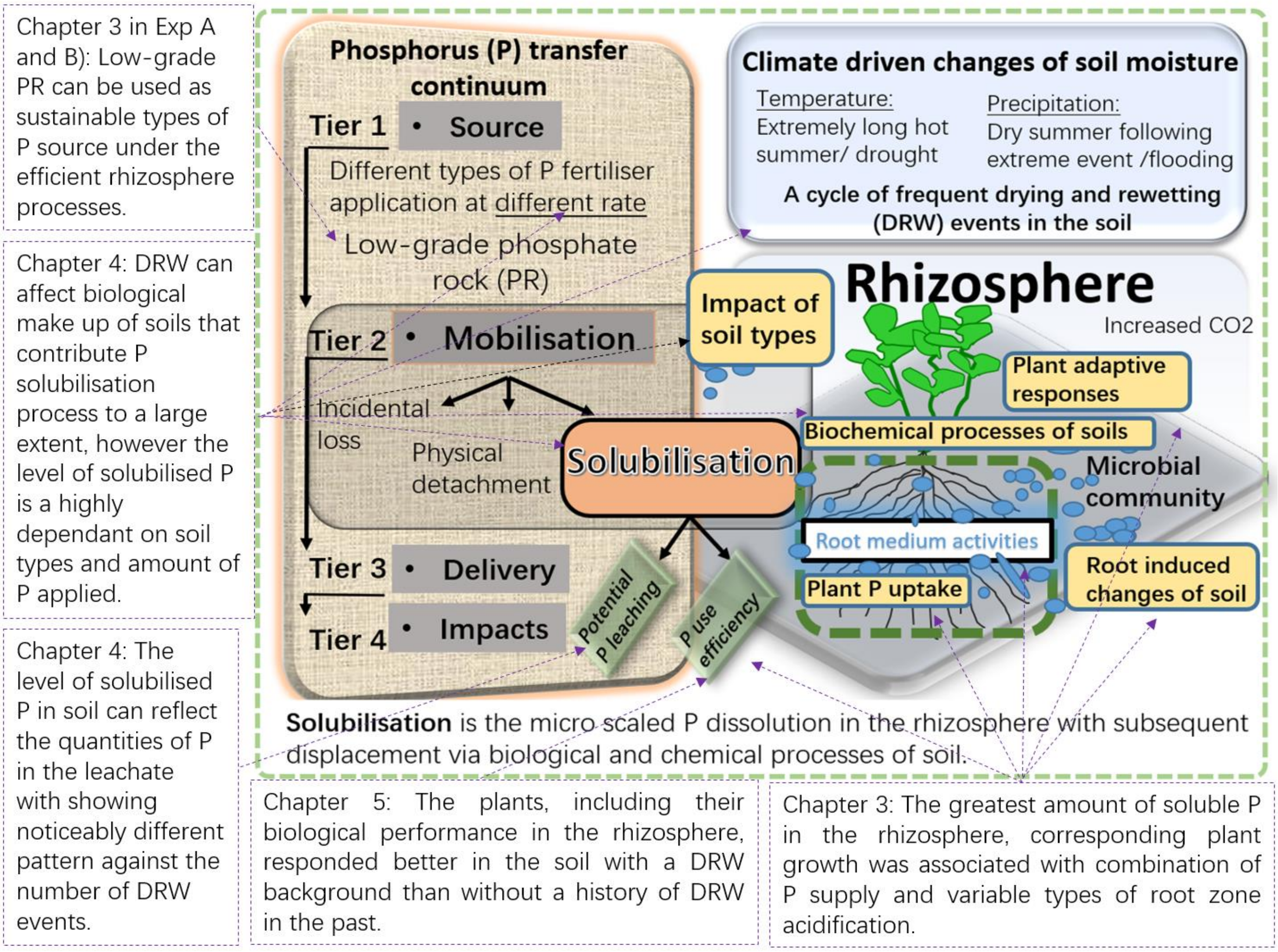
A large degree of variability in the P solubilisation and potential leaching, was noted among different soils under a climate influence (Djodjic et al., 2004). In this thesis this was addressed by using 4 different soils (Cambisol, Lithosol, Haplic Phaeozems and Luvic Kastanozems), with the inherently different properties, with differing bio-physio-chemical characteristics under variable rates of P supply. These soils are also representative of agricultural areas that are susceptible to frequent drying and rewetting occurrences, located in the contrasting agricultural regions with a wide geographical spread, with variable P solubilisation and leaching consequences.

