

**Soil drying and re-wetting effects on phosphorus  
availability and plant yields**

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B.Sc. (Hons) Physical Geography (Australasia), Lancaster University

M.Sc. Sustainable Agriculture and Food Security, Lancaster University

Thesis submitted for the degree of Ph.D. at Lancaster University

March 2018

## **Declaration**

I declare that the contents in this thesis are my own work and have not been submitted in the same form for the award of a higher degree elsewhere. The data presented in Chapter 4 resulted from joint research with collaborators working with AfricaRice, and the Laboratoire des Radio-Isotopes and FOFIFA (Madagascar). Input from collaborators and published work are acknowledged throughout.

Hannah Wright

Lancaster University, March 2018

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### Abstract

Water and phosphorus (P) are essential resources for crop production, yet both are increasingly limited, threatening global food security. Soil drying and re-wetting (DRW) has reportedly increased P availability in numerous soils, whilst intermittent irrigation that applies less water than conventional full irrigation can maintain crop yields. To test the hypothesis that DRW could release P at soil water potentials (SWPs) that can support plant growth, thereby increasing crop P use efficiency and yields, experiments at different scales aimed to evaluate P release and plant uptake. Laboratory studies using three low-P UK soils determined that available P (sodium bicarbonate extractable P) significantly increased (by 4-7 mg kg<sup>-1</sup>) as SWP decreased (to a minimum of -212 MPa). A significant change point (releasing 2 mg P kg<sup>-1</sup>) occurred at -2.9 MPa. A pot study showed that surface soil drying to this change point did not increase P availability over one or two DRW cycles, suggesting laboratory results could not be scaled up spatially and temporally. Initially air-drying (to -38 MPa) and re-wetting soil prior to planting *Brachypodium distachyon* in pots significantly increased available P at transplanting (by 1 mg kg<sup>-1</sup>) and doubled grain yields independent of P fertiliser application. In a field trial in central Madagascar in low-P, highly P-fixing soil, applying alternate wetting and drying (AWD) or post-anthesis soil drying to *Oryza sativa* hardly altered P uptake or yields. Thus, DRW increased P availability and yields in pots, but plants did not benefit during a cropping cycle in the field. Applying P fertiliser (10 or 25 kg ha<sup>-1</sup>) had a greater effect than AWD, with optimal harvest index (HI) and P use efficiency (PUE) at the intermediate rate. Further research to determine locally-relevant management techniques, stimulating P release at appropriate spatial and temporal scales to allow plant uptake, is urgently required.

*Key words: phosphorus, drying and re-wetting, soil water potential, phosphorus use efficiency, yields.*

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\*\*\*

“...Good luck and Good work for the happy mountain raindrops...some... creep out of site to  
the grass roots... seeking and finding their appointed work.”

-John Muir, 1911, *My first summer in the Sierra.*’

\*

“I cannot over-emphasize the importance of Phosphorus not only to agriculture and soil  
conservation but also to the physical health and economic security of the people of the  
nation.”

-Franklin D. Roosevelt, 1938, *Message to Congress on Phosphates for Soil Fertility.*’

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## Abbreviations

<b>Term</b>	<b>Abbreviation</b>
<b>Moisture status</b>	
Gravimetric water content	GWC
Soil water potential	SWP
Plant permanent wilting point	PWP
Dry weight equivalent	DWE
Field-moist	FM
Air-dried	AD
Drying and re-wetting, or dried and re-wet	DRW
Continuously flooded	CF
Alternate wetting and drying	AWD
<b>Phosphorus measurement</b>	
Phosphorus	P
Water-extractable total P	TP
Water-extractable molybdate reactive P	MRP
Water-extractable molybdate unreactive P	MUP
Sodium bicarbonate extractable P	NaHCO <sub>3</sub> -P
Anion exchange (resin) membrane P	Resin-P
Diffusive gradients in thin films P	DGT-P
Microbial biomass P	MBP
<b>Agronomic efficiency</b>	
Harvest Index	HI
Phosphorus use efficiency	PUE
Water use efficiency	WUE
Water productivity	WP
<b>Concepts</b>	
Deficit irrigation	DI
Sustainable intensification	SI

## **Chapter 1: General Introduction**

### **Global water and phosphorus use in agriculture**

The supplies and management of water and phosphorus are two of the most important issues related to global agricultural production and food security. Water is fundamental to the growth of crop plants as it is required for structure, photosynthesis and solute transport. Irrigation comprises the major component of global water use, although increases in demand alongside uncertain impacts of climate change are predicted to cause severe water stress to agricultural productivity and food security (van Dijk et al., 2013; Wang et al., 2016). Severe water scarcity is already experienced by 2.4 billion people, comprising 36 % of the global population, and predicted to affect 52 % of the global population by 2050 in the absence of effective mitigation strategies (von Grember et al., 2016). As well as geo-political and socio-economic controls on water supplies, climate change will likely create more variable and extreme rainfall and drought events (NAS, 2016). Linking crop and water simulation models with different scenarios of socio-economic and climatic change showed that future water scarcity will likely limit cereal crop production by up to 40 % from current levels by the 2040s. This is due to increased crop water requirements with elevated temperatures associated with climate change as well as increased water demand from other sectors (Wei et al., 2009). Already, there are many examples of these effects. Madagascar's diminished rice harvest in 2013, which left four million people food insecure, was attributed to erratic weather characterised by flooding followed by a period of drought (FAO/ WFP, 2013). More than 18 million people in Somalia, Ethiopia and Kenya experienced acute food insecurity in 2017, largely caused by declines in crop yields due to drought and flooding; for example, cereal yields were 87 % below average in northwest Somalia due to late onset and early cessation of seasonal rains (USAID, 2017). Increasing the efficiency of water use in agriculture at the global scale is paramount to current and future food security.

Nutrients are also vital for crop growth. Phosphorus (P) is an essential macronutrient which cannot be substituted, forming adenosine triphosphate (ATP) which is crucial to metabolism

and growth for all forms of life. The green revolution dramatically increased crop yields, partially enabled by the increased application of mined rock phosphate as a primary source of P in chemical fertilisers (Elser and Bennett, 2011). However, rock phosphate is a non-renewable resource and P sustainability in agriculture has gained increasing attention in recent years. Following a price spike of 700 % in 2008, Cordell et al. (2009) cautioned of the risk of global “peak phosphorus” due to a high dependency causing demand to outweigh supply, with strong economic and geo-political implications due to inequitable control of, and access to, phosphate mines. Several comprehensive reviews have highlighted the need to reduce dependency on rock phosphate due to environmental and economic risks, and to instead seek opportunities to sustainably utilise P resources in food production (Cordell et al., 2009; 2014; 2015; Haygarth et al., 2014; George et al., 2016).

Although water and phosphorus are essential resources for food production, both are limited and increasingly threatened. Moreover, integrated guidelines on water and nutrient management (as in Thompson et al., 2005) are limited. There is increasing understanding that water availability affects P availability and it is the central aim of the studies herein to further characterise how they interact and their agronomic relevance. Since approximately 40 % of global food production depends on irrigation (Turrall et al., 2011), and 90 % of global phosphorus demand is for food production (Cordell et al., 2009), focusing on agricultural food crops is a key priority. Although a diversity of food crops is essential to human nutrition and health, the majority of calories are supplied by cereal crops, principally paddy rice, wheat and maize (Fischer et al., 2014) which use the most water (Mekonnen and Hoekstra, 2010), land (Fischer et al., 2014) and nutrient fertilisers (Heffer, 2013). Thus reducing water and fertiliser inputs in these systems without reducing yields would be beneficial.

### **Increasing the efficiency of water use in agriculture**

As demand for food increases, there is a need to enhance crop yields using limited water. The “sustainable intensification” (SI) of agriculture aims to increase yields without converting more

non-agricultural land into production, and without depleting resources or causing adverse environmental impacts (Pretty and Bharucha, 2014). Consistent with the SI concept, understanding water-limited crop yield as the product of water use, water use efficiency (biomass production / water use; WUE) and harvest index (harvested biomass / total biomass; HI) is central to realising opportunities to maximise yields (Passioura, 1980; 1996). Irrigation water productivity (crop yield per unit of applied irrigation water; WP) is also a useful agronomic measure (Sadras, 2009). A key practical challenge of limiting irrigation volumes is to maintain a sufficient mass of the root system within soil that is maintained above the point of soil drying beyond which plants fail to recover, known as permanent wilting point (PWP). This can be judged by the soil water potential (SWP) and is generally considered to be  $-1.5$  MPa (Figure 1.4). This value depends on soil type (Czyż and Dexter, 2013) and the tolerance of different plant species and genotypes to soil moisture deficit, yet remains a useful reference point for severely water-limited conditions.

Various techniques and technologies aiming to reduce irrigation inputs have been developed for different crop production systems globally, and are employed by commercial enterprises. In numerous studies, deficit irrigation approaches (where water is provided at rates below requirements for full crop evapotranspiration) can yield equally to fully irrigated crops, often with improvements in crop quality, despite reduced water use (Feres and Soriano, 2007). Deficit irrigation (DI) techniques allow water supply to be regulated according to crop requirements, accounting for changes in water demand with phenological development. Water application is thereby decreased temporally by reducing irrigation frequency, though can also be decreased spatially by supplying water to only part of the rootzone alternately, termed partial rootzone drying. Differences in physiological responses and agronomic outcomes of these temporal and spatial approaches have been reviewed (Dodd, 2009; Sadras, 2009; Dodd et al., 2015). In addition to saving water, DI techniques can have agronomic benefits. For example, not watering wheat plants after anthesis induced a mild water deficit and promoted vegetative senescence, which increased carbon (C) translocation from the stem to the grain thereby

increasing HI and yields (Yang et al., 2000; Yang and Zhang, 2006). Whether WUE and WP can be increased while maintaining or enhancing yields compared to conventional full irrigation, depends largely on the extent of the water deficit and when it is imposed (Dodd et al., 2015). Therefore agronomic impacts depend on crop tolerance to reduced SWP, regulated by soil and plant factors.

A unique form of deficit irrigation, developed by the International Rice Research Institute (IRRI) (Bouman et al., 2007) for lowland irrigated rice, is termed “alternate wetting and drying” irrigation (AWD). Lowland irrigated rice is traditionally cultivated under continuously flooded (CF) conditions, to suppress weeds and pests and increase yields (Bouman et al., 2007), whereas AWD creates intermittent flooding. Managing AWD “safely” allows the surface soil to dry but prevents the water table dropping lower than 15 cm below the soil surface. This management reduces crop water use and increases the crop WUE, whilst ensuring access to water via deeper roots (Bouman and Lampayan, 2009). In addition to reduced water use, AWD can have various agronomic benefits including increased WP, improved rooting systems and thereby reduced lodging (Bouman et al., 2007) and increased HI (reviewed in Price, 2013). A meta-analysis of 56 studies, involving 528 comparisons of adjacent CF and AWD treatments, found that adherence to safe AWD guidelines did not limit yield while reducing water use by 23.4 %. However, more severe drying ( $SWP < -0.02$  MPa) reduced yields by 22.6 % compared to CF, which was exacerbated when AWD occurred throughout the crop life cycle (Carrijo et al., 2017). Therefore relating soil water deficits to degrees of drying (SWPs) is important.

The mechanisms underpinning plant responses to soil drying and re-wetting are uncertain, though likely reflect a combination of altered plant water relations, soil nutrient availability, and root-to-shoot phytohormonal responses (Dodd et al., 2015; Wang et al., 2017). Beneficial nutritional effects depend on maintaining xylem flow and phloem function and the recovery of photosynthesis after re-wetting the soil (Yang and Zhang, 2006). Improved understanding of the physiological responses to different controlled soil drying and re-wetting regimes, such as

AWD, is needed to optimise management techniques to improve WUE, HI and yields and reduce water use.

### **Increasing the efficiency of phosphorus use in agriculture**

The need for more sustainable P use has become apparent and urgent in recent years (Cordell et al., 2009). Critical soil P levels for optimal crop yields, and to avoid environmental losses, vary according to soil type and properties, as well as the crop species (Bai et al., 2013). In regions in which mineral P fertilisers are available, over-application is inefficient and causes economic losses. For example, dairy and tillage farms in the Republic of Ireland over-applied inorganic P fertiliser by an average of 2.9 to 3.5 kg P ha<sup>-1</sup> in 2008, which was similar to P losses via runoff (Buckley and Carney, 2013). Contrary to the SI concept, loss to non-agricultural land and water disrupts adjacent and downstream environments, and soil nutrient availability can alter numerous measures of ecosystem functioning (Laliberté and Tylianakis, 2012). The edges of remnant New Zealand forests adjacent to pasture had plant-available P (Olsen P; Figure 1.1) levels that were 500-5000 % higher than reference forest sites (Didham et al., 2015). Leachate P concentrations from arable soils have been related to soil P concentrations, with P solubilisation suddenly increasing at “change points” indicating threshold values for P leaching (Fortune et al., 2005). Various legislation aims to increase efficiency and avoid polluting effects associated with nutrient management, including P standards for rivers under the Water Framework Directive (WFD, 2013). There are also regulations for P fertiliser application in Northern Ireland (DAERA, 2014) and specific guidelines for farmers on nutrient application rates and best management practices, such as the Fertiliser Manual (RB209) prepared by the Department for the Environment and Rural Affairs in the UK (DEFRA, 2017). Challenges persist such as the limitations of spatially and temporally restricted soil P tests and their suitability for different soil types, and the relatively high costs of equipment aiding precision agriculture approaches; yet progress towards increasing P efficiency is needed for the long-term sustainability of P management in systems receiving inorganic fertilisers.

Perhaps more scientifically challenging is the search for opportunities to increase P efficiency in regions where P fertilisers cannot be accessed. An estimated one-sixth of the world's farmers lack the financial resources to access fertiliser markets (Cordell et al., 2014). Thus, crop yields are limited by insufficient plant-available (largely inorganic) soil P. Plants acquire P via roots as orthophosphate ions, primarily  $\text{H}_2\text{PO}_4$  and also  $\text{HPO}_4$  (Johnston and Syres, 2009). However, organic P may contribute a large proportion of soil total P that is potentially available to plants (Oberson et al., 2006; Richardson et al., 2011; Nash et al., 2014) and harnessing it is essential to crop nutrition in P-limited soils (Stutter et al., 2012). Soils under rice cultivation with more organic matter had higher organic P concentrations (Turner, 2006), requiring greater understanding of the role of organic P in crop P nutrition. Whilst some plant species can access organic P (Stutter et al., 2012), inputs from substrates such as straw and manure partially depend on microbial mineralisation to become available; therefore complementary agronomic practices must support this process. Most studies have focused on the role of adding organic matter to soil, though some have simultaneously investigated other management factors including water. For example, available P (resin-P; Figure 1.1) was compared in soils with and without straw addition in both aerobic and anaerobic soils from rice fields in Madagascar, with organic matter addition immediately prior to flooding increasing P availability (Rakotoson et al. 2015). Studies combining various approaches, common in studies of the "system of rice intensification" (SRI) which integrates different planting, nutrient amendment and irrigation practices (discussed in Chapter 4) are valuable for determining the outcomes of soil process interactions. However, there remains the need to isolate water management effects on soil P availability, especially since the drying and re-wetting cycles imposed with AWD (and as an aspect of SRI) may also drive the turnover of organic P due to microbial cell lysis (Turner, 2006). Further study of these effects on soil P availability is required.

Improving agricultural P efficiency should also occur at the plant level. Crop P use efficiency (PUE) is often agronomically defined as grain yield per unit of P uptake (in the above-ground biomass) (Rose et al., 2013). Whilst there are many breeding efforts towards improving

physiological PUE (total shoot biomass produced per unit of P in shoots) (Rose et al., 2013), altering irrigation scheduling may also regulate P supply and uptake. For example, limiting water supply to rice substantially reduced tissue P content, with little effect on N (Somaweera et al., 2016). In cereal crops, more than 70 % of the total P content at maturity is typically stored in grains (Rose et al., 2013), which is removed from the field at harvest and thereby unavailable to subsequent crops. Avoiding luxury P uptake by the crop beyond levels contributing to increased yields, thereby limiting soil P depletion, is a valuable goal towards increasing PUE, which is affected by P availability and other environmental conditions (Vandamme et al., 2016).

### **Soil nutrient availability responses to soil drying and re-wetting: processes**

Since water and P efficiencies should be increased whilst maintaining crop yields, it is vital to better understand the impacts of deficit irrigation techniques, imposing soil drying and re-wetting, on soil P availability to crop plants. Drying and re-wetting (DRW) is also a very common abiotic stress in soils, which is experienced at different magnitudes and durations according to geographical location and associated climatic and seasonal variations in water supply.

#### **The “Birch effect”**

The contribution of soil DRW cycles to increased mineral nutrient availability, first reported by Birch (1958), is well documented. The effect describes a pulse of organic matter decomposition and mineralisation of P and N upon re-wetting a dried soil, with a decline in rate over time. Different interacting soil processes regulate the occurrence and magnitude of the “Birch effect” in determining nutrient availability. Briefly, more intense drying (to lower water contents and water potentials, such as occurring at high temperatures or for long durations) induces more mineralisation upon re-wetting. Recurrent DRW cycles may increase mineralisation overall, compared with continuously moist soils (Jarvis et al., 2007), emphasising the importance of soil moisture fluctuations in driving the underlying nutrient cycling processes. However, whether these fluctuations can be actively managed to enhance soil nutrient provision to plants has

received little attention. Given the difficulty of defining “plant-available” P, key forms and soil P tests are outlined in Figure 1.1 and discussed (this chapter).

Soil DRW affects the transfers and transformations of P between the four pools outlined in Figure 1.1, which is the foundation to understanding how biological and physical processes cause and regulate the “Birch effect”, summarised in Figure 1.2 and further discussed.

POOL	AVAILABILITY	SOIL P TESTS
<p>SOIL SOLUTION P</p> <p>↓</p> <p>↑</p> <p>ADSORBED P (SURFACES)</p> <p>↓</p> <p>↑</p> <p>ABSORBED P (STRONGLY BONDED OR ORGANIC)</p> <p>↓</p> <p>↑</p> <p>INACCESSIBLE P (VERY STRONGLY BONDED)</p>	IMMEDIATE	<ul style="list-style-type: none"> <li>• WATER-EXTRACTABLE MOLYBDATE REACTIVE P</li> <li>• SODIUM BICARBONATE EXTRACTABLE P (OLSEN P)</li> <li>• ANION EXCHANGE MEMBRANE (AEM)/ RESIN P</li> <li>• DGT-P</li> </ul>
	READY	<ul style="list-style-type: none"> <li>• SODIUM BICARBONATE EXTRACTABLE P (OLSEN P)</li> <li>• ANION EXCHANGE MEMBRANE (AEM)/ RESIN P</li> <li>• DGT-P</li> </ul>
	LOW	<ul style="list-style-type: none"> <li>• WATER-EXTRACTABLE MOLYBDATE UNREACTIVE P</li> <li>• MICROBIAL BIOMASS P</li> </ul>
	VERY LOW	<ul style="list-style-type: none"> <li>• TOTAL P</li> </ul>

Figure 1.1: Phosphorus forms commonly analysed and availability to plants.

Sources: Johnston and Syres, 2009; Six et al., 2013.

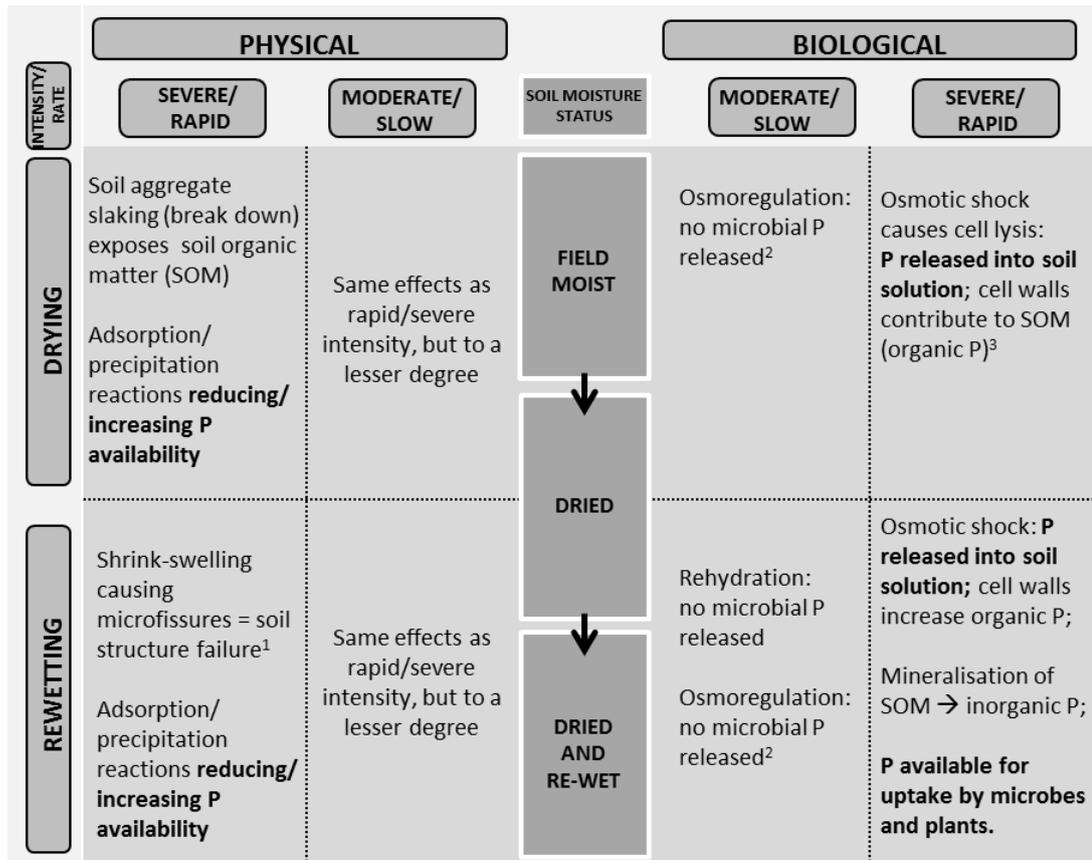


Figure 1.2: Effects of soil drying and re-wetting on phosphorus availability, driven by physical and biological processes across different intensities (degrees) and rates.

Sources: <sup>(1)</sup> Cosentino et al. (2006); <sup>(2)</sup> Fierer and Schimel (2003); <sup>(3)</sup> Gordon et al. (2008).

Published in: Dodd et al., 2015.

Biological processes contributing to the “Birch effect” include the death of soil microbes due to desiccation upon drying and cell lysis upon rapid re-wetting (caused by osmotic shock), releasing their nutrient constituents into the soil solution (Figure 1.2). This contributed the majority of the increased water-extractable total P (TP) (88 %) and molybdate unreactive P (MUP)<sup>1</sup> (95 %) from two fertilised pasture soils following DRW (Turner et al., 2003). In many studies, the quantities of organic P released into solution after drying were greater than those of inorganic P, due largely to the contributions from the microbial biomass and soil organic matter (SOM) (Turner and Haygarth, 2001, 2003; Turner et al., 2002, 2003; Blackwell et al., 2009). For example, across 29 permanent grassland soils in England and Wales, varying in P concentrations (9 – 48 mg kg<sup>-1</sup> sodium bicarbonate extractable P, NaHCO<sub>3</sub>-P) and C and clay contents, water-soluble TP increased by 1.9 – 19-fold following DRW. This was primarily (56–100 %) water-soluble MUP, and the positive linear correlation with soil MBP concentration suggested a direct release from the microbial biomass (Turner and Haygarth, 2001). Therefore the microbial biomass size and composition can substantially determine the significance of the “Birch effect” to nutrient availability in different soils (Jarvis, 2007; Gordon et al., 2008; Blackwell et al., 2010).

Different soil properties regulate microbial responses to DRW and the release of P for plant use. Soil microbial processes are driven by the availability of organic C (Oberson et al., 2006), such as straw and manure additions in arable soils, which largely determines the energy supply to microbes, thereby regulating mineralisation rates and P release following DRW (Sparling et al., 1985). Microbial cell walls also become incorporated into the SOM, and mineralisation releases inorganic P which is available for plant uptake and immobilisation by both surviving and new microbes (Blackwell et al., 2010; Figure 1.2). Nevertheless, microbial immobilisation of P

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<sup>1</sup>Water-extractable total P (TP) is the sum of water-extractable molybdate-unreactive and –reactive P (MUP and MRP, respectively). MUP is adsorbed P (strongly bonded or organic) and therefore has low availability to plants, whilst MRP easily transfers to soil solution P and is immediately available (Figure 1.1). Water-soluble TP, MRP and MUP are measured via the same method although extracts are more finely filtered (0.45 *cf.* 2–3 µm) prior to analysis (Haygarth and Sharpley, 2000; Turner et al., 2002). References are to the water-extractable forms and unless “soluble” or “dissolved” is specified, references to TP, MRP and MUP hereafter refer to the water-extractable (2–3 µm filtered) fraction.

released by DRW, indicated by the strongly reduced C:P ratio of the microbial biomass in the DRW soils compared with control soils, can result in some soils showing no change in reactive P concentrations following DRW (Butterly et al., 2011b). Furthermore, soil DRW increased  $\text{NaHCO}_3\text{-P}$  in soils from regions with a lower annual water deficit ( $< 400$  mm) to a greater extent than soils with a higher annual water deficit, suggesting microbial adaptation only to moderate soil drying (Sparling et al., 1987). Thus, the degree of soil drying is an important control on biological P release. Following rapid re-wetting of dry soil, the microbial biomass can recover ATP synthesis over relatively short time scales (within 6 hours) (De Nobili et al., 2006). Since soil microbes and plants take up the same forms of P, the organisms compete; therefore enhanced microbial assimilation of P suggests that any benefits to plants from increased availability of P following DRW events may be short-lived (Chepkwony et al., 2001). Soil oxygen status is also important: under anaerobic conditions, microbial turnover and mineralisation rates are usually low compared to aerobic conditions, reducing the rate and extent that nutrients are mineralised to plant-available forms (Stoop et al., 2011; Turner, 2006) (Figure 1.3). Allowing the soil to become aerobic with intermittent irrigation can increase mineralisation and therefore P availability, depending on the degree of soil drying.

Physical processes during DRW events disrupt soil aggregates, which can be an important process for nutrient release, often interacting with biological processes. Soil DRW induces structural changes which release SOM from microaggregates. No longer protected, this SOM is more readily mineralised by soil microbes (Chepkwony et al., 2001; Navarro-Garcia et al., 2012). After drying clay soils, fractions of plant-available P (both MRP and  $\text{NaHCO}_3\text{-P}$ ) increased (as a proportion of total P), due to structural changes in SOM and physical disturbance of microbial cells (Soinne et al., 2010). Furthermore, aggregate stability tests and sodium (Na)-facilitated dispersion tests suggested that microbial P release during DRW (based on increased MRP and MUP concentrations) was sourced from within microaggregates (where it was trapped) and became water-extractable when combined with soil dispersion (Bünemann et al. 2013). Navarro-García et al. (2012) also concluded that soil DRW responses are driven by the

physical associations of mineral particles and substrates with biological organisms. Thus, physical and biological processes causing and regulating the “Birch effect” are coupled. A further physical process caused by DRW is slaking (physical breakdown caused by air compression, which is trapped during wetting; Bünemann et al., 2013) which can release MRP (largely inorganic) and MUP (largely organic) P (Soinne et al., 2010) (Haygarth et al., 1998) (Figure 1.2). Following aggregate stability tests, slaking was identified as the most important form of physical disruption to soil structure potentially releasing P (Bünemann et al., 2013). Compared to a constantly moist soil, significant (up to 44%) increases of resin-P concentration following DRW of a sterilised soil further emphasised the physical processes (Bünemann et al., 2013). Sterilisation removed potential biological effects, although may have affected the soil physical properties. Furthermore, DRW can physically detach soil colloids thereby increasing MRP and MUP in leachate due to shrinkage and swelling of soil aggregates (Chepkwony et al., 2001; Blackwell et al., 2009). These physical processes can significantly contribute to P release following DRW (Bünemann et al., 2013; Sun et al., 2017a).

The effects of DRW on soil P transfers and transformations, in terms of the magnitude of increase in P availability and its potential for utilisation by plants, depend on other soil properties; principally the availability of soil organic matter, and the cation exchange capacity (CEC) and concentrations of iron (Fe), aluminium (Al) and manganese (Mn) oxides, which are affected by soil pH (Amery and Smolders, 2012; Rakotoson et al., 2014). The initial soil P concentration is also important. The reduction of Fe in particular has gained attention since concentrations are often high in tropical soils, where P availability is often low. Fink et al. (2016) comprehensively described Fe/P chemical reactions in soils and their interactions with organic matter. Despite increases in P availability immediately following DRW, soil P can be strongly adsorbed to clay minerals and Fe and Al oxides (Gérard, 2016), becoming unavailable to plants. Thus, the degree to which DRW increases P availability to plants can be highly variable according to soil type.

Soil P reactions can be profoundly affected by anaerobic conditions created by flooding, and alternation of anaerobic and aerobic conditions, in which P interactions with other soil properties are distinct (Figure 1.3). In a laboratory experiment with floodplain sediments exposed to alternate drying and submergence, soil total P release was greater from sediments with longer dry periods before re-wetting and with greater soil drying (soil water content decreased by 80 %) (Schonbrunner et al., 2012). The differences between the final and initial concentrations (during wetting) of TP and  $\text{Fe}^{3+}$  were significantly positively correlated, suggesting the role of the reduction of Fe hydroxides in the simultaneous release of  $\text{Fe}^{3+}$  with TP (Schonbrunner et al., 2012). Similarly, Surrige et al. (2007) attributed P release (MRP) following the flooding of a wetland soil to the reductive dissolution of Fe, as occurs in paddy rice fields. The same effect has been reported for paddy rice soils, with dependency on other soil properties. Flooding can increase pH in acidic soils, affecting P availability (Amery and Smolders, 2012). Soils with larger CEC have more cation sorption sites which can scavenge  $\text{Fe}^{2+}$  in the soil solution, preventing precipitation of new P sorbing Fe(II) minerals, thereby decreasing potential P sorbing sites; therefore there are more free (available) P ions in the soil solution (Amery and Smolders 2012). Although submergence has increased soil P availability in many studies, not all soils have higher P concentrations under anaerobic conditions, especially soils with high Fe content (due to re-adsorption); furthermore, applying organic matter stimulated the reduction of Fe only if CEC was high (Amery and Smolders, 2012). Since P deficiency has been reported in field trials with irrigated rice in various tropical soils characterised by high Fe contents, it is likely that flooding soils does not solubilise sufficient P to meet crop demand (Dobermann et al., 1998), suggesting that AWD should be further investigated as an alternative mechanism for increasing P availability.

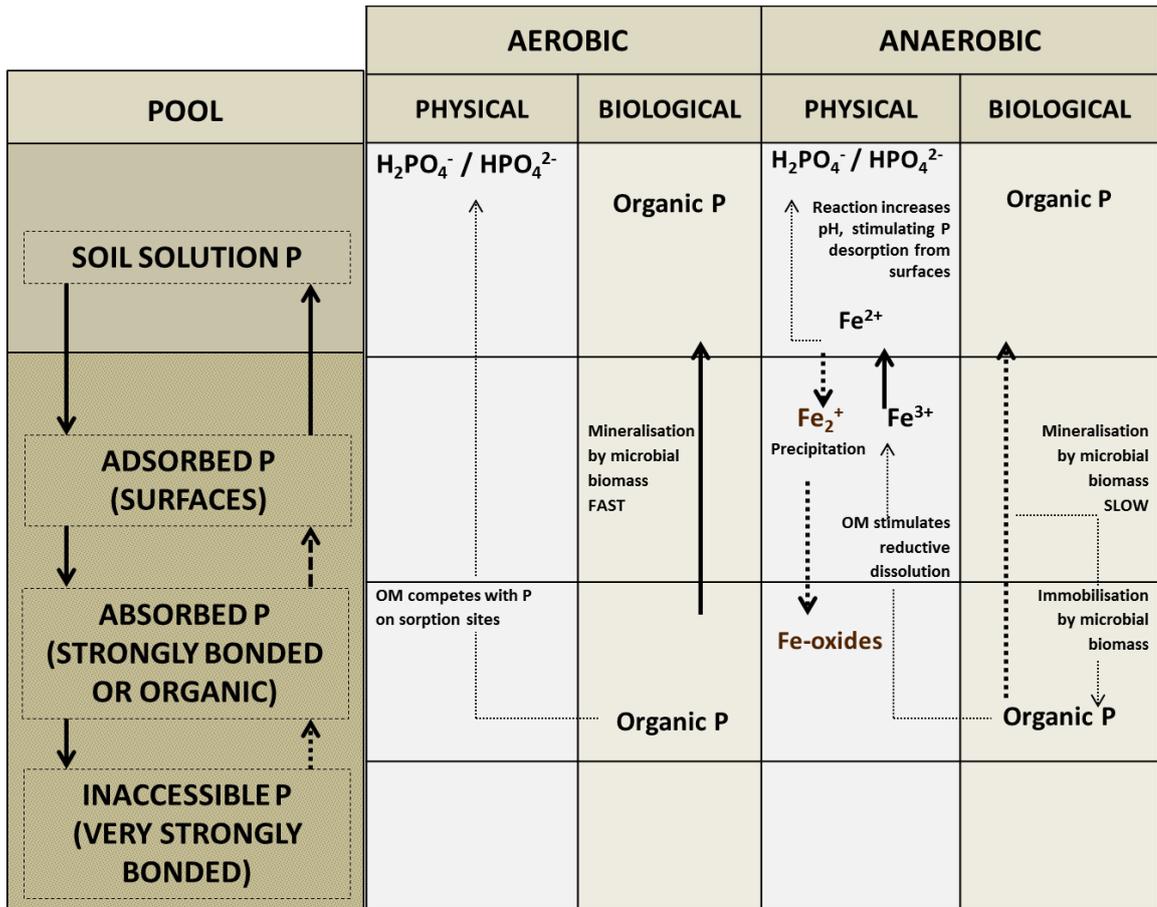


Figure 1.3: Effects of aerobic and anaerobic conditions on reactions affecting soil P availability.

Sources: Amery and Smolders, 2012; Rakotoson et al., 2014; Fink et al., 2016.

## **Soil nutrient availability responses to soil drying and re-wetting: responses and applications**

Interacting biological and physical soil dynamics under DRW pose a challenge to determining the effects of irrigation management on nutrient availability. This is further complicated by the large variability in soil properties and DRW methodologies, but also nutrient analyses, across different studies. To utilise the P released following DRW and manage the response via controlled soil drying, the fundamental question is whether soil drying and re-wetting releases sufficient P to improve crop P nutrition and yields. To answer this, the key issue is whether soil P availability increases to a sufficient level at water contents supporting crop growth.

Soil water potential (SWP) is the crucial measure of soil water availability to plants (discussed later in this chapter), though is often omitted from “soil-exclusive” studies. Studies demonstrating changes in P availability in dried compared to moist soils have often only reported the drying method (typically temperature and duration) and not a SWP or gravimetric water content (GWC) relating to changes in P availability (Venterink et al., 2002; Turner et al., 2003; Turner and Haygarth, 2003; Soenne et al., 2010; DeLonge et al. 2013). Some studies reported the GWC only (e.g. Sparling et al., 1987; Butterly et al., 2011a and b; Sun et al., 2017a) or even percentage of the water holding capacity (WHC) (Sun et al., 2017b), but these cannot be explicitly compared in the absence of SWP values. Whilst DRW can increase P availability, hydraulic flow (therefore nutrient uptake) is limited during the drying period; this highlights the need for soil (and root) water potential measures to confirm plant water uptake. Furthermore, soil microbial activity responds to drying intensity (Figure 1.2) as changes in SWP. In two soils, decreasing SWP from an optimal level for microbial activity ( $\geq -0.7$  MPa) to  $-2.0$  MPa reduced the microbial biomass by 35-50 %, and the proportion of remaining (adapted) microbes survived at lower SWPs (as low as  $-6.8$  MPa) but with reduced activity (respiration) (Chowdhury et al., 2011). This suggested that drying soil to  $-2.0$  MPa would cause organic P release due to microbial cell lysis whilst maintaining mineralisation rates, increasing P availability, whilst drying to lower SWPs releases the same amount of organic P but reduces mineralisation to

plant-available forms due to the lower microbial activity. Although many studies have reported increased P availability following soil DRW and release from the microbial biomass, greater unity is needed to clarify the water potentials to which soils were dried and the amounts of P made available to plants.

Several studies have assigned changes in P concentrations to SWP values. For example, drying loamy/ sandy forest soil samples to  $-100$  MPa, whilst maintaining control soil samples at 50 % WHC, significantly increased water-soluble P concentrations. Responses varied according to soil type and horizon with a maximum increase in water-soluble MRP of  $72.6$  mg P  $\text{kg}^{-1}$  and water-soluble MUP of  $29.0$  mg P  $\text{kg}^{-1}$  (Dinh et al., 2016). Measuring P concentrations immediately after, and prior to, re-wetting would provide further insights. In a different study, air-drying a clay grassland soil from  $-0.4$  MPa (24.2 % GWC) to  $-78$  MPa (0.9 % GWC) significantly increased  $\text{NaHCO}_3\text{-P}$  by 72 % ( $8.9$  mg  $\text{kg}^{-1}$ ) (Blackwell et al., 2009). However, P release at intermediate water potentials was not measured. Gradual P release may have occurred as SWP decreased, although “change points” have described the relationship between leachate P concentrations and transfer to surface waters with soil P concentrations, with sudden increases in P solubilisation indicating threshold values for P release, which vary between soils (Fortune et al., 2005). Whether this concept applies to P release in response to threshold SWP values is unknown. Thus, further work should prioritize determining whether these P responses occur following re-wetting of soils dried to lesser degrees. These two studies demonstrate the value of reporting SWP values associated with the P concentrations released by DRW, and represent very different soil types and ecological systems. Crop system studies would benefit from the same approach to determine the agronomic significance of attained SWPs and available P concentrations.

Temporal dynamics are important to determining whether increased soil P availability following DRW is enhanced, sustained or diminished over multiple cycles, and reported responses are variable. For example, three additional DRW cycles did not further increase water-soluble MRP after an initial increase (Dinh et al., 2016), implying that the initial increase

was sustained although not enhanced. In contrast, soil P availability increased more after two ( $\text{NaHCO}_3\text{-P}$ ; Chen et al., 2016), three (resin-P; Butterly et al., 2009) or four (MRP; Scalengheh et al., 2012) cycles than after the first, likely due to microbial and non-microbial effects and suggesting cumulative benefits of multiple DRW cycles to P availability. However, the corresponding SWPs reached by drying were not reported. Determining the effects of multiple DRW cycles (to the same SWPs) on soil P availability is necessary to identify the duration (as well as magnitude) of potential benefits to plants during a crop cycle. If P availability increases following initial DRW and does not rapidly decline, imposing a DRW cycle prior to planting (such as during a fallow period) may be considered (Tsujimoto et al., 2010; Bünemann et al., 2013).

Determining the SWPs at which P availability increases following DRW in different soils would indicate whether plants would survive soil drying and benefit from increased P nutrition. The spatial variability in drying intensity and potential P release within a soil profile should also be considered. Vertical soil moisture gradients exist with drier soils at the surface due to evaporation (Figure 1.4). As discussed, surface drying occurs naturally without continuous irrigation in both field and pot experiments (Bünemann et al., 2013). Since P release following soil DRW likely depends on the degree of drying, it is likely that more P is released within the (drier) surface soil. Whether released P is taken up by plant roots, rapidly assimilated by the microbial biomass, sorbed to soil particles or leached to deeper layers is not clear. Determining the agronomic relevance of these processes requires that the amounts of P released following soil DRW are related to SWPs, considering the spatial variability within the soil profile.

### **Analysis of available water and available phosphorus**

#### **Soil water potential**

Although fluctuating soil water status can profoundly affect biogeochemical cycling, it is necessary to determine the specific soil water status at which transformations occur. The total SWP is the most appropriate measure of soil water availability to plants and microbes. Total

SWP ( $\Psi_t$ ) comprises three components: gravitational potential ( $\Psi_g$ ), osmotic potential ( $\Psi_o$ ) and matric (pressure) potential ( $\Psi_m$ ). It describes the forces on water associated with soil particles, determining plant water uptake according to gradients between soil and root water potentials (Whalley et al., 2013). The total SWP also determines the accessibility of water to soil microbial communities, which are important regulators of the “Birch effect” (Fierer and Schimel, 2003). For example, drying soil to -1.5 MPa decreased microbial respiration to 5-8 % of the maximum, occurring at *c.* 0 MPa (Fischer, 2009), suggesting that the low SWP (plant PWP) was detrimental to the microbial biomass, but not completely biocidal. Soil microbial resistance to decreasing SWP varies widely according to species (Swift et al., 1979). Furthermore, the SWP describes water availability to plants and can be critical to understanding their growth and development (Whalley et al., 2013). Although measures of water potential *in planta* vary with light, temperature, evaporative demand and other environmental variables, root and leaf water potentials tend to equilibrate with the bulk soil water potential during the night when transpiration declines (Schmidhalter, 1997). Therefore the total SWP is the most appropriate measure of plant water availability. Correlating SWP values with plant responses is essential to determining the effects of given water deficits (and in some cases managing irrigation); critical ranges are summarised in Figure 1.4.

Soil water gradient	SWP (MPa)	Relevance
	< -1.5 MPa	Below the PWP, but common in surface soils in hot/ dry environments without continuous irrigation.
	-1.5 MPa to 0 MPa	From PWP to saturation: most crop plants thrive at soil water potentials in this range.
	-0.01 MPa to -0.03 MPa	Field-drained soil.
	0 MPa	Saturation: some plant species thrive in flooded conditions, principally lowland irrigated rice as a crop plant. As well as surface flooding, water exceeding the soil water holding capacity and unable to drain (e.g. held by a clayey plough pan) increases the water potential to this range, which may be detrimental to some species.

Figure 1.4: Important ranges of total soil water potential (SWP) and significance to plants, relating to the permanent wilting point (PWP).

The soil moisture release curve describes the relationship between the soil matric potential ( $\Psi_m$ , MPa) and GWC (%), and this varies according to soil texture and changes in structure (Whalley et al., 2013). Estimates of matric potential from GWC are inaccurate since the relationship is non-linear, and further complicated by the effect of hysteresis such that the relationship differs according to whether the soil is becoming progressively drier or wetter (Whitmore and Whalley, 2009). Thus soil matric or water potential needs to be measured directly using different instruments that vary in their accuracy across different  $\Psi_m$  ranges (Whalley et al., 2013). At present, the most accurate instruments ( $\pm 0.1$  MPa) to measure soil water potential across a wide range (-0.1 to -300 MPa) are potentiometers such as the WP4 Dewpoint Potentiometer (Decagon Devices, Inc., Pullman, WA, USA), especially where in-situ measurements are not practical due to small sample sizes in laboratory experiments. In contrast, tensiometers are more accurate in moist soils (between 0 to -0.08 MPa) and can provide continuously-logged in-situ measurements in larger soil volumes. Unless soil is constantly irrigated to maintain saturated or flooded conditions, SWP inevitably varies along a vertical soil moisture gradient, increasing with depth (Figure 1.4). Therefore a further advantage of measuring SWP using a WP4 instrument is the capability to measure approximately 6 cm<sup>3</sup> of soil, substantially more than some other instruments (e.g. thermocouple psychrometers) thereby enabling a more representative soil sample to be measured, incorporating the heterogeneity of particle size. Furthermore, the soil volume required is appropriately small to select samples from precise soil depths and at low SWPs (*cf.* tensiometers), again with multiple samples accounting for heterogeneity in soil moisture.

### **Soil phosphorus availability**

There are fundamental methodological difficulties in measuring “plant-available P” in different soils. A major challenge for accurately recommending P fertiliser rates is the variation in P-fixing and P buffering capacities of different soils according to their chemical properties (such as pH and concentrations of Al), which profoundly affect the amount of applied P that remains plant-available (Daly et al., 2015). Yet measuring P availability via different soil tests is

important to determine P concentrations in different soil pools (Moody et al., 2013) and to estimate or predict crop responses to P supply within cropping systems (Six et al., 2013; Speirs et al., 2013).

### **Laboratory processes**

The method of quantifying available soil P, as any other soil nutrient or property, is critical since methods of soil preparation and P extraction can affect the results and their interpretation. This is particularly problematic when measuring soil P dynamics following drying and re-wetting is the key interest. Air-drying soil is a preparatory step required for the common analysis for available P in the UK, Olsen P, a sodium bicarbonate ( $\text{NaHCO}_3$ ) extraction, and water-extractable TP, MRP and MUP. Because air-drying can increase the solubility of soil P (Soinnie et al., 2010), analyses of air-dried soils are not representative of P solubility in the field. Since the rapid re-wetting of dried soils increased both the Olsen extractable P (Turner and Haygarth, 2003) and the water-soluble P (Turner and Haygarth, 2001), soil P analyses requiring sodium bicarbonate, water or other extractant (re-wetting) using dried soils should be questioned.