There are uncertainties and challenges with differ types of experiments. In the field-based observations, Olsen-P may not represent the P solubilisation alone in the DRW experiments, as both Olsen P and the water-soluble P (Turner and Haygarth, 2003, Blackwell et al., 2009, Blackwell et al., 2013) have been shown to increase under the rapid rewetting of dried soils. It is also challenging switching interpretations between field and greenhouse experiments, the latter of which are highly mechanistic (Gordon et al., 2008). In my pot experiments, soil sampling was restricted or constrained due to the physical size of the growth pots. The pot size can potentially impact on solubilisation rates whilst the soil environment in a the field setting may reflect a more natural buffering capacity against P input (Goyette et al., 2018) and routes of P transport throughout the soil profile (Haygarth et al., 1999; Heckrath et al., 1995).

The fundamental sampling procedures and preparation stage, which may mask the true effect of DRW inevitably reveal a limitation of the experiments, such as the soil collection depth, storage time, sieving and sampling season. First, dried and sieved soil may not be a representative of the field soil (explained in chapter 4), as sieving can create homogeneity and more attachment later on. Secondly, the soil storage may be a key cause to inhibit the results. Turner and Romero (2010) found that soil stored for more than 2 weeks showed a significant decrease in microbial biomass phosphorus (MBP). In addition, the most appropriate depth to collect soil sample for MBP has been reported to be 10 cm from soil surface as this layer has been observed to represent the greatest MB (Turner and Haygarth, 2001; Blackwell et al., 2009; 2013). Further, the microbial burden and community composition of the soil related with selection of soil sampling period to collect the soil, specifically soil collected during the warmer period can build the entire base of progressive contribution in P leaching experiment. However, the record of soil sampling period of some soil types used has not been obtained for competence of experiment. The four soils used in these experiments were sampled up to 30 cm in depth and stored for more than 6 months, which may have affected P responses thereby restricting the soluble P concentration of in the soil solution under series of DRW.

Inevitably, there are some uncertainties about the use of Olsen P test which should be considered. The Olsen P is the one of most used soil extraction method over many countries around the world to assess available and labile form of P. This method can help to contextualise results under the variable purpose. In England and Wales, Olsen P method is used as a standard soil extractant. However, it is arguable that Olsen P may not reflect truly what is available in the soil, as this method has some potential limitations to interpret the results. Firstly, this method was originally developed for the analysis of alkaline soil. Therefore, effectiveness of Olsen P may vary among the soil types and its chemical properties. Secondly, Olsen P can only assess P availability in relatively short term, without capturing long term changes in P dynamics. A fraction of P released from sources, which may become available for plant uptake for over time related with organic matter or mineral P is the case in point. Thirdly, there are sensitivities of the Olsen P to variations in the test condition, such as pH, temperature and same amount of time to shake the soil solution. However, the research laboratory in China Agricultural University (CAU) employed under Rhizosphere Team uses Olsen P method as a highly prescribed and deeply controlled routine procedure in each experiment in accordance with a set of written instructions, in order to minimise variations between batches.

In my experiments, the duration of the drying time varied considerably across different cycles and soil types. This variable length of incubation period led to significant fluctuation in the total P levels observed in the leachate for the different soil types, which may have impacted by the adsorption/desorption and absorption/desorption of soil P (Olsen and Court, 1982, Shen et al., 2011, Hedley & McLaughlin., 2005, Pierzynski et al., 2005) and likely to characterised by aggregate size and composition (Chepkwony et al., 2001; Blackwell et al., 2009), soil organic matter content (Achat et al., 2012; Soinne et al., 2021) and physical properties of soil especially clay soils with shrink-swell characteristics (Whitmore and Whalley, 2009). The organic matter affects the soil's ability to retain moisture (Kallenbach et al., 2019; Vogelmann et al., 2013) and lead to leaching loss following rewetting, as proportion of hydrophobic and hydrophilic compounds of organic matter changes via van der Waals interaction during the incubation period (Doerr et al., 2007; 2000) under dry soil condition for example water content between 0.36 and 0.57 $cm^3\ cm^{-3}$ (Haber-Pohlmeier et al., 2021). Yet, in experiments the soil containing high organic carbon at 24.5 $g\ kg^{-1}$ (clay loam textured black Haplic Phaeozems) showed the highest amount of solubilised P in the soils with the lowest amount of transferred P in the leachate, as compared with other soil types, particularly sandy loam textured brown Cambisol soils. Sandy soil showed the leaching losses of the highest amount of total P throughout the experimental period. Therefore, it implied that soil texture and structure affect the hydraulic properties of soil during the prolonged dry spell, increasingly repelling soil water throughout the dry spell (Maxwell and Kollet, 2008).