Analytical results may also be distorted by filtering the soil and extractant, which occurs in some P analysis methodologies. Although the total amount of water-extractable and water-soluble total P (TP) was unchanged, air-drying increased the proportion of small-sized to large-sized MRP and MUP fractions (Soinnie et al., 2010). This was likely due to structural changes in SOM, suggesting that the larger-sized particles were a source of the increase in small-sized P. However, filtering removes the larger-sized fractions and therefore its proportion was likely underestimated (Soinnie et al., 2010). Alternative methods which do not require filtration also have constraints; for example anion exchange (resin) membranes mimic plant uptake (DeLonge et al., 2013), but the method can require soil wetting which could increase the mineralisation of organic P (Chepkwony et al., 2001). Therefore, other soil P tests have the caveat of requiring that a solution (or water) is added. Thus, the laboratory procedures for measuring soil P concentrations have important implications for assessing its availability. Ideally soil analyses should be carried out on soils at their sampled moisture contents to understand processes

occurring under field conditions (Turner and Haygarth, 2003; Styles and Coxon, 2006; Butterly et al., 2011b).

### Selecting soil phosphorus tests: intensity *versus* quantity indices

Soil tests measure the concentration of orthophosphate in soil solution (intensity) or the amount of available P sorption sites on the solid phase (quantity). Various methods are used to measure soil P availability (Pierzynski, 2000), and those considered most important are summarised in Table 1.1.

Table 1.1: Summary of common tests for available soil phosphorus

Soil test	Abbreviation	Type	Extractant/ membrane
Water-extractable total P	TP	Intensity	Deionised or milli-Q water.
Water-extractable molybdate reactive P	MRP	Intensity	Deionised or milli-Q water.
Water-extractable molybdate unreactive P	MUP	Intensity	Deionised or milli-Q water.
Sodium bicarbonate-extractable P (Olsen P if conform to analysing air-dried soil)	NaHCO <sub>3</sub> -P (or Olsen P)*	Intensity + Quantity	0.5 M Sodium bicarbonate (NaHCO <sub>3</sub> ), adjusted to pH 8.5 with sodium hydroxide (NaOH).
Anion exchange membrane P	Resin-P	Intensity (+ Quantity, depending on extraction time and extractant)	AEM/ resin membrane and deionised or milli-Q water.
Diffusive gradient in thin films P (DGT-P)	DGT-P	Intensity (+ Quantity, depending on extraction time and extractant)	Iron oxide membrane with diffusive layer.

\*Sometimes AEM-P but referred to hereafter as resin-P (Turner et al., 2018).

Water-extractable TP, MRP and MUP indicate different operationally defined forms of P and potential availability. Sodium bicarbonate (0.5 M) has a larger ionic strength than deionised water, collapsing larger soil particles and thus typically measuring more P (Soinne et al., 2010). Following a tradition initiated by Olsen et al. (1954), Olsen P remains the principle test used to determine plant-available P in the UK. Olsen P test results are used by farmers by consulting a Fertiliser Manual, RB209, produced by DEFRA (2017) which recommends fertiliser application rates for different crops by assigning indices based on the soil test P concentration. Recommendations are also based on soil test results from resin-P extractions, for grass and forage crops although not arable crops (DEFRA, 2018a,b). An important methodological note is that the indices are based on Olsen P concentrations in mg per litre and whilst the recommendations state that direct comparison can be made with soil test results (mg per kg; DEFRA, 2017), the comparison assumes a soil bulk density of 1. This applies to most mineral soils so the results are very similar (Poulton et al., 2013; Johnston et al., 2013), although may not be comparable for organic soils with lower bulk densities.

The suitability of soil P tests to determining plant-available P depends on the soil type and properties. The Olsen P test was developed for calcareous soils with relatively high pH, though it remains widely used across soil types (Johnston et al., 2013). In temperate soils which tend to have a lower P sorption capacity than tropical soils and where mineral fertiliser is available, measurements of available P based on quantity indices may largely explain crop P uptake and/or yields (e.g. Poulton et al., 2013; Johnston et al., 2013). In tropical soils however, with higher P sorption capacity and often lacking in mineral P fertiliser, the conventional soil P tests (developed for temperate soils) cannot accurately indicate soil P availability (Oberson et al., 2006). The DGT technique measures soil solution P and also re-supply from the solid phase, by binding solution P thereby establishing a diffusion gradient promoting re-supply, with a diffusive layer to limit the P flux similarly to P uptake by plant roots. A major benefit of the DGT technique is that critical DGT-P values for maize yields did not relate to the P buffering capacity, so soil types with different properties affecting P availability could be compared (Six

et al., 2013). Resin-P, another intensity measure (Table 1.1), better predicted rice yields in soils with low P availability than DGT-P (Six et al., 2013). Therefore it is necessary to measure different P pools via various analytical methods to gain a comprehensive understanding of how they are affected by DRW and the implications for P availability to plants. Another important process affecting soil P availability is the slow mineralisation of organic P (Blackwell et al., 2012), which may be suggested by changes in microbial biomass P (MBP) concentrations and determined through repeated sampling following a soil DRW event.

### **Thesis objectives**

Many questions regarding how soil drying and re-wetting affects P availability persist, limiting the ability to identify optimal irrigation regimes to exploit “windows of opportunity” for increased P availability to plants (Chepkwony et al., 2001). This thesis addresses questions considered crucial to determining the potential agronomic importance of soil DRW, particularly where water and P resources are limited. The aim was to evaluate soil P release and plant uptake to test the hypothesis that DRW could release P at soil water potentials (SWPs) that can support plant growth, thereby increasing crop P use efficiency and yields. Experiments comprised different spatial and temporal scales, from laboratory investigations of effects of soil water potential on P availability, through pot experiments to determine spatial variability in soil moisture and P availability within soil profiles and effects on plants, to a field trial to determine the impacts on P availability, uptake and yields in an extant environmental setting for rice cultivation where soil P is limiting.

**Five main questions were addressed:**

1. Firstly, whether increased soil P availability caused by DRW occurs at soil water potentials that can support plant growth (Chapter 2).
2. Secondly, whether the maximum P availability occurs when soil is air-dried and re-wet initially, and/ or with multiple DRW cycles (Chapter 3).
3. Thirdly, whether soil P availability following DRW (from saturated conditions) or AWD (from flooded conditions) varies spatially within a soil profile in accordance with variation in SWP (Chapter 3).
4. Fourthly, whether soil DRW increases plant P uptake and yields due to increased soil P availability (Chapters 3 and 4).
5. Finally, whether controlled soil drying, via AWD or post-anthesis drying, decreases grain P concentrations and increases P use efficiency whilst maintaining yields (Chapter 4).

**Chapter 2** explores the change in P forms following air-drying and re-wetting in contrasting soils and the likelihood of microbial regulation (Experiment 1); and furthermore determines the soil water potentials at which soil P availability significantly increases at thresholds or along a continuum between “field-moist” and “air-dried” conditions (Experiment 2).

The hypotheses are that greater increases in available P following DRW occur in the soils with higher microbial biomass P (MBP) concentrations (Experiment 1); and increased P availability following DRW is correlated with decreased SWP (Experiment 2).

**Chapter 3** scales up laboratory experiments to determine the change in P availability following DRW and AWD at different soil depths (and thus SWPs) within a pot (Experiment 1); and furthermore determines the impacts of whole-pot and surface soil DRW on P uptake, growth and yields in the model cereal crop *Brachypodium distachyon* L. (Experiment 2).

The hypotheses are that the greatest soil P availability will occur within the dried and re-wet or re-flooded treatments and in the surface soil due to lower soil water potentials upon drying. Furthermore, soil P availability will be highest after two DRW cycles compared to one cycle, and will be higher in initially DRW compared to continuously moist soil (Experiment 1). Initial soil DRW will increase available P concentrations compared to continuously moist soil, and reducing irrigation frequency will have additive effects; and the highest P uptake, biomass and grain yields will occur in plants grown in soils which were dried and re-wetted prior to planting, and irrigated less frequently (Experiment 2).

**Chapter 4** scales the laboratory and pot studies to a field trial, determining the impacts of AWD on soil P availability and P uptake, growth and yields in the globally-important cereal crop *Oryza sativa* L.

The hypotheses are that under low P supply, soil P availability, plant biomass production and grain yields will be higher under AWD compared to CF; and grain P concentrations will be reduced under post-anthesis soil drying compared to CF, increasing PUE.

**Chapter 5** summarises the key results from these studies and draws conclusions on the likely benefits of applying DRW/ AWD to improve water and P efficiencies in crop production, highlighting the further work required.

## **Chapter 2: Characterising soil drying and re-wetting effects on phosphorus availability.**

### **Abstract**

**Context:** Whilst soil drying and re-wetting has previously been shown to increase soil phosphorus (P) availability, the degree of drying necessary to release P amounts relevant to plant uptake remains unknown.

**Hypotheses:** Two experiments aimed to determine whether: greater increases in available P following drying and re-wetting (DRW) occurred in soils with higher microbial biomass P (MBP) concentrations; and increased P availability following DRW is correlated with decreased soil water potential (SWP).

**Strategic approach:** Three soils with different MBP concentrations were gradually air-dried, and available P concentrations were measured across soil moisture gradients ranging from -0.1 to -212 MPa.

**Conclusions:** Plant-available sodium bicarbonate extractable P ( $\text{NaHCO}_3\text{-P}$ ) concentrations significantly increased at similar SWPs between the soils, which were below plant permanent wilting point (PWP) but which can occur in surface soils under field conditions. This suggested that soil DRW can potentially increase plant P nutrition due to surface soil drying and/or when applied during a fallow period.

### **Introduction**

#### **Soil drying and re-wetting effects on P availability: importance of the microbial biomass**

Studies of the “Birch effect” have included different soil types from around the world, and the increase in P availability following DRW is caused primarily by combined soil physical and biological processes (Figure 1.2). Numerous studies have confirmed the role of the microbial

biomass in regulating the P response to DRW. For example, across 29 permanent grassland soils in England and Wales, varying in P concentrations ( $9\text{--}48\text{ mg kg}^{-1}\text{ NaHCO}_3\text{-P}$ ), water-soluble TP increased by 1.9–19-fold following DRW. This was primarily (56–100 %) water-soluble MUP, and the positive linear relationship with soil MBP concentration suggested a direct release from the microbial biomass (Turner and Haygarth, 2001). Furthermore, the potential contribution of bacterial cell lysis to released TP was at least 88 %, and to released MUP was 95 % (Turner et al., 2003). Although MBP concentrations were not measured, the potential contribution was measured by direct bacterial cell counts following extraction, then calculating total P contents by assuming a mean (bacterial) volume and P content (Turner et al., 2003). Thus air-drying released water-soluble TP, MRP and especially MUP largely due to lysis of bacterial cells, indicating the important role of the microbial biomass. More recently, Blackwell et al. (2009) also suggested greater release of organic than inorganic P into solution following DRW, although the data were not specifically related to MBP concentrations. Therefore despite evidence for the important role of the microbial biomass in regulating the “Birch effect”, attempts to compare changes in P availability following DRW between different soil types with different MBP concentrations in the same study are otherwise limited.

### **Effects of the degree of soil drying on P availability following re-wetting**

Whilst previous research has shown that P availability is often increased following DRW, determining the potential contribution of this response to plant P nutrition requires soil P availability to be related to soil water availability. Greater soil drying (with lower minimum SWPs before re-wetting) increases microbial mortality and potentially mineralisable P upon re-wetting (Blackwell et al., 2012), while also affecting soil structure and other physical properties regulating P availability. Whilst various studies have compared changes in soil biogeochemistry in “air-dried” and “field-moist” soils, little attention has been given to biogeochemical processes and effects at intermediate water contents. An exception is a study of incremental re-wetting on the “Birch effect” which measured the responses of respiration, microbial biomass C and net N mineralisation (Lado-Monserrat et al., 2014). Rather than observing responses at different

stages of drying, responses to several DRW events were monitored. Although it was not possible to reproduce SWP values due to hysteresis occurring upon multiple DRW cycles (with different relationships between GWC and SWP during drying and wetting processes), GWC determined the water status (volumes of water added). Thus, observing effects during a single drying event would instead allow SWP measurements. The SWPs were estimated separately for the samples before re-wetting, for a sandy loam soil (-0.004 MPa to -1.189 MPa with two samples at intermediate SWP) and a clay loam soil (-0.078 MPa to -40 MPa or lower with three samples at intermediate SWP). The sensitivity of the soil microbial biomass to drying and re-wetting intensity (defined as the amount of water added per day; Borcken and Matzner, 2009) was related to substrate availability and was soil dependent (Lado-Monserrat et al., 2014). Thus it was difficult to separate the effects of lower SWP from other soil properties such as texture and organic C contents. Furthermore, the two soils were sampled from a pine forest, so their responses to DRW may differ from soils under grassland or other agricultural management. Whilst this study quantified effects of DRW on microbial dynamics, it did not provide information on P availability. More measurements at intermediate SWPs are necessary to determine whether increases in P availability can occur within the ranges above PWP that do not cause plant mortality.

Previous studies of the effects of the degree of soil drying on P availability have often measured soil moisture as changes in GWC, rather than SWP, making comparisons difficult. After drying a silty clay soil from an initial GWC of 40 % to different degrees (2, 5, 10, 15, 20 and 40 % GWC) and then re-wetting to 40 % GWC, resin-P significantly increased after drying to 10 % GWC or lower, with the maximum increase (by nearly 1000 %,  $6.9 \text{ mg kg}^{-1}$ ) occurring in soils that had dried to 2-5 % GWC (Bünemann et al., 2013). Similarly, drying a silt loam from an initial GWC of 30% to different degrees (2, 5, 10, 15, 20 and 30 % GWC), then re-wetting to 30 % GWC, significantly increased  $\text{NaHCO}_3\text{-P}$  (by *c.* 16 %,  $1 \text{ mg kg}^{-1}$ ), but only when soil had dried to 2 % GWC (Sun et al., 2017a). Furthermore, in five different soils (two loams, sandy loam, silt loam and silty clay loam), the greatest increases in  $\text{NaHCO}_3\text{-P}$  (by 26.3 – 48.1 %)

occurred when soils were dried from 50 % to 5 % WHC, and generally did not increase until dried to 10 % WHC (Sun et al., 2017b). Thus, although the degree of soil drying affected  $\text{NaHCO}_3\text{-P}$  concentrations following re-wetting, significant increases only occurred in the driest soils. However, GWC and WHC are of limited value in understanding plant water uptake, which is determined by water potential gradients between soil and roots. There remains a need to determine how  $\text{NaHCO}_3\text{-P}$  and other measures of P availability change as SWP decreases.

With few studies reporting how the degree of soil drying affects P release upon re-wetting, it is uncertain whether the relationship between P availability and soil moisture can be described by a linear, logarithmic or “change point” response (Figure 2.1). Characterising the relationship between leachate P concentrations with soil P concentrations has utilised the concept of “change points”, with sudden increases in P solubilisation indicating threshold values for P leaching, which vary between soils (Fortune et al., 2005).

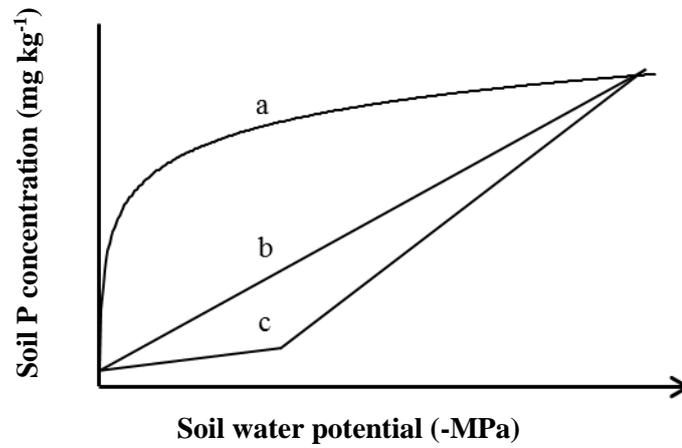


Figure 2.1: Hypothetical change in soil P concentrations with decreasing soil water potential. The relationship may be characterised by a logarithmic (a), linear (b), or change point (c) response.

In studying the effects of the degree of soil drying, the microbial impacts of the “Birch effect” (e.g. respiration) could not be related to an individual threshold value of SWP, due to their non-linear decrease with the pre- re-wetting SWP (Lado-Monserrat et al., 2014). However, for P, change points may have occurred at the lowest GWC and WHC measured, since P concentrations only significantly increased from FM levels at those points (Bünemann et al., 2013; Sun et al., 2017a, b). Nevertheless, more data from different soils at a range of water contents is needed to test this proposition. The form of the relationship between SWP and P availability (Figure 2.1), and whether significant change points exist, have yet to be determined. Knowing whether such soil drying occurs under field conditions, either during or prior to initiating a cropping cycle, will inform whether irrigation management may stimulate increased P availability, considering the PWP and P requirements of the crop.

### **Objectives and hypotheses**

Two experiments aimed to determine the role of the microbial biomass and the relationship between the degree of soil drying and the magnitude of P release following DRW in five different soils, identifying SWPs at which P availability increased upon DRW, by testing two hypotheses:

- 1. Greater increases in available P following DRW occur in the soils with higher microbial biomass P (MBP).** This is because a substantial component of newly available P is the rapidly mineralised P derived from organic P, released from the microbial biomass due to cell lysis caused by osmotic shock upon re-wetting.
- 2. Increased P availability following DRW is correlated with decreased SWP, and the SWP at which P availability increases varies according to soil.** Greater increases in available P occur with more intense drying measured as GWC (Sun et al., 2017a,b), and thus SWP. Since P availability and the response to DRW depend on numerous soil

properties (e.g. MBP concentration), and changes in SWP relate to texture (particularly clay and organic matter contents), threshold SWP values will differ according to soil type and properties.

## **Materials and Methods**

### **Site, sampling and preparation**

Five different soils were collected from south-west England. One soil was collected from 0-10 cm depth from the Tadhams Moor SSSI in Somerset, UK, and two soils were collected from 0-10 cm depth at Rothamsted Research, North Wyke in Devon, UK, during May-June 2014. Two further soils were collected from 0-20 cm depth (also used for larger experiments; Chapter 3) from the Rowden and Whiddon Down sites at North Wyke in October 2014 and February 2017, respectively. Soil characteristics are outlined in Tables 2.1 and 2.4. Samples were stored at field moisture contents at 4 °C until preparation and analysis. All samples were sieved to 2 mm; then the field-moist (FM) gravimetric water contents (GWC) were measured (maintained from sampling) and the soils were again stored at 4 °C. Soils were air-dried by spreading them thinly on clean trays in an oven at 35 °C until reaching constant weight, then sealing the air-dried (AD) soils in plastic bags. For Experiment 1, soil analyses were carried out on FM and AD soils, although microbial biomass P was analysed for FM samples only. For Experiment 2, soil analyses were also carried out for an intermediate soil moisture range. These experiments compared FM to dried then re-wet (DRW) soils, since the analytical procedures require re-wetting the soils with a fixed volume of extractant for a specified period of time. Analyses on FM soils were carried out at similar percentage of water holding capacity (% WHC) (within 14%) despite the variation in GWC (with a range of 41%) across soil types (Table 2.1).

Table 2.1: Soil characterisation. Field moisture contents (GWC) and percent of the water holding capacity (% WHC) are means ( $\pm$  SE) ( $n = 3$ ). The % WHC was calculated as: ((mass of water in saturated soil / dry weight of soil) \* 100).

Reference (location)	Soil series	Texture	Management	GWC (%)	% WHC
Tadham Moor	Altcar 1 <sup>a</sup>	Peat	Low-input ungrazed grassland reserve	63 (1.3)	56 (0.7)
Little Burrows	Halstow	Clay	Grazed grassland	27 (0.4)	64 (0.2)
Joseph's Carr	Fladbury	Clay	Low-input ungrazed woodland	38 (0.5)	60 (0.3)
Rowden	Hallsworth <sup>b</sup>	Clay	Low-input ungrazed grassland	37 (0.3)	66 (0.6)
Whiddon Down	Hallsworth	Clay	Grazed grassland	22 (0.2)	70 (1.4)

<sup>a</sup>Evans *et al.*, 2017; <sup>b</sup>Harrod and Hogan, 2008.

### Experimental design

To test the hypothesis that microbial effects regulate soil P availability following DRW, the MBP concentrations were measured in three FM soils, and water-extractable TP, MRP and MUP concentrations determined for these soils in FM and DRW conditions (Experiment 1).

For Experiment 2, three soils were selected for a drying threshold experiment:

- Tadham Moor since it had the highest MBP concentration;
- Rowden since it was used previously (Blackwell et al., 2009; 2012), with sufficient volume available for larger experiments (Chapter 3);
- Whiddon Down, selected from a site acquired by Rothamsted Research, North Wyke during the development of Experiment 1, since it is similar to the Rowden soil but more intensively managed (Table 2.1), and also for its availability for scaled-up experiments (Chapter 3).

The experiment was repeated twice: initially with the Rowden soil (T1) and later to also include the Tadham Moor and Whiddon Down soils, and analyse a broader range of P forms (T2).

Field-moist soils were gradually dried in an oven at 35 °C, with sub-samples taken at different times to measure P availability at different degrees of drying. For T1, FM soils were weighed into 200 mL extraction bottles (with approximate dimensions of 120 mm height x 60 mm diameter) which were placed directly and randomly in the oven, with each bottle containing one of the triplicate samples for P analysis. Additionally, one bottle per sample time was included to determine soil moisture characteristics. For T2, to increase the drying rate and within-sample uniformity, soils were instead dried in randomised tin trays which had a greater horizontal surface area (with approximate dimensions of 30 mm height x 120 mm length x 80 mm width). Sample times were determined by a preliminary trial that frequently recorded the GWCs for each soil type within 48 hours. For T1, soil was sampled at 0, 2, 4, 8, 16 and 24 hours. For T2, the Rowden and Whiddon Down soils were sampled at 0, 0.25, 0.5, 0.75, 1, 1.5, 2 and 24 hours

and the Tadham Moor soil (with a higher initial GWC and slower drying rate) was sampled at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours. Samples taken during T1 were extracted immediately to determine  $\text{NaHCO}_3\text{-P}$ , whereas samples taken during T2 were stored in sealed plastic bags at 4 °C until analysis of  $\text{NaHCO}_3\text{-P}$  and water-extractable TP and MRP.

### **Soil moisture characteristics**

Soil moisture contents (%) were determined gravimetrically with a two decimal point balance. Soil water potential was determined using a WP4-T Dewpoint Potentiometer (Decagon Devices Inc., USA). This method measures the temperatures of the soil sample and of a mirror, which is cooled until water condenses on its surface, i.e. at the dewpoint. The relative humidity is then calculated which relates to soil water potential according to the Kelvin equation (see Whalley et al., 2013).

### **Analytical procedures for soil phosphorus**

All analyses were carried out in triplicate following standard procedures as described. All P concentrations are expressed on a dry weight equivalent (DWE) basis. Analytical quality controls (AQC) were included with each analytical procedure. A solution and a reference soil each with a known concentration of orthophosphate were included with each analytical run so it could be confirmed that these known concentrations were measured. Repeats of the blanks, AQC and calibration standards were included within sample runs (within as well as between plates), and some repeat samples were also analysed. The limits of detection (LOD) and quantification (LOQ) were determined by measuring ten matrix blanks and calculating three times and ten times the standard deviation of the blanks, respectively. Samples reading below the LOQ were excluded from the results.

### **Microbial biomass phosphorus (MBP)**

Soils were incubated at room temperature (approximately 25 °C) for 24 hours prior to MBP analysis (Blackwell et al., 2012). MBP was measured according to Brookes (1982), via

chloroform fumigation for 24 hours to lyse microbial cells followed by sodium bicarbonate extractable P (NaHCO<sub>3</sub>-P) measurement according to Olsen et al. (1954) and adapted for moist soils by Snars et al. (2006). Briefly, the analysis included fumigated and non-fumigated samples and a non-fumigated sample spiked with a known concentration of orthophosphate (PO<sub>4</sub>-P), such that the difference could be calculated allowing for P sorption during extraction for each individual sample. The NaHCO<sub>3</sub> extractant was adjusted to pH 8.5 with sodium hydroxide (NaOH). A soil: extractant ratio of 1:20 was maintained, in this case with soil weight of 10 g (DWE) and NaHCO<sub>3</sub> volume of 200 mL. Samples were agitated on a reciprocating shaker at 150 rpm for 30 minutes, then filtered through pleated 2-3 µl filter papers (Whatman plc., UK or VWR International Inc., UK). Orthophosphate was measured within 24 hours according to Murphy and Riley (1962) using an Aquakem 250 Photometric Analyzer (Thermo Scientific, UK) at the Rothamsted Research North Wyke analytical laboratory. Incorporating the P recovery factor, MBP concentration (mg kg<sup>-1</sup>) was calculated as:

$$(25 * (C_f - C_u)) / (0.4 * (C_s - C_u))$$

Where:

25 is the concentration of the P spike (mg kg<sup>-1</sup>);

C<sub>f</sub> is the concentration of P in the fumigated sample (mg kg<sup>-1</sup>);

C<sub>u</sub> is the concentration of P in the unfumigated sample (mg kg<sup>-1</sup>);

0.4 is a conversion factor assumed to represent the fraction of MBP extracted following fumigation;

C<sub>s</sub> is the concentration of P in the spiked sample (mg kg<sup>-1</sup>).

For the Whiddon Down soil, MBP was measured via hexanol fumigation and extraction with anion exchange resin membranes (Kuono et al., 1995; Bünemann et al., 2013). Chloroform and hexanol were equally effective as biocides, with hexanol preferred because it is not carcinogenic (McLaughlin et al., 1986), and the hexanol fumigation-resin extraction method was considered to be more effective for saturated soils (Chapters 3 and 4). Resin membranes were prepared by

shaking at 100 rpm in 1.5 M NaHCO<sub>3</sub> for 1 hour, rinsing in Milli-Q water and repeating. Six samples of 2.5 g DWE soil were then shaken at 150 rpm for 16 hours in 20 mL Milli-Q water with one resin membrane per sample, with the addition of 0.8 mL hexanol to three sub-samples for fumigation. Resin membranes were then recovered in 8 mL of 0.1 M hydrochloric acid (HCl) and shaken at 150 rpm for 1 hour. Finally the resin membranes were removed and eluates analysed using malachite green reagent and a plate reader (Multiskan™ GO Microplate Spectrophotometer, ThermoFisher Scientific, USA) at 625 nm absorbance, with sample P concentrations determined from a standard curve. For this method, the P recovery factor was determined for four samples by adding 1 mL of 5 mg P L<sup>-1</sup> P spike solution (and reducing the Milli-Q water volume to 19 mL), then applied to the sample results.

P recovery was calculated as:

$$\mathbf{P\ Recovery = ((C_s - C_f) * 100) / C_s}$$

Where:

C<sub>s</sub> is the concentration of P in the spiked sample (mg kg<sup>-1</sup>);

C<sub>f</sub> is the concentration of P in the fumigated sample (mg kg<sup>-1</sup>).

The mean P recovery was 46.4 % (SE = 2.6) for the Whiddon Down soil; thus MBP was calculated as:

$$\mathbf{MBP = ((C_f - C_u) * 0.008) / (SW * 0.464)}$$

Where:

C<sub>f</sub> is the concentration of P in the fumigated sample (mg kg<sup>-1</sup>);

C<sub>u</sub> is the concentration of P in the unfumigated sample (mg kg<sup>-1</sup>);

0.008 is the volume of HCl in litres;

SW is the DWE weight of the soil sample in kilograms;

0.464 is the recovery factor.

**Water-extractable total phosphorus (TP), molybdate reactive phosphorus (MRP) and molybdate unreactive phosphorus (MUP)**

Water-extractable TP and MRP were measured according to the verified method for the North Wyke laboratory (Williams, 2007), with appropriate adaptations as advised (M. Blackwell, pers. comm.). Soil was extracted with Milli-Q water in a 1:4 DWE soil: water ratio on a reciprocating shaker for 30 minutes. Suspensions were centrifuged at 4600 rpm for 5 minutes, then supernatants were filtered through 2-3  $\mu\text{m}$  filter papers. For TP, sub-samples of the filtrates were taken and the non-molybdate reactive forms (organic, condensed and colloidal P) were oxidised with acidified potassium persulphate in an autoclave, thereby converted to orthophosphate. Orthophosphate in each set of sub-samples for TP and MRP was measured according to Murphy and Riley (1962). All TP samples were measured with an Aquakem 250 Photometric Analyzer (Thermo Scientific, UK) at the Rothamsted Research North Wyke analytical laboratory. The MRP samples were measured with an Aquakem 250 Photometric Analyzer (Thermo Scientific, UK) at the Rothamsted Research North Wyke analytical laboratory for Experiment 1; an AutoAnalyser (AA3, SEAL Analytical, Porvair Sciences) for Experiment 2, T1; and a plate reader (Multiskan<sup>TM</sup> GO Microplate Spectrophotometer, ThermoFisher Scientific, USA) at 880 nm absorbance for Experiment 2, T2. Unreactive P is generally considered to be organic P (Haygarth et al., 1998) and was calculated as the difference between TP and MRP.

**Sodium bicarbonate extractable phosphorus ( $\text{NaHCO}_3\text{-P}$ )**

Sodium bicarbonate extractable P ( $\text{NaHCO}_3\text{-P}$ ) was measured as described for the determination of MBP, according to Olsen et al. (1954) and adapted for moist soils by Snars et al. (2006). Soil was extracted with 0.5 M  $\text{NaHCO}_3$ , adjusted to pH 8.5 with NaOH, in a 1:20 DWE soil: extractant ratio on a reciprocating shaker for 30 minutes. Suspensions were then centrifuged at 4600 rpm for 5 minutes, then supernatants were filtered through 2-3  $\mu\text{m}$  filter papers. Filtrates were neutralised by adding 0.5 mL of 1.5 M sulfuric acid ( $\text{H}_2\text{SO}_4$ ) to 2.5 mL

of sample and swirling until the reaction was complete. Orthophosphate was measured within 24 hours according to Murphy and Riley (1962) using an AutoAnalyser (T1) and plate reader (T2) as described for water-extractable P.

### **Statistical analysis**

Statistical analyses were performed using the software SPSS version 23 (IBM, 2014). In all cases, a confidence threshold of 0.05 was applied.

For Experiment 1, significant differences in mean MBP concentration between the soils were determined using Welch's one-way ANOVA and a Games-Howell pairwise test. For water-extractable TP, MRP and MUP, data were transformed (log base 10) prior to carrying out two-way ANOVA to determine the effects of soil type, air-drying and their interaction. To determine whether air drying significantly increased water-extractable TP, MRP and MUP concentrations compared to field-moist samples, independent sample t-tests were carried out, except for the MRP data for the Joseph's Carr soil which required a Welch-Satterthwaite t-test (due to unequal variance of the standardised residuals). Linear regression analysis was carried out to determine whether the positive relationships between MBP and the percent increases in TP, MRP and MUP were significant, where non-significant changes following air-drying were assumed have no increase.

For Experiment 2, significant differences between P concentrations according to water status were determined by ANOVA as next described, using drying time (hours) as the dependent variable associated with water status. Differences between GWC were determined using Welch's one-way ANOVA with Games-Howell pairwise tests (Rowden, T1 and Whiddon Down and Tadham Moor, T2) and one-way ANOVA with Tukey's pairwise test (Rowden, T2). Differences between SWP were determined using Welch's ANOVA with Games-Howell pairwise test (all soils, T1 and T2). Differences in  $\text{NaHCO}_3\text{-P}$  were determined by one-way ANOVA with Tukey's pairwise test (Rowden, T1) and Welch's ANOVA with Games-Howell pairwise test (all soils, T2). Differences in TP and in MUP were determined by Welch's

ANOVA with Games-Howell pairwise test (Rowden and Whiddon Down, T2; data unavailable for Tadham Moor). Differences in MRP were determined by Welch's ANOVA with Games-Howell pairwise test (Rowden and Tadham Moor soils, T2) and by one-way ANOVA with Tukey's pairwise test (Whiddon Down, T2).

For T1 and T2, logarithmic regression analysis was performed on the means of the water status variables and  $\text{NaHCO}_3\text{-P}$  to characterise the relationships and their significance. ANCOVA was carried out to determine whether the effect of (log) SWP on  $\text{NaHCO}_3\text{-P}$  differed according to soil type (Rowden and Whiddon Down). Piecewise regression was used to determine the significance of two fitted linear regressions at assumed change points for SWP, with the original SWP values and the differences from assumed change points as independent variables and  $\text{NaHCO}_3\text{-P}$  as the dependent variable in linear regression models.

## **Results**

### **Experiment 1**

#### **Soil microbial biomass phosphorus concentration**

The MBP concentration differed significantly across a 10-fold range between five soils ( $p < 0.001$ ; Table 2.2).

Table 2.2: Microbial biomass P concentrations in five soils. Different upper case letters indicate significant differences between the three soils reported in Experiment 1 ( $p = 0.003$ ) and different lower case letters indicate significant differences between the five soils reported in Experiments 1 and 2 ( $p < 0.001$ ). Significant differences are according to Welch's ANOVA with Games-Howell pairwise test (measured once for each soil;  $p \leq 0.05$ ). Data are means ( $\pm$  SE) ( $n = 3$ ).

Soil	Microbial biomass P (mg kg <sup>-1</sup> )	Experiment
Tadham Moor	361.1 (38.1) A	1, 2
Little Burrows	40.0 (3.2) C b	1
Joseph's Carr	82.9 (0.8) B a	1
Rowden	32.8 (4.3) b	2
Whiddon Down	45.3 (0.9) b	2

### Effects of air-drying on TP, MRP and MUP concentrations

Air-drying affected P concentrations differently, according to P fraction and soil (Table 2.3; Figure 2.2 a-c). For the Tadham Moor soil, air-drying significantly increased all measured forms of P compared to concentrations in FM soils. Mean TP increased by > 1000 % (11.26 mg kg<sup>-1</sup>) ( $p < 0.001$ ), within which the proportions of mean MRP increased the most substantially, by > 10000 % (1.14 mg kg<sup>-1</sup>) ( $p < 0.001$ ) despite lower overall concentrations, whilst mean MUP, the dominant form by mass, increased by 912 % (10.12 mg kg<sup>-1</sup>) ( $p < 0.001$ ). For the Little Burrows soil, air-drying significantly increased TP by 148 % (2.85 mg kg<sup>-1</sup>) ( $p = 0.001$ ). MRP decreased by 30 % (0.07 mg kg<sup>-1</sup>) ( $p = 0.008$ ) following air-drying, whereas MUP increased by 171 % (2.91 mg kg<sup>-1</sup>) ( $p = 0.001$ ). Soil drying did not significantly increase TP of the Joseph's Carr soil. Although air-drying increased MRP significantly by 329 % (0.23 mg kg<sup>-1</sup>) ( $p = 0.002$ ), MUP (which was the dominant P form) did not significantly increase.

Table 2.3: Significant effects of soil type and moisture status (air-dried compared to field-moist samples) and their interaction for water-extractable total P (TP), molybdate reactive P (MRP) and molybdate unreactive (MUP) according to two-way ANOVA following transformation (log base 10) ( $n = 3$ ).

Model term	p-values		
	TP	MRP	MUP
Soil	0.027	0.021	0.023
Moisture	< 0.001	< 0.001	< 0.001
Soil * Moisture	< 0.001	< 0.001	< 0.001

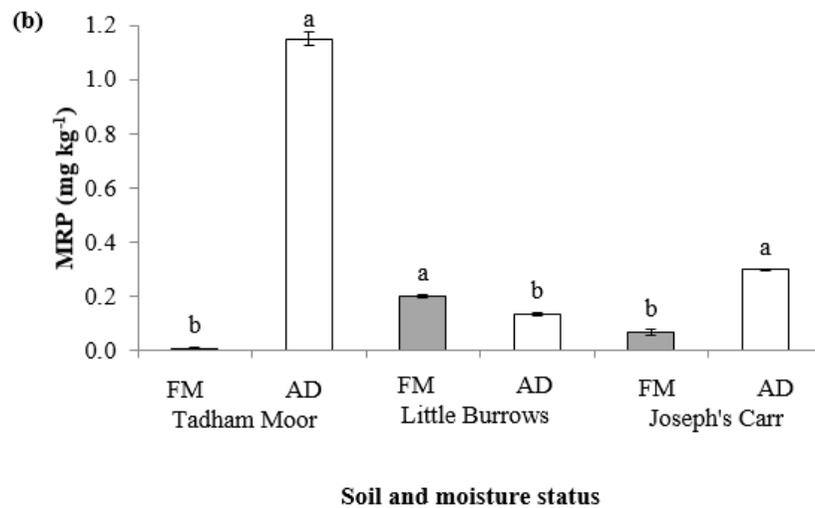
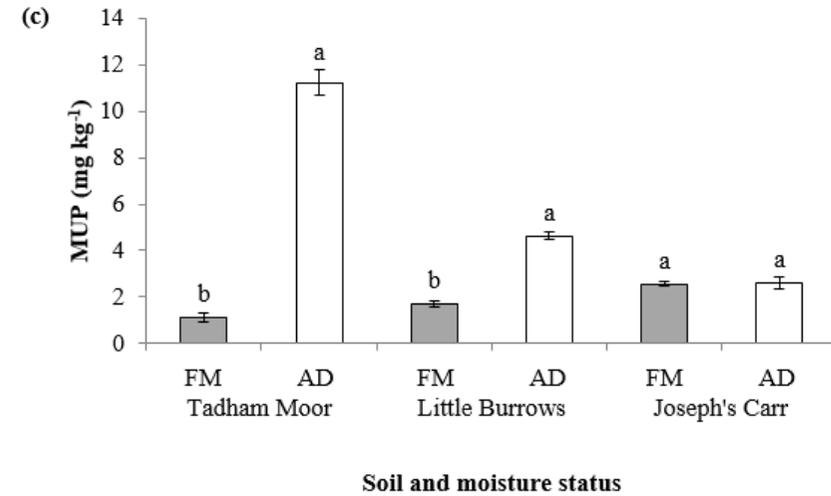
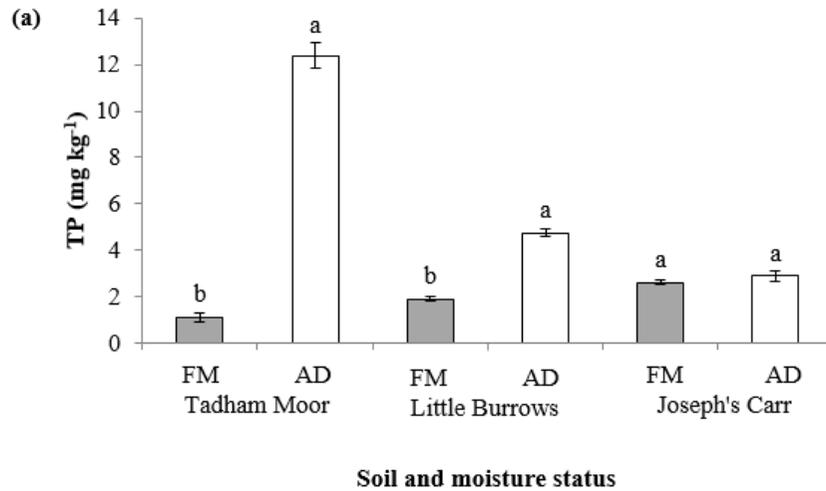


Figure 2.2: Water-extractable (a) total P, (b) MRP and (c) MUP concentrations in three soils, at field-moist (FM) and air-dried (AD) moisture contents and expressed on a dry-weight basis. Data are means ( $\pm$  SE) and  $n = 3$  (except Little Burrows, AD:  $n = 2$ ). Different letters above the columns indicate significant differences ( $p \leq 0.05$ ) between the FM and AD moisture status for each soil, according to independent samples t-tests (except Joseph's Carr, MRP: Welch-Satterthwaite t-test).

**Effects of air-drying on phosphorus availability related to MBP concentration**

For the Tadhams Moor, Little Burrows and Joseph's Carr soils, the average increases in TP, MRP and MUP following air-drying were strongly positively ( $R^2 = 0.933, 0.991$  and  $0.913$ , respectively;  $n = 3$ ) though not significantly related to the average MBP concentrations.

In summary, MUP was the dominant form by mass in all FM soils, comprising 90–99 % of TP. Air-drying significantly increased TP, MRP and MUP in the Tadhams Moor soil, and TP and MUP in the Little Burrows soil whereas only MRP in the Joseph's Carr soil (Figure 2.2). The effects of air-drying were distinct for the Tadhams Moor soil for increasing TP overall as well as both the more and less available MRP and MUP fractions, respectively. Although the greatest increases in P caused by air-drying occurred in the Tadhams Moor soil, which also had the highest MBP concentration (Table 2.2), regressions between the increases in TP, MRP and MUP with MBP after air-drying were not significant.

**Experiment 2**

The major soil properties for the Rowden, Whiddon Down and Tadhams Moor soils are outlined in Table 2.4.

Table 2.4: Major soil physical and chemical properties for the three soils used in Experiment 2.

Analyses were carried out by NRM Laboratories, UK and Rothamsted Research, UK.

Soil property	Result			Units	Method
	Rowden	Whiddon Down	Tadham Moor		
<b>Texture class</b>	Clay	Clay	Peat		Particle size distribution via laser diffraction
<b>Sand</b>	13	8	7	% w/w	
<b>Silt</b>	37	41	35	% w/w	
<b>Clay</b>	50	51	58	% w/w	
<b>Organic Matter</b>	11.5	7.6	No data	% w/w	Loss on ignition (LOI)
<b>pH</b>	4.9	4.9	5.9		In water (1:2.5)
<b>Total C</b>	4.79	2.97	28.58	% w/w	Combustion catalytic oxidation
<b>Total N</b>	0.48	0.32	1.99	% w/w	
<b>C:N Ratio</b>	10.0:1	9.3:1	14.36:1		
<b>Total P</b>	674	640	1026	mg kg <sup>-1</sup>	Aqua-regia soluble elements : HCl and HNO <sub>3</sub> digestion with analysis via ICP-OES
<b>Total K</b>	1572	1450	3064	mg kg <sup>-1</sup>	
<b>Total Mg</b>	730	1570	4141	mg kg <sup>-1</sup>	
<b>Total Mn</b>	986	239	647	mg kg <sup>-1</sup>	
<b>Total Fe</b>	39336	37283	19066	mg kg <sup>-1</sup>	
<b>Available Fe</b>	171	181	7578/ 7970	mg L <sup>-1</sup>	DTPA extraction, or dithionite/ ammonium oxalate extraction (Tadham Moor)

For the first sampling time (T1), air-drying the Rowden soil significantly decreased GWC ( $p = 0.002$ ) and SWP ( $p = 0.046$ ) over time, reaching minimum values of 6.4 % and  $-34.4$  MPa respectively after 24 hours. The NaHCO<sub>3</sub>-P concentrations significantly increased as GWC and SWP decreased ( $p = 0.001$  and  $p = 0.009$  respectively). NaHCO<sub>3</sub>-P increased by 518 % over this period (by  $7.09$  mg kg<sup>-1</sup>;  $p < 0.001$ ), with significant differences from FM soil detected after

16 hours (when GWC and SWP had decreased to 16.3 % and -3.0 MPa) (Table 2.5). Therefore air-drying significantly increased  $\text{NaHCO}_3\text{-P}$  when SWP was -3.0 MPa or lower.

Table 2.5: Effect of drying time on GWC and SWP ( $n = 2$ ) and  $\text{NaHCO}_3\text{-P}$  ( $n = 3$ ) in T1. Data are means ( $\pm$  SE). Different letters indicate significant differences ( $\leq 0.05$ ) within each column according to one-way ANOVA with Tukey's or Games-Howell pairwise tests.