Solubilisation is the micro-scaled P dissolution in the rhizosphere with subsequent displacement via biological and chemical processes of soil.

Chapter 3 in Exp A and B): Low-grade PR can be used as sustainable types of P source under the efficient rhizosphere processes.

Chapter 4: DRW can affect biological make up of soils that contribute P solubilisation process to a large extent, however the level of solubilised P is a highly dependant on soil types and amount of P applied.

Chapter 4: The level of solubilised P in soil can reflect the quantities of P in the leachate with showing noticeably different pattern against the number of DRW events.

Chapter 5: The plants, including their biological performance in the rhizosphere, responded better in the soil with a DRW background than without a history of DRW in the past.

Chapter 3: The greatest amount of soluble P in the rhizosphere, corresponding plant growth was associated with combination of P supply and variable types of root zone acidification.

Figure 0.2 Summary of the key findings of this thesis including the four experiments dealt with chapters 3, 4 and 5.

6.4 Wider implications

The finding of studies reported in Chapter 3, 4 and 5 has contributed an integrated understanding of P solubilisation in the rhizosphere (potential leaching transfer) under extreme climate event scenarios. However, much more work is needed in future to explore the new findings of the role of the rhizosphere in the P transfer continuum, especially in the DRW susceptible arid regions. The implications provided are as follows:

Alternative sources of phosphorus fertiliser

It is suggested that sustainable type of low-grade PR can be used as an alternative P source (Van Kauwenbergh et al., 2013) is expected in near future. The changes in biochemical processes of the soil, specifically acidifying exudative behaviours of rhizosphere, promote the alteration of surrounding soil pH, which were used in this experiment as rhizosphere engineering approach to manipulate the rhizosphere setting. The effects of this approach, coupled with inter-cropping and chemical acidifiers, increased the effectiveness of low-grade PR for plant P benefits.

Plant uptake

The effect of delayed DRW of soil (with varying frequencies, intensities and duration) on the root and microbial activities (Wang et al., 2012; Zhang et al., 2017) can mimic in-soil "rhizosphere engineering" that affects soil nutrient availability through provoking bio-physiochemical change of soil and plant. DRW induced changes of soil may potentially lead to improve the plant P uptake, through the increased rhizosphere process (Blackwell et al., 2012, Chepkwony et al., 2001) and plant root growth (Dodd et al., 2015). These have implications for reduction of P transfer in turn, as modes of mobilisation tiers of the P transfer continuum are affected:

(a) The phytohormonal responses of plants to naturally occurring DRW affect the bio-chemical mechanisms to solubilise P in the root medium (Davies (Alvarez et al., 2008; Davies and Zhang, 1991; Hansen and Dörffling, 2003) are highly likely to result in significant proportion of P uptake through the soil root interface in at the rhizosphere (Chepkwony et al., 2001). Consequently, it can reflect an amount of P to solubilise and leach horizontally throughout the soil profile alongside root channelling under rewetting, due to the positioned plants' uptake.

(b) Improved plant growth under recurrent DRW increase root function particularly percentage of fine root thereby reducing a soil erosion (Bochet and García-Fayos, 2015; Burylo et al., 2014; De Baets et al., 2009). The soil erosion-reducing potential of root function is highly dependent on root diameter. Suggested that reduction in soil erosion would in turn limit the amount of P being mobilised via physical detachment.

(c) Sustainable approaches to managing phosphorus should consider the potential of better-utilising stored “legacy” P. DRW and the subsequent “rhizosphere engineering” approach to modify the rhizosphere surroundings and to increase plant use of P (Haygarth et al., 2018; Menezes-Blackburn et al., 2018), if this could be achieved, it would mean that reduced rates of P fertiliser application were potentially required (Sattari et al., 2012).