Drying time (hours)	GWC (%)	SWP (MPa)	$\text{NaHCO}_3\text{P}$ ( $\text{mg kg}^{-1}$ )
0	31.2 (0.04) <b>a</b>	-0.1 (0.24) <b>a</b>	1.37 (0.27) <b>cd</b>
2	31.4 (0.00) <b>a</b>	-0.1 (0.94) <b>a</b>	1.00 (0.18) <b>d</b>
4	29.5 (0.02) <b>ab</b>	-0.2 (0.06) <b>a</b>	1.96 (0.12) <b>c</b>
8	27.4 (0.05) <b>a</b>	-0.6 (0.36) <b>a</b>	1.93 (0.12) <b>c</b>
16	16.3 (0.66) <b>b</b>	-3.0 (0.57) <b>a</b>	3.02 (0.17) <b>b</b>
24	6.4 (11.62) <b>c</b>	-34.4 (0.65) <b>a</b>	8.46 (0.16) <b>a</b>

For T2, air-drying the Rowden soil significantly decreased GWC and SWP over time ( $p < 0.001$ ), reaching minimum values of 5.3 % and -142.9 MPa respectively after 24 hours.  $\text{NaHCO}_3\text{-P}$  significantly increased as GWC and SWP decreased ( $p = 0.001$  and  $p < 0.001$  respectively; Figure 2.3).  $\text{NaHCO}_3\text{-P}$  increased by 252 % over this period (by  $3.88 \text{ mg kg}^{-1}$ ;  $p < 0.001$ ), with significant differences from FM soil detected after 2 hours (when GWC and SWP had decreased to 12.1 % and -26.3 MPa) (Table 2.6). However, a significant change point for  $\text{NaHCO}_3\text{-P}$  concentration occurred at -2.9 MPa. Above and below this change point, slopes of the regressions were  $0.014$  and  $0.995 \text{ mg kg}^{-1} \text{ MPa}^{-1}$  respectively (change =  $-0.981$ ,  $p = 0.013$ ). At the higher SWP of -1.9 MPa, the change in slope was not significant ( $p = 0.052$ ) suggesting that a change point may have occurred between these values but was first detected at -2.9 MPa. After 24 hours, air-drying significantly increased soil TP (by 1100 %), MRP (by 71 %) and MUP (by 2540 %) ( $p < 0.001$ ; Table 2.6). These regressions remained significant even after removing the data at 24 hours, though not 2 hours (for TP, MRP and MUP,  $p = 0.006$ ,  $p = 0.016$ ).

and  $p = 0.008$  respectively), suggesting that P increased when SWP decreased to -26.3 MPa (Table 2.6). Therefore air-drying increased TP, MRP and MUP only when SWP was -26.3 MPa or lower, but  $\text{NaHCO}_3\text{-P}$  when SWP was -2.9 MPa or lower.

Air-drying the Whiddon Down soil significantly decreased GWC and SWP over time, reaching minimum values of 1.3 % and -150.9 MPa respectively after 24 hours ( $p < 0.001$ ).  $\text{NaHCO}_3\text{-P}$  significantly increased as GWC and SWP decreased ( $p < 0.001$ ; Figure 2.3).  $\text{NaHCO}_3\text{-P}$  increased by 257 % over this period (by  $4.71 \text{ mg kg}^{-1}$ ;  $p < 0.001$ ), with significant differences from FM soil detected after 0.5 hours (when GWC and SWP had decreased to 14.7 % and -2.3 MPa) (Table 2.6). A significant change point occurred for  $\text{NaHCO}_3\text{-P}$  at -13.1 MPa, above and below which slopes of the regressions were  $0.020$  and  $0.178 \text{ mg kg}^{-1} \text{ MPa}^{-1}$  respectively (change =  $-0.158$ ,  $p = 0.004$ ). However, whether the significant increase in  $\text{NaHCO}_3\text{-P}$  occurred when SWP was -2.3 MPa (Table 2.6) could not be tested because there were only two data points above that value. The change at -3.1 MPa was not significant; therefore  $\text{NaHCO}_3\text{-P}$  significantly increased when SWP was -2.3 MPa (Table 2.6) but more data are required for SWPs below -3.1 MPa, and between -2.3 MPa and -13.1 MPa, to clearly distinguish any change point(s). TP increased by 589 % ( $1.06 \text{ mg kg}^{-1}$ ;  $p < 0.001$ ), with significant differences from FM soil detected only after 24 hours (Table 2.6). MRP concentrations did not significantly increase with drying, whilst MUP increased by 3433 % ( $1.03 \text{ mg kg}^{-1}$ ;  $p < 0.001$ ), detected only after 24 hours (Table 2.6). However, overall TP and MUP significantly increased as GWC ( $p < 0.001$ ) and SWP ( $p = 0.001$ ) decreased over 24 hours, and also as SWP decreased over 2 hours ( $p = 0.002$ ) and 1.5 hours (TP:  $p = 0.023$ , MUP:  $p = 0.022$ ) though not 1 hour, suggesting that P increased when SWP decreased to -56.9 MPa (Table 2.6). Therefore air-drying increased TP and MUP only when SWP was -56.9 MPa or lower, but  $\text{NaHCO}_3\text{-P}$  when SWP was -2.3 MPa or lower.

Air-drying the Tadham Moor soil significantly decreased GWC and SWP over time ( $p < 0.001$ ), reaching minimum values of 9.8 % and -212.3 MPa respectively after 24 hours.  $\text{NaHCO}_3\text{-P}$  significantly increased as GWC and SWP decreased ( $p < 0.001$ ).  $\text{NaHCO}_3\text{-P}$  increased by 106 % after 24 hours although by 201 % after 8 hours (by  $4.25 \text{ mg kg}^{-1}$ ;  $p < 0.001$ ), with significant

differences from FM soil detected after 6 hours (when GWC and SWP had decreased to 35.3 % and -2.6 MPa). No significant change point was detected indicating a consistent rate of P release as the soil dried. MRP significantly increased as SWP decreased over 24 hours (by 310 %;  $p < 0.001$ ) and 8 hours (by 103 %;  $p = 0.010$ ) though not 7 hours, suggesting that MRP increased when SWP decreased to -7.0 MPa (Table 2.6). Therefore air-drying increased MRP only when SWP was -7.0 MPa or lower, but  $\text{NaHCO}_3\text{-P}$  when SWP was -2.6 MPa or lower.

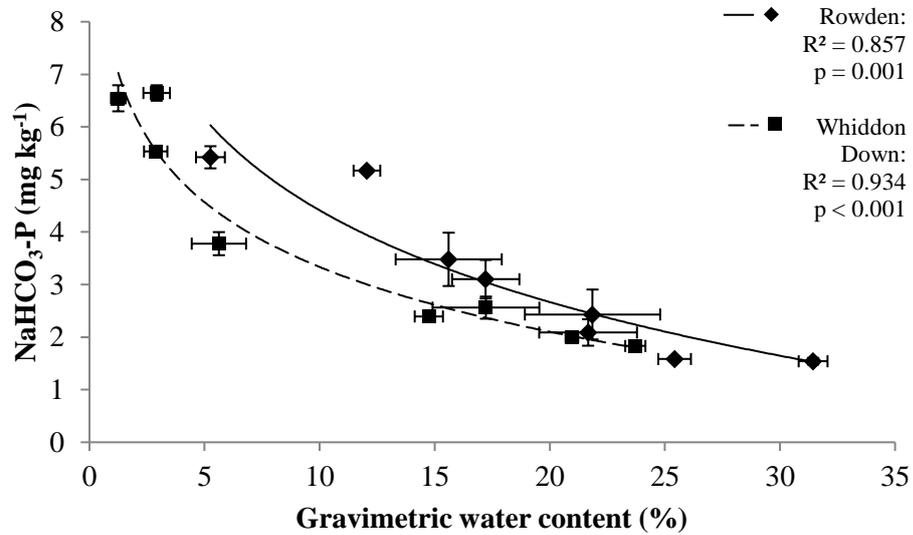
Table 2.6: Effect of drying time on GWC and SWP and  $\text{NaHCO}_3\text{-P}$  and water-extractable TP, MRP and MUP (all  $n = 3$ ) in T2. Data are means ( $\pm$  SE). Data were not available for TP and MRP for the Tadhams Moor soil. Different letters indicate significant differences ( $\leq 0.05$ ) within each water status and P concentration variable for each soil according to one-way ANOVA with Tukey's or Games-Howell pairwise tests. Asterisks indicate the highest P concentrations that yielded significant logarithmic regression *versus* SWP when included in the analysis, showing significant increases over time.

Drying time (hours)	GWC (%)		SWP (MPa)		$\text{NaHCO}_3\text{-P}$ ( $\text{mg kg}^{-1}$ )		Water-extractable TP ( $\text{mg kg}^{-1}$ )		Water-extractable MRP ( $\text{mg kg}^{-1}$ )		Water-extractable MUP ( $\text{mg kg}^{-1}$ )	
<b>Rowden</b>												
0	31.4	(0.63) <b>a</b>	-0.6	(0.02) <b>a</b>	1.54	(0.07) <b>b</b>	0.12	(0.01) <b>a</b>	0.07	(0.00) <b>a</b>	0.05	(0.01) <b>a</b>
0.25	25.4	(0.71) <b>ab</b>	-0.8	(0.05) <b>a</b>	1.58	(0.04) <b>b</b>	0.14	(0.01) <b>a</b>	0.06	(0.00) <b>a</b>	0.08	(0.01) <b>a</b>
0.5	21.7	(2.12) <b>bc</b>	-1.4	(0.31) <b>a</b>	2.09	(0.25) <b>b</b>	0.12	(0.02) <b>a</b>	0.09	(0.00) <b>a</b>	0.03	(0.01) <b>a</b>
0.75	21.9	(2.94) <b>bc</b>	-1.9	(0.73) <b>a</b>	2.43	(0.48) <b>ab</b>	0.15	(0.02) <b>a</b>	0.07	(0.00) <b>a</b>	0.09	(0.02) <b>a</b>
1	17.2	(1.46) <b>ce</b>	-2.9	(0.54) <b>a</b>	3.10	(0.37) <b>ab</b>	0.14	(0.04) <b>a</b>	0.08	(0.01) <b>a</b>	0.06	(0.04) <b>a</b>
1.5	15.6	(2.30) <b>cd</b>	-5.2	(2.28) <b>a</b>	3.48	(0.51) <b>ab</b>	0.34	(0.23) <b>a</b>	0.09	(0.01) <b>a</b>	0.24	(0.22) <b>a</b>
2	12.1	(0.58) <b>def</b>	-26.3	(11.93) <b>a</b>	5.17	(0.05) <b>a</b>	0.89	(0.53) <b>a*</b>	0.11	(0.02) <b>a*</b>	0.78	(0.52) <b>a*</b>
24	5.3	(0.63) <b>f</b>	-142.9	(6.54) <b>b</b>	5.42	(0.21) <b>a</b>	1.44	(0.50) <b>a*</b>	0.12	(0.01) <b>a*</b>	1.32	(0.50) <b>a*</b>

[Continues]

Drying time (hours)	GWC (%)	SWP (MPa)	NaHCO <sub>3</sub> -P (mg kg <sup>-1</sup> )	Water-extractable TP (mg kg <sup>-1</sup> )	Water-extractable MRP (mg kg <sup>-1</sup> )	Water-extractable MUP (mg kg <sup>-1</sup> )
<b>Whiddon Down</b>						
0	23.7 (0.44) <b>a</b>	-0.7 (0.00) <b>a</b>	1.83 (0.02) <b>ef</b>	0.18 (0.01) <b>b</b>	0.14 (0.01) <b>ab</b>	0.03 (0.02) <b>b</b>
0.25	21.0 (0.28) <b>a</b>	-1.2 (0.09) <b>ac</b>	2.00 (0.09) <b>de</b>	0.12 (0.01) <b>b</b>	0.12 (0.02) <b>abc</b>	0.00 (0.02) <b>b</b>
0.5	14.7 (0.61) <b>b</b>	-2.3 (0.17) <b>bc</b>	2.39 (0.05) <b>cd</b>	0.12 (0.01) <b>b</b>	0.08 (0.00) <b>c</b>	0.04 (0.00) <b>b</b>
0.75	17.2 (2.32) <b>abc</b>	-3.1 (0.60) <b>ac</b>	2.56 (0.21) <b>cde</b>	0.13 (0.01) <b>b</b>	0.10 (0.01) <b>bc</b>	0.03 (0.02) <b>b</b>
1	5.6 (1.18) <b>c</b>	-13.1 (3.48) <b>ac</b>	3.78 (0.22) <b>cf</b>	0.30 (0.06) <b>b</b>	0.15 (0.02) <b>ab</b>	0.15 (0.05) <b>b</b>
1.5	2.9 (0.51) <b>c</b>	-56.9 (4.90) <b>d</b>	5.53 (0.11) <b>b</b>	0.80 (0.14) <b>ab*</b>	0.12 (0.01) <b>abc</b>	0.68 (0.15) <b>ab*</b>
2	2.9 (0.58) <b>c</b>	-129.8 (5.45) <b>e</b>	6.65 (0.15) <b>a</b>	0.98 (0.26) <b>ab*</b>	0.16 (0.01) <b>ab</b>	0.82 (0.26) <b>ab*</b>
24	1.3 (0.32) <b>c</b>	-150.9 (5.00) <b>e</b>	6.54 (0.25) <b>ab</b>	1.24 (0.03) <b>a*</b>	0.17 (0.01) <b>a</b>	1.06 (0.04) <b>a*</b>
<b>Tadham Moor</b>						
0	67.5 (1.26) <b>a</b>	-0.6 (0.04) <b>a</b>	2.11 (0.04) <b>b</b>		0.31 (0.06) <b>a</b>	
1	65.4 (0.94) <b>a</b>	-0.4 (0.09) <b>a</b>	2.27 (0.06) <b>b</b>		0.29 (0.15) <b>a</b>	
2	61.9 (1.12) <b>a</b>	-0.6 (0.04) <b>a</b>	2.33 (0.12) <b>b</b>		0.16 (0.00) <b>a</b>	
3	55.3 (3.12) <b>ab</b>	-0.3 (0.05) <b>a</b>	2.97 (0.36) <b>b</b>		0.19 (0.01) <b>a</b>	
4	46.2 (3.66) <b>ab</b>	-1.2 (0.44) <b>a</b>	4.06 (0.79) <b>ab</b>		0.21 (0.02) <b>a</b>	
5	44.8 (7.57) <b>abc</b>	-2.3 (1.47) <b>a</b>	3.82 (0.73) <b>ab</b>		0.34 (0.21) <b>a</b>	
6	35.3 (1.34) <b>b</b>	-2.6 (0.48) <b>a</b>	5.57 (0.26) <b>a</b>		0.23 (0.04) <b>a</b>	
7	32.5 (2.95) <b>bc</b>	-5.9 (2.69) <b>a</b>	6.28 (0.75) <b>ab</b>		0.61 (0.20) <b>a</b>	
8	31.3 (0.99) <b>b</b>	-7.0 (2.66) <b>a</b>	6.36 (0.37) <b>a</b>		0.63 (0.06) <b>a*</b>	
24	9.8 (0.42) <b>c</b>	-212.3 (6.01) <b>b</b>	4.35 (0.52) <b>ab</b>		1.27 (0.16) <b>a*</b>	

(a)



(b)

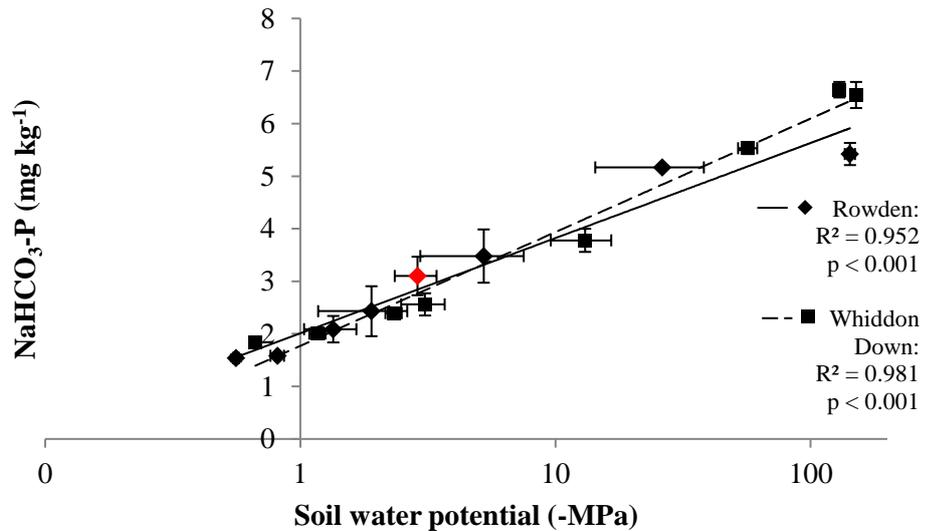


Figure 2.3: Significant logarithmic regression between (a) GWC (%) and  $\text{NaHCO}_3\text{-P}$  ( $\text{mg kg}^{-1}$ ); and (b) SWP (-MPa) and  $\text{NaHCO}_3\text{-P}$  ( $\text{mg kg}^{-1}$ ) for the Rowden and Whiddon Down soils (T2). The significant change point (-2.9 MPa) for the Rowden soil is illustrated by a red marker (b). Data are means ( $\pm$  SE) ( $n = 3$ ). The slopes for SWP did not significantly differ between the soil types ( $p = 0.332$ ).

In summary, air-drying significantly increased soil P concentrations. The magnitude of the response depended on the P form and (to a lesser extent) the soil type, as well as the degree of drying. Most notably, (plant-available)  $\text{NaHCO}_3\text{-P}$  consistently increased with decreasing GWC and SWP in both experiments (T1 and T2) and the different soils, increasing by the same magnitude with the same decrease in SWP for the Rowden and Whiddon Down soils as shown by the same regression slopes (Figure 2.3). The increase in TP (for Whiddon Down) was dominated by the MUP proportion, suggesting air-drying caused changes in the organic P fraction. Threshold water potentials at which P concentrations significantly increased above FM levels varied according to the soil type. Most notable were the similar SWPs for  $\text{NaHCO}_3\text{-P}$  at -3.0 MPa averaged across T1 and T2 for Rowden, -2.3 MPa for Whiddon Down and -2.6 MPa for Tadham Moor; lower at -7.0 MPa for MRP for Tadham Moor; whilst much lower at -56.9 MPa for TP and MUP for Whiddon Down. Therefore air-drying significantly increased P availability in three different soils, although only when SWP was -2.3 MPa or lower. The key results are summarised in Figure 2.4.

Figure 2.4: Summary of key results illustrating the percentage change in soil P concentrations following air-drying and re-wetting in relation to the hypotheses.

**Percent increases in water-extractable TP, MRP and MUP in air-dried compared to field-moist soils (Experiment 1).**

Percent change (Experiment 1)	Increase	Decrease
10000 +		
500 - 9999		
100 - 499		
1 - 99		
No significant difference from field-moist soil		
NA		

**1.**

**Hypothesis:**

Greater increases in available P following DRW occur in soils with higher MBP.

**Consistent with the hypothesis:**

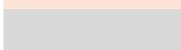
Soil DRW significantly increased TP and MUP in the Tadham Moor and Little Burrows soils, and MRP in the Tadham Moor and Joseph’s Carr soils. The greatest increases occurred in the Tadham Moor soil which had the highest MBP concentration.

**Contrary to the hypothesis:**

Soil DRW decreased MRP in the Little Burrows soil and did not affect TP and MUP in the Joseph’s Carr soil. Regressions between the increases in TP, MRP and MUP with MBP after air-drying were not significant.

Soil	Water-extractable P		
	TP	MRP	MUP
<b>Tadham Moor</b>			
<b>Little Burrows</b>			
<b>Joseph’s Carr</b>			

**Percent increases in water-extractable TP, MRP and MUP, and plant-available NaHCO<sub>3</sub>-P, in soils dried to the SWP threshold (for increased NaHCO<sub>3</sub>-P) and the minimum SWP, compared to field-moist concentrations (Experiment 2).**

Percent change (Experiment 2)		Increase
	1000 +	
	500 - 999	
	200 - 499	
	100 - 199	
	1 - 99	
	No significant difference from field-moist soil	
	NA	

**2.**

**Hypothesis:**

Increased P availability following DRW is correlated with decreased SWP, and the SWP at which P availability increases varies according to soil.

**Consistent with the hypothesis:**

Soil P availability increased as SWP decreased in three soils. The SWP at which P availability (TP, MUP) increased (threshold) varied between the soils.

**Contrary to the hypothesis:**

The SWP at which P availability (NaHCO<sub>3</sub>-P) increased (threshold) was similar between the three soils.

Soil	Degree of drying	SWP (MPa)		Water-extractable P			Plant-available P
		TP, MRP, MUP	NaHCO <sub>3</sub> -P	TP	MRP	MUP	NaHCO <sub>3</sub> -P
<b>Rowden T1</b>	Threshold		-3.0				
	Minimum		-34.4				
<b>Rowden T2</b>	Threshold	-26.3	-2.9				
	Minimum	-142.9	-142.9				
<b>Whiddon Down</b>	Threshold	-56.9	-2.3				
	Minimum	-150.9	-150.9				
<b>Tadham Moor</b>	Threshold	-7.0	-2.6				
	Minimum	-212.3	-212.3				

## Discussion

### **Soil drying and re-wetting effects on phosphorus availability: importance of the microbial biomass**

Microbial biomass P (MBP) concentrations differed significantly between the three soils reported in Experiment 1, likely reflecting their different types and land uses (Table 2.1). A similar range in MBP concentrations across an order of magnitude occurred in 29 UK permanent grassland soils (31 - 239 mg kg<sup>-1</sup>) (Turner et al., 2001). Although MBP concentrations for the Joseph's Carr and Rowden soils fell within this range, the Tatham Moor soil exceeded it, probably associated with its organic texture and low-input management (Evans et al., 2017). Joseph's Carr had twice the MBP concentration as Rowden and although both have clay textures, the soils were under woodland and grassland respectively, so microbial community structure and composition, as well as biomass P concentrations, likely differed. For example, across 32 UK soils the MBP concentration was an order of magnitude higher in soils from moorland and woodland sites compared to grassland or arable soils, demonstrating that land use as well as soil type influences the abundance of different P forms (Stutter et al., 2015). Whether these differences in MBP concentration caused variation in P responses to DRW was tested, to determine the likely magnitude of DRW response in these particular soils and to aid in soil selection for larger-scale experiments.

For the three soils, the increases in water-extractable TP, MRP and MUP following air-drying were strongly positively ( $R^2 > 0.91$ ) though not significantly ( $p = 0.060$  to  $0.191$ ) related to MBP concentration. Therefore, contrary to the hypothesis, there was no evidence that a greater increase in available P following DRW occurred in soils with higher MBP concentration. In contrast, water-soluble TP (which was mostly MUP) was significantly positively correlated with MBP concentration across 29 UK soils (Turner and Haygarth, 2001). Measuring more soils in the present study may have resulted in significant regressions between different P fractions and MBP. Alternatively, the lack of significant regression may genuinely indicate that the MBP

concentration was not the most important driver of P responses to DRW, or reflect that the effects can be highly variable in different soils. As well as the greater sample number, an important difference was that Turner and Haygarth (2001) measured water-soluble P; separating this fraction from combined soluble and particulate water-extractable P (via finer filtering) may alter the results (and relationship) provided that the microbial biomass had a stronger effect on dissolved P forms. For example, a different study showed that soil DRW increased TP and MUP in leachate in the water-soluble forms, but there were no consistent effects on the particulate forms (Blackwell et al., 2009). However, the same study found that increases in P could not be related to decreases in MBP caused by drying, and similarly the greatest increase in water-extractable TP and MUP in leachate following DRW occurred in the soil with the lower MBP concentration (Blackwell et al., 2012), suggesting that other (non-microbial) sources were important to P release.

The greatest increase in water-extractable P following DRW occurred for the Tadham Moor soil, dominated by the increase in the MUP form (by mass; Figure 2.2), suggesting P release from an organic source, likely the soil organic matter (SOM) (Butterly et al., 2009). This can be released from microaggregates by DRW (Chepkwony et al., 2001; Navarro-Garcia et al., 2012) depending on aggregate stability (Bünemann et al., 2013). The results were consistent with other studies showing greater quantities of water-extractable or –soluble MUP released after drying than MRP. Thus DRW produced MUP concentrations that were up to four times higher than MRP (Bünemann et al., 2013), attributed to release of MUP from the microbial biomass (Turner and Haygarth, 2001; Turner et al., 2003). Nevertheless, sterilising soils indicated non-microbial contributions to the P increases with DRW, with up to 44 % higher resin-P following DRW compared to a constantly moist soil, whereas non-sterilised soil showed a smaller change of 34 % (Bünemann et al., 2013). Thus microbial effects contributed more than physical effects. Similarly, increased  $\text{NaHCO}_3\text{-P}$  following DRW was not consistent with reductions in MBP and occurred regardless of whether the soil was initially sterilised, emphasising the influence of non-microbial factors (Sun et al., 2017). Therefore a combination

of microbial and soil structural sources of MUP was likely important, as well as the greater immobilisation of released MRP by microbes and fixation by soil minerals (Blackwell et al., 2009; Butterly et al., 2011b; Bünemann et al., 2013), particularly Fe/ Al oxides (Figure 1.2). Also, soil C content was positively associated with greater microbial P release following DRW (Sparling et al., 1985). Thus, P release from organic sources in the Tadhams Moor soil (with comparatively high MBP) likely caused the greater increase in MUP compared to MRP, and compared to the other soils, following DRW. Measuring MBP following re-wetting would be useful, where a decrease could suggest that lysed cells were the source of released P whereas a stable concentration could suggest either alternative sources or rapid recovery (Chapter 3).

The substantial proportional increase in MRP (> 100-fold) following DRW in the Tadhams Moor soil should not be overlooked. Tadhams Moor was the only soil for which TP, MRP and MUP increased following DRW, suggesting mineralisation of released organic P, or direct release of inorganic P. The Little Burrows and Joseph's Carr soils released only MUP or MRP respectively, perhaps suggesting lower mineralisation in the Little Burrows soil due to its significantly lower MBP concentration. However, mineralisation may not have contributed to MRP over the experimental duration (re-wetting occurred during a 30 minute extraction period), and a higher contribution of MUP due to microbial cell lysis would be expected for the Joseph's Carr soil, with higher MBP. Similar to the response of the Little Burrows soil, MRP decreased following DRW despite increases in TP and MUP in two (of five) soils in a distinct series, which were the only clay soils (Turner et al., 2002; 2003). Soil texture may have been the most important factor, because the soil was highly P-fixing and the decrease in MRP was thought to result from increased soil sorption capacity for orthophosphate following drying (Turner et al. 2002; 2003; Haynes and Swift, 1985). Similar to the response of the Joseph's Carr soil, a lack of change in water-extractable TP following DRW was attributed to microbial immobilisation of the released P, due to the reduced C:P ratio of the microbial biomass in the DRW soils compared with control soils (Butterly et al., 2011b). In contrast, increased water-soluble MRP following DRW was positively correlated with microbial biomass C concentration (Dinh et al.,

2016). High soil organic C concentration ( $> 20 \text{ g kg}^{-1}$ ) was considered necessary for DRW to increase P availability (Sparling et al., 1985). Therefore soil properties and land use (pasture or cropping) had a greater effect than water regime on P availability (Butterly et al., 2011b), likely explaining the different responses of the three presently studied soils.

### **Effects of the degree of soil drying on phosphorus availability following re-wetting**

Re-wetting stimulated greater increases in  $\text{NaHCO}_3\text{-P}$  concentrations of drier soils in all cases, and TP, MRP and MUP concentrations in two soils, consistent with previous studies (Bünemann et al., 2014; Lado-Monserrat et al., 2014; Sun et al., 2017a,b). The SWPs at which water-extractable P forms increased varied between soils, although were very similar for  $\text{NaHCO}_3\text{-P}$ . The increase in  $\text{NaHCO}_3\text{-P}$  with decreasing SWP was characterised by significant change points for two soils, suggesting that P release occurred at thresholds of SWP rather than gradually. This was similar to relationships between leachate P concentrations with soil Olsen P concentrations (Fortune et al., 2005). The change points indicated threshold values for SWP at which changes in  $\text{NaHCO}_3\text{-P}$  from the concentrations in FM soils became significant, which were  $-2.9 \text{ MPa}$  (Rowden) and  $-13.1 \text{ MPa}$  (statistically) although more likely  $-2.3 \text{ MPa}$  (Whiddon Down) but this could not be tested. Since the Rowden and Whiddon Down soils showed highly significant regressions between  $-0.6$  and  $-150.9 \text{ MPa}$  (Figure 2.3), further studies should measure P concentrations more frequently during drying, especially between  $-2.3 \text{ MPa}$  and  $-13.1 \text{ MPa}$  (Whiddon Down soil) and between  $-0.6 \text{ MPa}$  and  $-3.0 \text{ MPa}$ , to more precisely determine change points (Figure 2.3). This is particularly important since the range includes  $-1.5 \text{ MPa}$ , commonly the plant permanent wilting point (PWP). Whereas the similar changes in  $\text{NaHCO}_3\text{-P}$  with SWP between the Rowden and Whiddon Down soils (Figure 2.3b) was expected since the soils are of the same series and share similar properties (Table 2.4), the Tadham Moor soil was of a different series and was a peat soil with comparatively high total C content and MBP concentration (by an order of magnitude; Table 2.4 and Table 2.2 respectively), slower drying rate, and lacked a significant change point. The logarithmic relationship for the Tadham Moor soil suggested more gradual P release with reducing SWP,

although more measures of  $\text{NaHCO}_3\text{-P}$  concentrations below  $-2.6$  MPa may discriminate a significant change point.

In all soils, water-extractable TP, MRP and MUP significantly increased at lower and more variable SWPs than  $\text{NaHCO}_3\text{-P}$ . Consistent with the present study (Table 2.6), air-drying soil from the Rowden site (to  $-78$  MPa) and re-wetting increased  $\text{NaHCO}_3\text{-P}$  (by  $8.9 \text{ mg kg}^{-1}$ , 72 %) whilst MRP in leachate did not change (Blackwell et al., 2009). This suggested that P was released but became fixed, so would not be measured in the soil solution until all adsorption sites became occupied or an equilibrium concentration was reached (Blackwell et al., 2009), related to the P buffering capacity of different soils. Compared to water-extracts,  $\text{NaHCO}_3\text{-P}$  was higher because the extraction increases the solubility of calcium phosphates, extracting approximately half the surface-sorbed P whilst minimising secondary adsorption reactions (Olsen et al., 1954). Change points could not be distinguished for TP, MRP and MUP because increases from FM soil occurred at the lowest two or three SWPs (Table 2.6). Although soil drying for different durations caused change points (with time) for increased soluble MRP in leachate for three grassland and arable UK soils, they were not detected for changes in SWP (Forber et al., 2017). This result occurred despite soils drying within a similar SWP range ( $-0.24$  to  $-158$  MPa) as the present study, likely because the greatest decrease in SWP occurred after the first two or three sampling points (to between  $-139$  and  $-146$  MPa). Therefore this relationship remains unknown. Whilst Experiment 2 has determined change points for plant-available P, further studies within the most relevant SWP ranges identified are needed.

### **Soil drying and re-wetting effects on phosphorus availability according to the magnitude of drying: agronomic relevance**

#### **Measuring soil water potential in agricultural fields**

Determining the SWP values at which DRW significantly increased P availability was fundamental to identifying whether plants may benefit. Since P availability increased as SWP decreased, the greatest increase occurred in the driest soils ( $-34.4$  to  $-212.3$  MPa), far lower than

plant PWP. However, significant increases in  $\text{NaHCO}_3\text{-P}$  first occurred when soils dried to -2.3 to -2.9 MPa, much nearer to the PWP (Table 2.6; Figure 2.3b). Previous studies of the effects of soil DRW on P availability have primarily compared P concentrations in field-moist soils to those from soils dried to very low SWPs, without exploring agriculturally relevant ranges. For example, DRW significantly increased water-extractable TP and MUP in leachate from soil dried to -78 MPa (0.9 % GWC) whilst the field-moist controls were -0.4 MPa (24.2 % GWC) (Blackwell et al., 2009). However, air-drying (to 8 or 11% of the field-moist GWC) was considered representative of surface soils during the southern Australian summer, and therefore the field conditions of their sample sites (Butterly et al. 2011b). Similarly, Sun et al. (2017a) based the lowest GWC (5 %) in their study on field measurements of as low as *c.* 3 % at the sampling site. Based on soil moisture release curves for most agricultural soils, these low GWCs indicate water potentials below PWP. Numerous studies have shown that once established, plants can survive mild water deficits (e.g. -0.02 MPa; Carrijo et al., 2017) if imposed spatially (vertically or horizontally) or temporally (avoiding the most sensitive phenological growth stages, typically anthesis) such that sufficient roots maintain access to water (Dodd, 2009; Dodd et al., 2015; Carrijo et al., 2017). Therefore the identified threshold SWPs for increased plant-available  $\text{NaHCO}_3\text{-P}$  suggested that soil DRW could benefit plants, depending on the spatial and temporal management (Chapter 3).

Whether P release following soil DRW is beneficial depends on the effects on microbial communities, as well as plants. Soil water potential affects soil microbial processes, partially driving P release in response to DRW. Fischer (2009) questioned how dry a soil must be to stimulate respiration following re-wetting, measuring SWP as the key determinant of water availability to microbes. After drying to below -0.63 MPa, re-wetting substantially increased microbial respiration. At plant PWP (-1.5 MPa), respiration reduced to 5-8 % of the maximum (occurring at > -0.001 MPa), indicating that the reduced SWP diminished microbial function yet was not completely biocidal (Fischer, 2009). Specific mineralisation (mineralised P / total P) declines with decreasing SWP, indicating that lower water potentials limit P mineralisation

(Grierson et al., 1999). Thus changes in SWP, and not only the absolute SWP values, cause cell lysis upon DRW (Fischer, 2009). Microbial effects on P release depend on the resilience of the microbial community to low SWPs, and the soil moisture history (Evans et al., 2012), which vary according to soil type and cropping system. Therefore soil P availability increased following DRW at agriculturally relevant SWPs if they are carefully managed, and determining the effects on microbial biomass P at these SWPs is important (Chapter 3).

### **Magnitude of increase in phosphorus and plant relevance**

In addition to the SWP range, the other key question was whether soil P concentrations increased sufficiently to potentially benefit plant P nutrition and yields. Crop P requirements vary according to species and genotype, availability of other nutrients and anticipated yields, and many other biotic and abiotic factors. The target for arable, grassland and forage crops in the UK is 16-25 mg L<sup>-1</sup> NaHCO<sub>3</sub>-P (Index 2; DEFRA, 2017). In context, from the minimum SWP, DRW increased NaHCO<sub>3</sub>-P to total concentrations of 5.42 to 8.46 mg kg<sup>-1</sup>. The maximum increases in NaHCO<sub>3</sub>-P were by 7.09 mg kg<sup>-1</sup> (T1) and 3.88 mg kg<sup>-1</sup> (T2) for the Rowden soil, when soil was dried to -34.4 MPa and -142.9 MPa, respectively; 4.71 mg kg<sup>-1</sup> when SWP was -150.9 MPa for the Whiddon Down soil; and 4.24 mg kg<sup>-1</sup> when SWP was -212.3 MPa for the Tadham Moor soil. These suggested substantial increases in absolute concentrations of available P as well as large proportional increases (up to 518 %). Nevertheless, for all soils, even when the soil was air-dried (to < -34.4 MPa) as in conventional soil P tests and classification into indices, the NaHCO<sub>3</sub>-P concentrations remained within the lowest range, for Index 0 soils (0-9 mg kg<sup>-1</sup>). These values show the maximum potential increases in P availability which, given the very low SWPs, could be achieved provided DRW is imposed prior to planting. Lesser degrees of drying (-2.3 to -3.0 MPa) could potentially be carefully controlled spatially and/ or temporally to support plant growth (Chapter 3). These more moderate reductions in SWP significantly increased NaHCO<sub>3</sub>-P by 1.65 and 1.56 mg kg<sup>-1</sup> (to 3.02 and 3.10 mg kg<sup>-1</sup>), 0.56 mg kg<sup>-1</sup> (to 2.39 mg kg<sup>-1</sup>) and 3.46 mg kg<sup>-1</sup> (to 5.57 mg kg<sup>-1</sup>) in the Rowden (T1 and T2), Whiddon Down and Tadham Moor soils, respectively (Tables 2.5 and 2.6). Whilst these

increases were more conservative than occurring after greater degrees of drying, benefits to plant P uptake and growth in P-limited conditions may be detected by plants (explored in Chapters 3 and 4).

Overall, air-drying significantly increased  $\text{NaHCO}_3\text{-P}$  by similar absolute values across all soils. The increase for Rowden at T1 seemed unusually high compared to T2 and the other soils. Compared to the effects of DRW (to  $-78$  MPa) on P availability previously reported for the Rowden soil (Hallsworth series; Blackwell et al., 2009), at T1  $\text{NaHCO}_3\text{-P}$  increased by a greater magnitude (518 % rather than 72 %) although by a similar absolute concentration (7.08 rather than  $8.90 \text{ mg kg}^{-1}$ ). A later study similarly showed that following DRW (to  $-117$  MPa)  $\text{NaHCO}_3\text{-P}$  increased by 70 % ( $7.79 \text{ mg kg}^{-1}$ ) (Blackwell, 2012). All these studies (including Experiments 1 and 2) took soil from the Rowden site but from plots under different fertiliser management regimes, and sampled in different months (and seasons) and from different depths, explaining different  $\text{NaHCO}_3\text{-P}$  concentrations following DRW. The greater magnitude of P increase following DRW at T1 compared to T2 was most likely because of the shorter storage time after soil was collected from the field. Soil microbial phosphorus concentrations decreased after storage at  $4^\circ\text{C}$  for more than two weeks (Turner and Romero, 2010). Therefore the soil microbial biomass likely declined by T2, diminishing P release from DRW due to a lesser contribution from microbial cells, although this should be confirmed by measuring MBP at both time points. As well as its relevance to P fertiliser recommendations (DEFRA, 2017), the greater sensitivity of  $\text{NaHCO}_3\text{-P}$  to changes in SWP, by extracting P from exchange surfaces (where released P becomes sorbed, e.g. Ca-P, FeO-P) as well as the soil solution (Moody et al., 2013), suggested its usefulness at detecting P release following DRW.

Changes in water-extractable P concentrations gave insight into changes in P forms following DRW. Increases in water-extractable TP in the soil solution result from inorganic P (MRP) released from soil surfaces, and organic P (MUP) released from organic matter and the microbial biomass (Figure 1.1). Thus an increase in TP enhances P availability, provided the MRP fraction increases, although some plants can also utilise organic P and sorbed inorganic P

by excreting organic acids (Stutter et al., 2012; Nash et al., 2014). Despite large proportional increases, water-extractable P remained low in an agronomic context, except for an increase in MUP in the Tadham Moor soil by 10.12 mg kg<sup>-1</sup> (Experiment 1), which could potentially enhance plant P nutrition. Further studies should determine the SWP (change point or otherwise) at which MUP increased, and the impacts of that SWP threshold on soil-, microbe- and plant- mediated mechanisms regulating the plant-availability of organic P.

## Conclusions

Greater increases in available P following DRW tended to occur in soils with higher MBP concentrations, although other soil factors likely contributed to the different responses between soils and warrant further study. Further research should combine more soil types (as Turner and Haygarth, 2001), and multiple techniques such as direct bacterial counts (Turner et al., 2001) and sterilisation experiments (Bünemann et al., 2013; Sun et al., 2017). Moreover, soils should be sampled at different times of year to understand how P responses to DRW change seasonally, along with other regulatory soil properties. Studying soils from contrasting soil moisture regime histories would enrich this understanding (Evans et al., 2012).

Consistent with the hypothesis, increased P availability following DRW was correlated with decreased SWP, indicating greater P release in drier soils. Plant-available NaHCO<sub>3</sub>-P increased with decreasing SWP with significant change points occurring at -2.9 MPa and -13.1 MPa (although likely -2.3 MPa if it could be tested) in the Rowden and Whiddon Down soils respectively. The SWPs at which NaHCO<sub>3</sub>-P significantly increased above FM levels were similar across soil types (-2.3 to -3.0 MPa), whilst the water-extractable TP, MRP and MUP forms increased at lower SWPs (-7.0 to -56.9 MPa). The SWP thresholds for increased P availability indicated that careful soil moisture management at larger scales could increase soil P availability to plants. Drying soils to lower SWPs (-34.4 to -212.3 MPa) would maximise P release, but would need to occur prior to planting to prevent seedling mortality, such as during a fallow period. Although the maximum NaHCO<sub>3</sub>-P release (3.88–7.08 mg kg<sup>-1</sup>) from a single

DRW event did not increase the soil classification above Index 0 (DEFRA, 2017), it is uncertain whether multiple DRW events have greater effects. Moreover, the effects of vertical soil moisture gradients on soil P availability requires further study within the context of crop P nutrition.

## **Chapter 3: Effects of variation in soil water potential on phosphorus availability in soil, uptake by plants, and impacts on yields.**

### **Abstract**

**Context:** The spatial and temporal dynamics of phosphorus (P) release following soil DRW need to be determined, to potentially benefit crop plants via strategic irrigation management.

**Hypotheses:** It was hypothesised that drying and re-wetting (DRW) would increase soil P availability, with more intense soil drying and multiple DRW cycles having the greatest effect.

**Strategic approach:** A first experiment dried and re-saturated or re-flooded soils to determine the spatial and temporal effects on soil P concentrations. A second experiment initially air-dried soil to -38 MPa, then measured the effects on growth and yields in *Brachypodium distachyon* under different levels of P supply.

**Conclusions:** More intense drying at the soil surface did not maximise P availability following DRW, likely due to vertical P leaching occurring within the soil profile. Nevertheless, initial DRW doubled grain yields in *Brachypodium*, implying an important agronomic benefit.

### **Introduction**

In laboratory experiments, soil drying and re-wetting increased P availability at soil water potentials that can occur in agricultural fields (Chapter 2). Determining the magnitude and duration of increases in P availability following DRW at a larger scale is essential to understand potential benefits to plants.

#### **Increased phosphorus release from drier soils: effects of initial air-drying**

In Chapter 2, P availability increased with soil drying with maximum increases of 4 to 7 mg kg<sup>-1</sup> of plant-available NaHCO<sub>3</sub>-P occurring at the minimum SWPs of -34 to -212 MPa. If similarly intense drying occurred between consecutive crops, re-wetting the soil profile prior to planting

may benefit plant P nutrition. To interpret maximum potential increases in P availability following DRW, pot experiments have studied the effects of fully air-drying soil prior to planting on P availability and uptake. When a Cambisol from permanent grassland was air-dried from 40 % to 4 % GWC, resin-P increased from 0.5 to 4.2 mg kg<sup>-1</sup> (740 %) and remained at least 50 % higher than continuously moist soil for 22 days (Bünemann et al. 2013). Maize plants grown in unfertilised soil exposed to this initial DRW cycle had significantly higher biomass than plants grown in fertilised (5 or 10 mg P kg<sup>-1</sup>) continuously moist soil (by approximately 70 % and 20 % respectively for each fertiliser rate), corresponding to higher shoot P concentrations (by approximately 90 % and 30 % respectively). Thus, intense soil drying prior to planting increased available P, enhancing plant P uptake and yields above fertilised levels. Similarly, using six highly weathered Ferralsols from irrigated rice fields in Madagascar, Tsujimoto et al. (2010) compared the effects of a transition from initial air-drying (GWC or SWP was not reported) to continuous soil flooding on P uptake by rice. In unfertilised soil that was initially air-dried, P uptake increased (by 250 %) and was positively correlated with soil P availability, suggesting initial air-drying caused both to increase. This effect was greater in soils with higher available P contents due to NPK fertiliser application, likely because of combined nutritional effects (Tsujimoto et al., 2010). Therefore air-drying soil before planting enhanced P nutrition in aerobically- and anaerobically-grown crops, likely at least in part because soil drying was sufficiently intense to release substantial quantities of plant-available P. However, neither experiment determined the effects of subsequent, less intense DRW cycles on P availability and uptake.

#### **Multiple cycles of soil drying and re-wetting: effects on phosphorus availability**

An initial flush of nutrient availability following DRW (the “Birch effect”) was associated with increased microbial respiration, indicating enhanced microbial activity stimulated by greater organic matter availability and mineralisation (Figure 1.2). However, whether the pulse of nutrient availability is sustained over time is uncertain. In two grassland soils, microbial respiration doubled following drying (to -5 MPa) and re-wetting compared to continuously

moist controls, and was highest initially (at one and three days after DRW) compared to later (up to 50 days) (Gordon et al., 2008). The flush of microbial respiration can be limited by substrate availability over multiple cycles of DRW (Yu et al., 2014; Shi and Marschner, 2017). This suggests that the increase in soil P availability following DRW, caused by P release from the microbial biomass and subsequent mineralisation, can also diminish with multiple cycles of DRW.