6.5 Future work

The DRW experiments demonstrated in this thesis, could be further extended through the careful selection of sampling sites. For instance, this expansion could involve opting for land with a recent history of well-documented recurrent DRW events, alongside areas with an extended historical record of long-term naturally occurring DRW. The latter land with a lengthy DRW background could be associated with progressive degradation, due to the intensifying heat (drought) and water (flooding) stress, driven by climate changes. The wetland can also be experimented in the controlled studies, as the wetland is the area of land where the soil is saturated with water (reviewed in chapter 2). The former land has experienced recent DRW serves as a control to seek an opportunity to utilise land no longer used for agricultural practice. During the experiment, every sampling location holds significant importance to establish soil P responses to DRW can be set up in the cropped and uncropped soil conditions. This can be achieved by conducting the lysimetric pot experiments. However, the regular soil sampling in the experiments discussed in this thesis was constrained, due to the physical size of the lysimeter pots. Concerning the lysimeter, future research endeavours should focus on utilising large sized tubes with more extensive intact soil cores gathered from the field, employing techniques to preserve the integrity of both soil profile and structure. The use of large sized monolith lysimeters equipped with sidewall viewing windows could facilitate enhanced observation in height, enabling better validation of the data generated previous field experiments. The application of DRW practices could potentially bring advantage to arid farmlands, especially if these practices are extended to a broader catchment context in parallel.

Different scenarios can help to increase land use for agriculture, coupled with experimental design to replenish soil solution P by actively promoting the soil environment to enable P desorption, solubilisation and mineralisation and to evolve the plant-based mechanisms by modifying plant root anatomy and physiology to cope DRW (Richardson et al., 2009; Vance et al., 2003). The detailed observation of microbial dynamics under DRW needs to be confirmed within the land type, having soil with different P saturation point, as priming by DRW can change the microbial biomass. More powerful molecular technological tools could be used in future experiment to ensure the strategies to engineer the rhizosphere are safe and beneficial to sustain agricultural productivity. In my view, whilst improving the level of soluble P for plant acquisition, this may also have a potential side effect of also promoting leaching losses via the root, so it remains complicated.

Given the finite nature of high-grade P resources, the problems of P efficiencies need to be addressed at the rhizosphere level for the benefit of the plant and sustainability. To reach the maximum achievable potential of bioavailable P in the soil for plant uptake, rhizosphere manipulations in this thesis were developed under direct application of low-grade PR, through the addition of various types of acidifiers such as chemicals and intercropping. A parallel study, using only commercially available PR application, showed a similar outcome, thereby allowing the low-grade PR to substitute for unsustainable types of P sources under a “rhizosphere engineering” approach. However, due to the hazardous and cost-effective nature of chemical materials used here, the future improvement of PUE needs to seek more natural soil and plant function, by managing biology themselves at the rhizosphere.

One approach would be an integration of low-grade PR application and naturally occurring DRW plus a co-cropping system, which can be used as a soil-based ecologically engineered method in the future, to help improve crop yield and mitigate P loss from agriculture. I believe that greatly increased bioavailable P in the root zone under DRW is less likely to leach into drainage in the rewetting condition, when plant P uptake is intensified by the processes of rhizosphere. An intensification of rhizospheric activities conceptually, at least, could lead to reduction of P input in the cropland, by stimulating the hydrolysis of the legacy stored P adsorbed with soil surface. Further, increased plant P uptake in this case could also generate increased plant growth, particularly root growth with increased root-shoot ratio, which may prevent soil erosion and reduce a physical movement of P loaded soil aggregates. However, to reduce the impact of soil moisture restriction on the plant growth, experimental designs should be built up again by manipulating the field capacity, to meet the plant tolerance at minimum water demand, this will be complicated and challenging. There is still much to study here in a range of environments, plants and soils in different systems and scales.

A cycle of DRW events has potential to modify the processes in the rhizosphere that can solubilise P naturally. The processes stimulated involve rhizosphere exudates, enzyme activities and possible role of soil microbial function with effective MBP. It is possible that an increase in PUE under DRW associated with current climate change events could reduce solubilised P transfer from the soil through the methods practiced in the crop production of agriculture. However, these studies here did not provide data to inform a direct effect of DRW on P transfer from the soil grown plants with low-grade PR application. Therefore, there is an opportunity for future work to test this, perhaps using field studies and lysimeters outdoors, as mentioned. There is great potential for these underlying processes of frequent DRW can create an ‘enabling environment’ to induce such rhizospheric activities for the improvement of P-efficiency, without any addition of chemicals.

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