Whilst the initial flush of available P following DRW has been widely documented, whether the same response occurs over more than one DRW cycle remains uncertain. Repeatedly drying a forest loam soil to  $-100$  MPa whilst control soils remained continuously moist (at 50 % WHC) increased soluble MRP but the number of DRW cycles (one, two or three) had no effect on P release (Dinh et al., 2016). In contrast, soluble MUP increased after the first, but not the second or third, DRW cycle. It was likely that the microbial biomass did not recover following the first DRW cycle, thereby decreasing the magnitude of P release following subsequent cycles; or that the microbial biomass adapted such that cell lysis was reduced following subsequent DRW cycles (Dinh et al., 2016). Within a sandy Luvisol, soluble MUP similarly increased after one but not two DRW cycles, although the greatest increase occurred after three DRW cycles (Butterly et al., 2009). In this soil, resin-P released by DRW was higher after the second and third than the first DRW cycle, whereas MBP decreased after one DRW cycle, but not after a second or third, suggesting non-microbial sources of released P. When a loam soil was incubated over 90 days, the MBP increased by *c.* 41 %, 9 % and 21 % over three consecutive drying cycles (to  $< 5$  % GWC; Chen et al., 2016). Soil DRW increased  $\text{NaHOC}_3\text{-P}$  by 10-18 % compared to soil maintained continuously moist (at 50 % WHC) and the greatest increase occurred after two DRW cycles, similarly to the response of resin-P reported by Butterly et al. (2009). Therefore MBP and  $\text{NaHOC}_3\text{-P}$  were affected differently after one, two and three DRW cycles, with the greatest increase in  $\text{NaHOC}_3\text{-P}$  corresponding with the greatest decrease in MBP suggesting that P was released from the microbial biomass (Chen et al., 2016). However, the duration of drying was similar (one day) for all cycles but whether equivalent SWPs were

reached was not specified. These studies provided contradictory evidence of whether multiple cycles of DRW reduced or enhanced P release compared to the effects of the first cycle. Whether different forms of P respond similarly to multiple cycles of DRW requires further investigation.

Soil P transformations are distinct under the flooded, anaerobic conditions unique to rice production (Figure 1.3), although less studied than the “Birch effect” in aerobic systems. Nevertheless, 12 soils with contrasting properties were exposed to 11 redox cycles (with 20 days of saturation followed by one day of oxidation and one day of further drying) over 220 days to determine the effects on P availability (Scalenghe et al., 2012). The maximum increase in MRP occurred after the fourth redox cycle (80 days). Compared to initial values, MRP concentrations increased by an order of magnitude additionally supplying 10 to 24 mg P kg<sup>-1</sup>, although changes were not detected in NaHCO<sub>3</sub>-P concentrations (samples were dried before analysis). Exceptionally, in soil collected from a paddy rice field, MRP increased 30-fold suggesting that the previous exposure to periodic redox cycles amplified the effects on P availability. This result contradicts the idea that the soil microbial community adapts to redox cycles, decreasing P release from microbial cell lysis compared to the other soils, suggesting P was released from non-microbial sources (Figure 1.3). Alternatively, perhaps the rapid oxidation and drying in laboratory experiments poorly represented field scenarios and thus rapid desiccation (rather than re-wetting) had a biocidal effect on these microbial communities, due to osmotic shock and cell lysis. Importantly, soil solution MRP concentrations were greater under pulsed rather than continuously reducing conditions, implying that fluctuating water contents caused P release. Whilst field drainage systems are important to avoid reducing conditions and P solubilisation to reduce P losses and pollution at the catchment scale (Scalenghe et al., 2012), redox cycles within alternate wetting and drying (AWD) irrigation may alter P availability to plants. Nevertheless, better understanding of the effects of multiple AWD cycles on soil P dynamics is needed.

**Differential soil drying with sampling depth**

Although more severe soil drying maximised increases in soil P availability following DRW, significant increases occurred at SWPs of -2.3 to -3.0 MPa, contributing 0.56 – 3.46 mg kg<sup>-1</sup> of NaHCO<sub>3</sub>-P (Tables 2.5 and 2.6). Whilst SWPs as low as -100 MPa may occur in forest floor soils during dry summer periods (Dinh et al., 2012), crop plants cannot survive such extreme water deficits within the entire root zone during their development. However, unless soils are maintained flooded or saturated, vertical soil moisture gradients exist with drier soils at the surface due to surface evaporation (Figure 1.4) and higher root length density in the upper layers that takes up water to support plant transpiration requirements (Dodd et al., 2011). Partial drying thus occurs naturally in rainfed and some irrigated systems, whilst is also managed via controlled vertical or horizontal water deficits. Although the spatial distribution of P in the soil profile under different irrigation regimes (furrow, surface drip and subsurface irrigation) has been quantified, P availability was not specifically related to corresponding SWPs (Yang et al., 2011), or determined at SWPs below -0.063 MPa (Wang and Zhang, 2010). Furthermore, in a pot experiment investigating DRW effects on P availability and plant growth, Bünemann et al. (2013) reported a soil moisture gradient of 4 to 17 % GWC from the surface 2 cm to the lower portion of soil in 480 ml pots which likely affected soil P availability and uptake. Thus quantifying P release needs to consider vertical changes in SWP by differentiating between surface and bulk soils in pot studies.

**Relevance of initial soil phosphorus concentrations to the magnitude of phosphorus release**

Initial soil phosphorus concentrations can affect the impact of DRW events on P release, although reports are inconsistent. For example, the magnitude of increased P extractability (Bray-P) following DRW was greater in soils supplemented with inorganic P (monocalcium phosphate) compared to unfertilised soils, most likely due to higher adsorption of applied P during soil drying such that newly mineralised P was less readily adsorbed (Chepkwony et al.,

2001). Similarly, the relative increases in soluble TP increased as soil  $\text{NaHCO}_3\text{-P}$  concentration increased, which was attributed to the corresponding increase in the degree of P sorption site saturation (Styles and Coxon, 2006). Furthermore, DRW caused greater P mineralisation in fertilised than unfertilised soils (Grierson et al., 1998). Changes in response to environmental variables or treatments (including soil DRW) have been expressed as the ratio of net mineralised P to total soil P concentration, termed specific mineralisation (Grierson et al., 1998; 1999). Therefore to determine whether increased soil P availability relative to initial concentrations is sufficient to increase plant P uptake and yields, especially under P-limiting conditions, absolute as well as proportional changes (which can exaggerate the relevance of the increase) should be reported. Similarly, to determine whether plant P uptake increases in response to newly released soil P where initial P availability and plant biomass differ, comparing both plant P concentrations and P contents (concentrations x biomass) is necessary.

#### **Temporal dynamics of re-wetting on soil phosphorus availability to plants**

Multiple DRW cycles have variable effects on soil P availability, with the time between re-wetting soil and sampling for P analysis critical. Whilst the “Birch effect” can initially stimulate P release from the microbial biomass, a lag can exist with no additional P mineralisation until sufficient microbial recovery mineralises soil organic matter (Grierson et al., 1998), and physical responses to DRW occur (Chepkwony et al., 2001; Blackwell et al., 2009). For example, dissolved MRP, MUP and resin-P concentrations peaked within two hours of re-wetting air-dried soil, but disappeared after 49 hours (Butterly et al., 2011a). Similarly, the maximum increase in soluble MRP concentrations in leachate occurred in air-dried soils that were re-wet over 1.5 hours, rather than sooner or up to 24 hours later (Blackwell et al., 2012), and DRW increased P availability when soil was sampled 1 hour after re-wetting (Bünemann et al., 2013). Sampling within this time is important to determine the optimal P release after DRW and potential to exploit these “windows of opportunity” for increased soil P availability to plants (Chepkwony et al., 2001).

### **Objectives and hypotheses**

To determine how soil moisture dynamics affect P release spatially and temporally, two experiments aimed to test five hypotheses:

- 1. Soil P availability will be higher in initially air-dried and re-wet soils compared to continuously moist soils**, because a lower SWP will be reached than for control soils or with subsequent DRW cycles.
- 2. Soil P availability will be higher in soils exposed to drying and re-wetting or re-flooding compared to soils maintained under continuously saturated or flooded conditions**, because soil P availability increases as SWP decreases, partially due to P release from the microbial biomass.
- 3. Multiple DRW cycles will cause a greater increase in soil P availability than a single cycle**, without plant uptake. The microbial biomass will recover after soil DRW, so increased P availability caused by a pulse of mineralisation will be additive over repeated DRW cycles.
- 4. Soil P availability will be higher in the surface compared to the bulk soil within the drying treatments**, because the surface soil will be exposed to lower SWPs upon drying.
- 5. Increases in soil P availability at the whole-pot level will be sufficient to increase plant P uptake, biomass and yields** in drying compared to continuously moist treatments.

## Materials and methods

### Experiment 1

#### Site, sampling and preparation of soil

Soil was sampled from 0-20 cm depth from three sampling pits within a grazed pasture at the Whiddon Down site at Rothamsted Research, North Wyke (50° 44' 8.67"N, -3° 50' 56.29"W), in January 2017 (ungrazed at the time of sampling). It is a slowly permeable clay of the Hallsworth series in the Soil Survey of England and Wales system (Clayden & Hollis, 1984) (Table 2.1). Soil physical and chemical properties are outlined in Table 3.5. The soil was passed through a 10 mm sieve to remove large stones and pieces of organic matter, and stored at field moisture content at approximately 10 °C before establishing the experiment. Soil taken from the three pits was thoroughly mixed to create one composite sample. Soil was pre-incubated at 25 °C in a controlled environment room for 24 hours prior to establishing the different treatments, to stimulate the microbial biomass following storage. Each bag of soil was closed with an elastic band and a breathable plug made from tissue, to allow air transfer but minimise moisture loss.

#### Experimental design

A 2 (P level, P) x 2 (initial water treatment, W) x 4 (irrigation regime, I) factorial design with sixteen different soil treatments was established, with four replications. The P levels were low-P (no P added) and high-P (110 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>); the initial water treatment was soil maintained field-moist (FM) or air-dried for 48 hours and re-wet (DRW); and the irrigation treatments were continuously saturated (CS), dried from saturation and re-saturated (DRW), continuously flooded to a water level of 1 cm above the soil surface (CF), and dried from flooding and re-flooded (AWD). In addition, soil from a field trial described in Chapter 4 was included, which was collected from a rice paddy field in central Madagascar (19°10'46.5"S, 47°29'49.4"E) and is a clay soil of the Gleysol group, characterised by saturation for extended periods resulting in

reducing conditions (IUSS Working Group WRB, 2015; Table 3.5). Figure 3.1 clarifies the difference between soil drying as part of the initial water treatment, W (initial DRW) and as part of the irrigation regimes, I (subsequent surface DRW).

### **Establishing treatments**

To establish the high P treatment,  $\text{KH}_2\text{PO}_4$  was applied at a concentration of  $4.0 \text{ g L}^{-1}$  and volume of 50 mL, to supply 200.3 mg per pot (11 cm diameter x 12 cm height; 1 L volume). This rate of  $48.0 \text{ kg P ha}^{-1}$  (based on the pot surface area) was equivalent to the RB209 recommendation for a cereal crop (of  $110 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ ; DEFRA, 2018b). The low P treatment comprised soil at the P content sampled in the field, to which the equivalent amount of potassium (K) as for the high P treatment was added as potassium chloride (KCl), at a concentration of  $2.2 \text{ g KCl L}^{-1}$  and volume of 50 mL per pot. Chloride dissolves in the soil solution and does not associate with soil minerals including P (Schulte, 1999). Nutrient solutions were applied by spreading eight pots of soil (6400 g) thinly over a plastic sheet and applying 400 mL nutrient solution evenly over the surface using a spray bottle and regularly mixing thoroughly. Soil was immediately placed in a labelled plastic bag. This process was repeated three times for each nutrient solution, with KCl applied first using a spray bottle which was then thoroughly cleaned with deionised water. Based on the adjusted water content, the new weight of field-moist (FM) soil added to each pot was approximately 800 g.

To impose the initially air-dried soil treatment (DRW), half the soil from each bag was spread thinly (to ensure even drying) across plastic sheets in four separate batches, keeping the two nutrient treatments separate and dried at  $25 \text{ }^\circ\text{C}$  for 48 hours. Sub-samples were taken and the SWP was determined as: LP  $-61.7 \text{ MPa}$  (SE = 2.71; n = 2) and HP  $-53.0$  (SE = 4.60, n = 2); the difference was not significant ( $p = 0.27$ ). The same batch was weighed before and after drying to calculate water loss, and this volume was replaced for each batch as deionised water applied slowly across the surface using a spray bottle and mixed regularly (as described by Jarrell et al., 1999). Meanwhile the FM soil was maintained in plastic bags each closed with an elastic band

and a breathable plug made from tissue, as during pre-incubation (Figure 3.1 a). Sub-samples were taken from each bag to measure initial moisture and P contents and stored in sealed plastic bags at 4 °C. At this stage, the four treatments had slightly different GWCs ( $p = 0.032$ ) but differences were not significant between the treatments (Table 3.1). The Madagascar soil was maintained at low-P and the soil was initially DRW ( $n = 3$ ).

Table 3.1: Initial gravimetric water contents (GWC) of soils after different P and water treatments (before irrigation regimes) were established. FM is maintained field-moist and DRW is air-dried and re-wet. Data are means ( $\pm$  SE);  $n = 2$ . The treatment differences were significant overall according to Welch's one-way ANOVA ( $p = 0.032$ ) although not distinguished according to a Games-Howell post-hoc test.

<b>Initial P</b>	<b>Initial water</b>	<b>GWC (%)</b>
Low	FM	<b>28.6</b> (0.8)
Low	DRW	<b>29.9</b> (0.0)
High	FM	<b>25.9</b> (0.4)
High	DRW	<b>26.9</b> (0.9)
Low: Madagascar	DRW: Madagascar	<b>26.1</b> (0.7)

To establish the irrigation treatments whilst setting up the pots, 800 g of FM soil (which included initial water for FM or replacement deionised water to the DRW treatment, plus added nutrient solution) was weighed into each labelled and pre-weighed pot, uniformly to homogenise bulk densities. For saturated treatments, soil was slowly brought to saturation with deionised water applied to the surface and the new weight recorded. This was repeated for the remaining saturated treatments, adding the same volume of water to ensure equivalent pot weight. For flooded treatments, the process was repeated but once saturated, the water level was brought to 1 cm above the surface. The flooded pot was re-weighed and other flooded treatments brought to the same weight. After recording initial weights, pots were placed in an incubator

(Sanyo MIR-553) at 35 °C in randomised blocks (separating the four replications across four shelves) and their positions and the time recorded (Figure 3.1 c). Pots were incubated in darkness to prevent algal growth and higher temperatures nearer to the light sources. Evaporation was only from the soil/ water surface (Figure 3.1 b).

### **Monitoring**

After approximately 24 hours, all pots were weighed and the flooded control treatments (CF) were re-watered with deionised water to reach water levels of 1 cm above the soil surface. Since many of the saturated soils maintained surface water (and thus were oversaturated), the ponded surface water was allowed to evaporate, thereby establishing new starting weights. Deionised water was then replaced in the saturated control treatments (CS) according to individual requirements. Thereafter, all pots were weighed and evaporated water replaced in the controls every two days (or three days, twice). Pots were replaced in the incubator in the same blocks but their positions changed randomly within each block, and the shelf allocated to each block changed every two days to avoid pseudoreplication.

### **Sampling**

Sufficient soil was sampled from the surface 0-2 cm for analysis, and from the remaining bulk soil (2 cm to approximately 8 cm depth). The degree of soil drying was determined by the SWP corresponding to target weights, based on the upper 2 cm reaching below -1.5 MPa, because increases in P availability are greater as SWP decreases (SWP < -2.3 MPa significantly increased available P of this soil; Table 2.6). Although soil was dried to a target SWP, this could only be directly measured by removing soil. Therefore whole pot GWC (from regular weighing) was correlated with SWP values. After a flooded soil was allowed to dry, the upper 2 cm reached -4.35 MPa while the bulk soil remained at -1.34 MPa. This pot had a water: soil ratio of 0.22 and whole pot GWC of 17.8 %. Thus whole pot GWC could inform soil sampling, calculated from the weights of remaining water and soil. All pots within a treatment were sampled at the same time, with pots reaching the target GWC before other replicates covered

lightly with lids to reduce further desiccation until all replicates could be sampled (as in Venterink et al., 2002; Dinh et al., 2016). Sampling after the first drying and re-wetting cycle (S1) occurred after 29 (CS, DRW) and 31 and 33 (CF, AWD) days of incubation. Sampling after the second cycle (S2) occurred after 49 (CS, DRW) and 57 and 59 (CF, AWD) days.

Sampling occurred according to the schedule outlined in Table 3.2. Samples were taken at the end of drying to determine GWC and SWP. Other samples were taken 1.5 hours after re-saturating or re-flooding the soil, to measure the reasonably immediate impact of re-wetting. Previous increases in available P following DRW were greatest initially after re-wetting and then declined with time, with maximum soluble MRP in leachate reached when soil re-wetting occurred over 1.5 hours (Blackwell et al., 2012) and peak dissolved MRP, MUP and resin-P concentrations occurring within two hours of re-wetting (Butterly et al., 2011a).

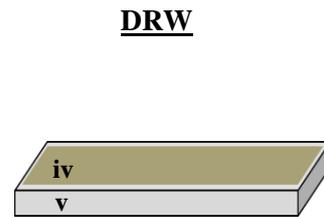
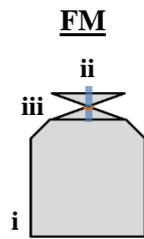
Table 3.2: Sampling schedule and analyses at the two sampling depths. Cells marked with “X” indicate the sampling time, or depth, associated with each analysis. The surface soil was 0-2 cm depth and the bulk soil was 2-8 cm depth.

<b>Analysis</b>	<b>End of first/ second drying</b>	<b>1.5 hours after first/ second re-wetting</b>	<b>Surface</b>	<b>Bulk</b>
<b>Whole pot GWC</b>	X	X	X	X
<b>Soil sample GWC</b>	X	X	X	X
<b>SWP</b>	X		X	X
<b>NaHCO<sub>3</sub>-P</b>		X	X	X
<b>Water-extractable MRP and TP</b>		X	X	X
<b>Microbial biomass P</b>		X		X
<b>Resin-P</b>				X

To sample saturated soil, an acid-washed syringe (50 mL) with the end removed was used (Figure 3.1 c, i). This was more effective in maintaining intact cores than a soil corer. Since sampling flooded cores disrupted the remaining soil, the surface water was first removed using

a syringe to allow core removal, and was then returned to the pot. The vacant spaces left by soil cores were each plugged with an acid-washed 12 cm section of 32 mm diameter PVC-U pipe (Figure 3.1 c, ii, iii), which surrounded the coring syringe (Figure 1 c, iv). Samples were sealed in plastic bags and stored at 4 °C until analysis (Figure 3.1 c, v). After the first sampling, new target weights for the second drying cycle were determined by subtracting the weights of cores removed and the pipes included from the original starting weights.

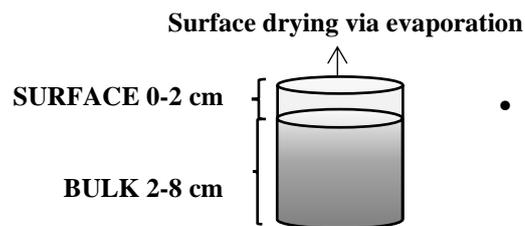
**(a) Initial water treatment**



- Soil maintained in bags (i).
- Bags closed with breathable plug (ii) to air exchange, held in place with rubber (iii).
- Soil spread thinly (< 1 cm deep) (iv) on plastic sheets (v).
- Maintained in stable environment for 48 hours, with frequent mixing to facilitate uniform drying.
- Re-wet by spraying evenly with deionised water.

**(b) Subsequent surface DRW**

**SOIL PROFILE**



- More intense drying at the exposed surface than the bulk soil.

**(c) Sampling procedures**



Figure 3.1: Summary of soil drying and re-wetting procedures as part of the initial water treatment (a) and the irrigation treatment (b), and the sampling procedure (c). FM is field-moist and DRW is dried and re-wet.

## Experiment 2

### Site, sampling and preparation of soil

Soil was sampled from 0-20 cm depth from three sampling pits within a 1-hectare field plot of permanent grassland on the Rowden experimental platform at Rothamsted Research, North Wyke in Devon, UK (50° 46' 47.12"N, -3° 54' 59.23"W), in October 2014. The soil is a clayey pelostagnogley of the Hallsworth series in the Soil Survey of England and Wales system (Clayden & Hollis, 1984), classified as a Dystric Gleysol in the FAO (2006) system (García-Marco et al., 2014). It is very similar to the Whiddon Down soil, though taken from low-input ungrazed grassland (Table 2.1; physical and chemical properties outlined in Table 3.10). The Rowden soil was selected for its moderate microbial biomass P content and increased P availability in response to DRW (Chapter 2), and also previous research which showed that DRW increased P availability and losses in leachate (Blackwell et al., 2009; 2012). After sampling, the soil was maintained in the field-moist condition during storage at 10–16 °C for 10 days and approximately 4 °C thereafter. It was passed through a 10 mm sieve to remove large stones and pieces of organic matter, and soil taken from the three pits was thoroughly mixed to create one composite sample. Soil was pre-incubated at 25 °C for 24 hours as described for Experiment 1.

A preliminary experiment mixed this soil with sand in different ratios (volumetrically) to determine the effects on plant growth (*Brachypodium distachyon* L.). Since maximal shoot dry weight and near-maximal grain number occurred when soil and sand was mixed in a 3:1 ratio (Table 3.3), this substrate (hereafter termed soil) was adopted for the main experiment to optimise plant growth.

Table 3.3: Shoot dry weights and grain number. Data are means ( $\pm$  SE) ( $n = 3$ ). Shoot dry weights and grain number were not significantly different according to one-way ANOVA with Tukey's pairwise test, and Welch's ANOVA with Games-Howell pairwise test respectively. Bold text indicates the selected composition.

<b>Soil: sand composition</b>	<b>Shoot dry weight (g)</b>	<b>Grain number</b>
100:0	0.022 (0.001)	5.3 (0.3)
<b>75:25</b>	<b>0.031</b> (0.004)	<b>7.7</b> (1.2)
50:50	0.027 (0.004)	8.0 (1.5)

### Crop species selection

The grass species *Brachypodium distachyon* L. (hereafter Brachypodium) was selected for its comparatively short cycle and small stature. It is a model grass for aerobic crop research in controlled environment studies due to these traits, in contrast to grasses such as rice which pose the challenges of large size (limiting high replication), long generation times and more demanding cultivation requirements including soil flooding (Brkljacic et al., 2011). Brachypodium has a similar genome to rice and is considered a typical grass (International Brachypodium Initiative, 2010). Therefore the selected species provides a model grass for the majority of plant traits including development, stress tolerance and yield (Brkljacic et al., 2011). Seeds of the standard sequenced line Bd21 were acquired from the John Innes Centre, UK.

### Experimental design

A 2 (P level, P) x 2 (initial water treatment, W) x 2 (irrigation frequency, I) factorial design with eight different treatments was established, with ten replications minus any failed plants. The P levels were: low-P (no P added) and high-P ( $16 \text{ mg P kg}^{-1}$ ); the initial water treatment was soil

maintained field-moist (FM) or air-dried for 48 hours and re-wet (DRW); and the irrigation frequencies were high-I (irrigated daily) and low-I (irrigated every three days).

### **Establishment of treatments**

Firstly, the initial water treatment was established by spreading the soil thinly and evenly on trays at 25 °C and air-drying for 48 hours. The GWC and SWP of the air-dried (AD) soil were determined (described below). At this stage the FM and AD soils had GWC of 20.3 % and 1.5 %, respectively, and the FM soil was maintained moist (*c.* 0 MPa) whilst the AD soil had dried to -37.6 MPa (SE = 0.56; n = 3). The AD soil was then re-wet to 40 % water holding capacity (WHC) by adding the precise volume of deionised water required to the surface (Jarrell et al., 1999), creating DRW soil. Meanwhile the remaining FM soil was maintained in plastic bags closed with a breathable plug and maintained at 25 °C. The FM soil was also brought to 40 % WHC. Air drying did not change the WHC (FM: mean = 69.8 %, SE = 1.0, n = 3; AD: mean = 69.4 %, SE = 1.7, n = 3) so the soils were brought to the overall mean WHC (69.6 %, SE = 0.9, n = 6). Thus the starting GWC was 27.8 % for both soil treatments. Sub-samples were taken from each bag to determine initial moisture and P contents and stored in sealed plastic bags at 4 °C. Soils were transferred to pots (12 cm top diameter and 8.5 cm bottom diameter x 11 cm height), uniformly to ensuring equal amounts and bulk densities, and the total weights of each pot and tray, identity label and the soil were recorded.

Brachypodium seeds were pre-germinated on moist filter paper at 4 °C for five days, then maintained in the dark at 25 °C for two days. Germinated seeds were selected for uniform root length and transplanted (with one seed per pot). An additional 10 mL of deionised water was then supplied to each pot to facilitate soil-root contact. After observing dry soil on the pot surface two days later, they were maintained at a higher % WHC. Using non-experimental unplanted pots, 30 mL was identified as the maximum volume of deionised water that could be easily applied to each pot without drainage, for both FM and DRW treatments, corresponding to new start weights at approximately 50 % WHC. Plants were grown in a controlled

environment room under LED lights (Valoya B100 (102 W) and B150 (144W), NS1spectrum, Valoya Oy, Helsinki) providing a 20 hour light/ 4 hour darkness photoperiod at 25 °C during light/ 18 °C during darkness (guided by Hong et al., 2011). The pot position on the bench was rotated daily in randomised blocks to avoid pseudoreplication.

The initial P concentrations comprised the low-P treatment. The high-P level was initiated eight days after transplanting (DAT), to ensure similar plant development between treatments at that time. High-P treatment plants were supplied with approximately 16 mg kg<sup>-1</sup> P, as 30 mL of a 2 mM solution of KH<sub>2</sub>PO<sub>4</sub>. This brought the soil from Index 0 to Index 2 for P nutrition for grasslands and arable crops according to RB209 and assuming a soil bulk density of 1 (since the recommendations are given according to volume rather than mass) (Defra, 2017). Unlike Experiment 1, the pot diameter varied with depth, so the applied P concentrations were not determined by the pot surface area. The low-P plants received the same amount of K as KCl (supplied as 30 mL of a 2 mM solution). However, NaHCO<sub>3</sub>-P analysed at 31 DAT indicated that the high-P treatment soils were at the lower end of the range for Index 1, suggesting depletion due to P uptake and/ or adsorption of some of the applied P. Therefore the same doses of KH<sub>2</sub>PO<sub>4</sub> and KCl were supplied again to the high- and low-P plants respectively at 40 days DAT (Figure 3.2).

Different irrigation frequencies were established at 54 DAT. Throughout the experiment, irrigation volumes were calculated for each pot to replace water loss via evapotranspiration (water use, WU) individually, or based on the mean of the same four plants representing both initial water treatments. To establish different irrigation frequencies, half of the plants continued to be watered daily with deionised water (high irrigation frequency) whilst half of the plants were allowed to partially dry and were irrigated every three days (low irrigation frequency), until drying before the final harvest.

**Monitoring**

Phenological development of each plant was monitored according to the BBCH scale for Brachypodium (Hong et al., 2011). This involved documenting principal and secondary growth stages, eight times during the first 38 DAT (leaf and tiller development) and twice thereafter (flowering and grain ripening). At 24 DAT, chlorosis was observed in *c.* 75 % of the plants in the initially FM soil treatment. Thus full Hoagland nutrient solution, without P, was supplied to all plants at this stage (supplying 30 mL per pot of solution containing 12 mM NO<sub>3</sub>, 1 mM NH<sub>4</sub>, 6 mM K, 3.5 mM Ca, 1 mM Mg and 1 mM SO<sub>4</sub>). It was first confirmed that soil electrical conductivity (EC, measured using a WET sensor, Delta-T Devices, UK) was within an acceptable range (0.52 – 1.20 dS m<sup>-1</sup>; n = 8) to avoid osmotic stress across all treatments (i.e. < 6.0 dS m<sup>-1</sup> tolerated by wheat; Maas and Hoffman, 1977).

**Sampling**

Soil was sampled from the surface 0-1 cm depth (to minimise disruption to roots compared to sampling to 2 cm depth, Experiment 1), once during crop growth and at the final harvest, occurring at 31 and 89 DAT. Bulk soil (1-8 cm depth) was also sampled at the final harvest, avoiding roots which were then carefully separated from adhering soil particles by gently washing with water over a sieve (1 mm). The sampling schedule related to phenological development is summarised in Figure 3.2, and the analyses conducted are summarised in Table 3.4.

Total water use (WU) was the total cumulative irrigation supplied, which was equivalent to evapotranspiration. Water use efficiency (WUE = total biomass / WU) and water productivity (grain yield / WU) were calculated after harvest based on water supplied (evapotranspiration).

Principal Growth Stage	0	1	2	3	4	5	6	7	8	9
	Germination	Leaf development	Tillering	Stem elongation	Booting	Inflorescence emergence, heading	Anthesis	Fruit development	Ripening	Senescence
High-I										
Low-I										
Schedule	<b>0 DAT:</b> Transplant (after initial DRW).	<b>8 DAT:</b> Initiate P level treatment.	<b>24 DAT:</b> Chlorosis observed, Hoegland nutrient solution supplied.	<b>31 DAT:</b> Soil sampling to check appropriate P supply.	<b>40 DAT:</b> Nutrient solutions supplied: KH <sub>2</sub> PO <sub>4</sub> (high-P) and KCl (low-P).		<b>54 DAT:</b> Initiate irrigation frequency treatment.			<b>89 DAT:</b> Harvest; Soil and plant sampling.

Figure 3.2: Irrigation treatments and schedule of treatments and sampling during the experiment, based on the phenological growth stages and Biologische Bundesantalt, Bundessortenamt and Chemische Industrie (BBCH)-identification keys of *Brachypodium distachyon* (Hong et al., 2011). High-I and Low-I refer to the high and low irrigation frequency treatments, respectively. DAT is days after transplanting.

*Note: Irrigated and drying periods are represented by shaded and open cells, respectively. Low-I treatment within a growth period is represented by occurrence of a shaded and open cell. Column width does not represent relative time of growth stage.*

Table 3.4: Sampling schedule and analyses for Experiment 2. Cells marked with “X” indicate the sampling time, or depth of soil where relevant, associated with each analysis. DAT is days after transplanting. The surface soil was 0-1 cm depth and the bulk soil was 1-8 cm depth.

Analysis	31 DAT		54 DAT		89 DAT	
	Surface	Bulk	Surface	Bulk	Surface	Bulk
NaHCO <sub>3</sub> -P	X		X		X	X
Water-extractable MRP and TP					X	X
Plant tissue TP					X	
Plant biomass					X	

## Experiments 1 and 2

### Soil and plant analyses

#### Soil moisture characteristics

Soil moisture contents (%) were determined gravimetrically, based on sample weights before and after oven-drying at 105 °C to constant weight. Soil water potential was determined using a WP4-T Dewpoint Potentiometer (Decagon Devices Inc., USA).

#### Soil phosphorus concentrations

For Experiment 1, separate analyses were conducted on soil samples taken after re-wetting from each depth, apart from MBP (Table 3.2), at the GWCs at which the soils were sampled. For Experiment 2, samples were air-dried at 35 °C for 24 hours to constant weight prior to analysis. Although air-drying increased NaHCO<sub>3</sub>-P for this soil (Figure 2.3), this was required to standardise the GWC between the drier samples taken from the surface and the wetter bulk soil samples. Otherwise, analysing soil at the GWC at which they were sampled would show higher NaHCO<sub>3</sub>-P at the surface because the soil sample was drier. This contrasted with Experiment

1, when soils were re-wet prior to sampling ensuring comparable water contents. For both experiments, all samples were passed through a 2 mm sieve and analyses were carried out in triplicate following standard procedures as described. All P concentrations are expressed on a dry weight equivalent (DWE) basis.

### **Microbial biomass phosphorus (MBP): Experiment 1**

Microbial biomass P (MBP) was measured via hexanol fumigation and extraction with anion exchange resin membranes, as described in Chapter 2. The mean P recovery for the Madagascar soil was 60.3 % (SE = 1.13); thus the P recovery factor was 0.603.

### **Resin-phosphorus: Experiment 1**

Resin-P was simultaneously determined with MBP as the P concentrations of unfumigated samples ( $\text{mg kg}^{-1}$ ), and calculated as:

$$(C_u * 8.00) / SW$$

Where:

$C_u$  is the concentration of P in the unfumigated sample ( $\text{mg kg}^{-1}$ );

8.00 is the volume of HCl in millilitres;

SW is the dry weight equivalent of the soil sample in grams.

### **Water-extractable total phosphorus (TP), molybdate reactive phosphorus (MRP) and molybdate unreactive phosphorus (MUP): Experiments 1 and 2**

Water-extractable TP and MRP were measured as described in Chapter 2, although MRP was measured with malachite green reagent because it is more sensitive in detecting orthophosphate in low concentrations (Irving and McLaughlin, 1990), using a plate reader at 625 nm absorbance. TP was measured at the Rothamsted Research North Wyke analytical laboratory.

**Sodium bicarbonate extractable phosphorus (NaHCO<sub>3</sub>-P): Experiments 1 and 2**

The NaHCO<sub>3</sub>-P concentrations were analysed as described in Chapter 2. For Experiment 1, samples were measured with malachite green reagent using a plate reader at 625 nm absorbance. For Experiment 2, orthophosphate was measured with molybdate blue reagent according to Murphy and Riley (1962) using an autoanalyser (AA3, Seal Analytical, UK) for samples taken at 31 and 54 DAT, and the plate reader at 880 nm absorbance for samples taken at the start of the experiment and at the final harvest (89 DAT). Malachite green is considered to be the more sensitive reagent in detecting low concentrations of orthophosphate.

**Shoot total phosphorus (TP) concentration: Experiment 2**

Plant tissue total P concentration was analysed with mature and immature (green) components combined per plant (since *Brachypodium* tends to produce new tillers after reaching maturity under favourable conditions; P. Nicholson, JIC, pers. comm. 04/08/2015), excluding roots. Grain and straw were oven-dried at 65 °C until constant weight, then ground using a ball mill (MM 400, Retsch, Germany) and analysed via acid digestion and ICP-OES (Thermo Scientific iCAP 6300 analyser) by research staff at the Lancaster Environment Centre. Briefly, approximately 0.1 g dry weight per plant was weighed into tubes and digested in 5 mL of 100 % HNO<sub>3</sub> in a microwave. Following appropriate dilution, samples were analysed via ICP-OES, then final concentrations were calculated based on the standard curves and dry weights.

**Plant biomass and yields: Experiment 2**

At maturity, plants were harvested and the total biomass was separated into roots, shoot (stems, leaves and husks = straw) and grains, and dried in an oven at 65 °C until constant weight to determine dry biomass and grain yield. Mature and immature components were combined per plant. The number of grains per plant was counted. The root-to-shoot ratio was calculated, and harvest index (HI) calculated as the ratio of grain weight to total biomass. Total dry biomass was used to calculate P concentrations and contents.

**Statistical analysis**

Data were analysed using SPSS version 23 (IBM, 2014) and R Studio. In all cases, a confidence threshold of 0.05 was applied.

**Experiment 1**

For samples taken at the start of the experiment, two-way ANOVA determined whether P concentrations differed according to P level and initial water treatment (with bootstrap analysis for NaHCO<sub>3</sub>-P and resin-P; n = 1000). For the samples taken at the first and second sampling times, the CS and CF controls were managed such that water contents did not change; therefore the GWCs were measured at re-wetting whilst SWP was assumed to be 0 MPa. To compare treatment effects on water and P responses, analyses were carried out separately on each response for each sampling time and depth; i.e. four separate ANOVA models were fitted for each response. Because of the overall difference in GWC before irrigation ( $p = 0.032$ ; Table 3.1), the initial GWC (mean for each P and W treatment) was included as a covariate in the analysis of variance models. Soil GWC and SWP were analysed across treatments separately to determine differences between the sample depths and sample times, using a paired samples t-test and a Wilcoxon matched-pair signed-rank test respectively. To determine differences between GWC and SWP between the irrigation treatments, 3-way ANCOVA with bootstrap analysis was performed (n = 1000) with Tukey's pairwise comparison. The same approach determined effects of P level, initial water treatment and irrigation regime and their interactions on all P forms at each sampling time and depth. To determine differences between P concentrations according to the irrigation regime at each P level, ANOVA with bootstrap analysis was performed (n = 1000) with Tukey's pairwise comparison. To determine differences between P concentrations in the surface and bulk soils, according to irrigation regime and P level but averaged across the initial water treatment and sample time, paired sample t-tests were carried out (n = 6-16). To determine differences between P concentrations at the two sampling times S1 and S2 according to irrigation regime, P level and initial water treatment but averaged

across the sample depths, paired sample t-tests were carried out ( $n = 8$  except for MBP and resin-P,  $n = 4$ ; and the Madagascar soil,  $n = 6$ ). TP and MUP were excluded due to low and uneven sample sizes due to limited filtrate volumes. Data from the Madagascar soil were analysed separately from the data from main experiment and described within the relevant results sections.

## **Experiment 2**

To determine differences in P concentrations at the start of the experiment (following initial DRW but before P application), Kruskal-Wallis independent samples tests were carried out. To determine treatment differences, 3-way ANOVA with Tukey's pairwise test was carried out for: soil P concentrations; plant biomass and yields; plant tissue TP concentrations; total P content of plant biomass (P concentration x biomass); P use efficiency (PUE: grain yield / total P content); cumulative water use (evapotranspiration), water use efficiency (WUE: biomass / water use) and water productivity (WP: grain yield / water use). Data were first transformed (log base 10) for:  $\text{NaHCO}_3\text{-P}$  (bulk soil), TP (surface and bulk) and MRP (surface); otherwise bootstrap analysis was performed ( $n = 1000$ ). To determine whether  $\text{NaHCO}_3\text{-P}$  and plant biomass differed between the three treatments, one-way ANOVA (with the treatment groups coded) with Tukey's pairwise test was carried out.

## **Results**

### **Experiment 1**

The major soil properties for the Whiddon Down and Madagascar soils are outlined in Table 3.5.

Table 3.5: Major soil physical and chemical properties for the two soils used in Experiment 1. Analyses were carried out by NRM Laboratories, UK and Rothamsted Research, UK.

Soil property	Result		Units	Method
	Whiddon Down	Madagascar		
Texture class	Clay	Clay		Particle size distribution via laser diffraction
Sand	8	27	% w/w	
Silt	41	33	% w/w	
Clay	51	40	% w/w	
Organic Matter	7.6	9.4	% w/w	Loss on ignition (LOI)
pH	4.9	4.8		In water (1:2.5)
Total C	2.97	2.10	% w/w	Combustion catalytic oxidation
Total N	0.32	0.16	% w/w	
C:N Ratio	9.3:1	13.1:1		
Total P	640	285	mg kg <sup>-1</sup>	Aqua-regia soluble elements : HCl and HNO <sub>3</sub> digestion with analysis via ICP-OES
Total K	1450	175	mg kg <sup>-1</sup>	
Total Mg	1570	392	mg kg <sup>-1</sup>	
Total Mn	239	65	mg kg <sup>-1</sup>	
Total Fe	37283	33667	mg kg <sup>-1</sup>	
Available Fe	181	115	mg L <sup>-1</sup>	DTPA extraction, or dithionite/ ammonium oxalate extraction (Tadham Moor)

### Summary of treatment effects on soil phosphorus availability

The P level, initial water treatment and irrigation regime, along with their interactions, had different effects on the different P forms (Table 3.6). Particularly notable were: the significant differences in P availability between the control (CS, CF) and drying (DRW, AWD) irrigation treatments according to the P level and initial water treatment; and differences in treatment effects between sampling times and depths.

Table 3.6: Main effects of P level (P), initial water treatment (W) and irrigation regime (I) and all 2- and 3-way interactions on P concentrations (results from ANCOVA with bootstrap analysis,  $n = 1000$ , and initial GWC as a covariate). S1 and S2 refer to sampling times 1 and 2, whilst S and B refer to surface (upper 2 cm) and bulk soil (2-8 cm depth) respectively. Treatment differences are: not significant (ns),  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*) and  $p \leq 0.001$  (\*\*\*). Data are for all treatments and replications ( $n = 64$ ).

Model	Significance of effect of model term on P concentration																			
	TP				MRP				MUP				NaHCO <sub>3</sub> -P				Resin-P		MBP	
	S1		S2		S1		S2		S1		S2		S1		S2		S1	S2	S1	S2
	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	B	B	B	B
<b>P</b>	*	*	ns	ns	**	***	***	**	ns	*	ns	ns	***	***	***	***	***	***	ns	ns
<b>W</b>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
<b>I</b>	***	***	*	***	***	***	***	***	***	***	*	***	ns	ns	ns	ns	ns	*	***	***
<b>P * W</b>	*	*	ns	ns	**	***	**	*	ns	*	ns	ns	***	***	***	***	***	***	*	ns
<b>P * I</b>	***	***	**	***	***	***	***	***	***	***	*	***	***	***	***	***	***	***	***	***
<b>W * I</b>	***	***	**	***	***	***	***	***	***	***	*	***	ns	ns	ns	ns	ns	*	***	***
<b>P * W * I</b>	***	***	ns	*	***	***	***	***	*	**	ns	*	***	***	***	***	***	***	ns	ns

**Effects of initial air-drying and re-wetting on phosphorus availability****Pre-irrigation phosphorus concentrations**

Adding dissolved  $\text{KH}_2\text{PO}_4$  at the start of the experiment to FM soil in the high-P treatment significantly increased all P forms by 42 to 1214 % (except for MUP which decreased) (Table 3.7). The water-extractable TP, MRP and MUP concentrations were low for both initial water treatments. Initial air-drying (to -57.4 MPa averaged across the P treatments) and re-wetting significantly reduced MRP by 0.02 and 0.06  $\text{mg kg}^{-1}$  (22 % and 23 %), and MBP by 15.43 and 30.87  $\text{mg kg}^{-1}$  (34 % and 48 %) at low-P and high-P respectively. In contrast, initial DRW increased pre-irrigation  $\text{NaHCO}_3\text{-P}$  and resin-P concentrations, although the magnitude depended on the P level (P\*W; Table 3.7). The greater proportional increases occurred at low-P for  $\text{NaHCO}_3\text{-P}$  (by 0.96  $\text{mg kg}^{-1}$ , 35 %) whereas high-P for resin-P (by 1.85  $\text{mg kg}^{-1}$ , 11 %). Thus, initial DRW affected the P forms differently, significantly increasing  $\text{NaHCO}_3\text{-P}$  and resin-P.

Table 3.7: Phosphorus concentrations at the start of the experiment by P level (P) and initial water treatment (W). For each P form, p-values indicate significant differences between P, W and P\*W according to two-way ANOVA. Data are means ( $\pm$  SE) and n = 3 except: HP, FM (MRP, n = 2) and HP, DRW (NaHCO<sub>3</sub>-P and MRP, n = 2; Resin-P and MBP, n = 1).

P form	P level	Mean ( $\pm$ SE) (mg kg <sup>-1</sup> )		p-values		
		Field-moist	Air-dried and re-wet	P	W	P*W
TP	Low	0.25 (0.03)	0.25 (0.01)	<b>0.018</b>	0.105	0.085
	High	0.36 (0.01)	0.27 (0.02)			
MRP	Low	0.09 (0.02)	0.07 (0.01)	<b>&lt;0.001</b>	<b>0.031</b>	0.170
	High	0.26 (0.02)	0.20 (0.01)			
MUP	Low	0.16 (0.03)	0.18 (0.02)	<b>0.021</b>	0.975	0.469
	High	0.10 (0.02)	0.08 (0.02)			
NaHCO <sub>3</sub> -P	Low	2.77 (0.05)	3.73 (0.05)	<b>&lt;0.001</b>	0.471	<b>&lt;0.001</b>
	High	16.33 (0.23)	19.59 (0.40)			
Resin-P	Low	1.29 (0.07)	1.34 (0.03)	<b>&lt;0.001</b>	0.405	<b>&lt;0.001</b>
	High	16.95 (0.44)	18.80 (NA)			
MBP	Low	45.32 (0.88)	29.89 (1.90)	<b>0.004</b>	<b>&lt;0.001</b>	<b>0.019</b>
	High	64.31 (2.73)	33.44 (NA)			

### Post-irrigation phosphorus concentrations

The initial water treatment had limited effects on P concentrations in isolation from the P level and irrigation treatments (Table 3.6). Averaged across the P levels and irrigation regimes, MBP was still significantly lower in initially DRW compared to FM soils, at S1 but not by S2 (Table 3.6) because the decrease was more pronounced at S1 (by 5.84 mg kg<sup>-1</sup>, 31 %). This reflected the greater decline in MBP between sample times in the FM soil (by 32 %) than the DRW soil (by 20 %), as well as the lower concentrations following initial DRW at both sampling times.

The effects of the initial water treatment otherwise depended on interactions with the P level and irrigation regime, and varied according to sample time and depth (Table 3.6). The increases in NaHCO<sub>3</sub>-P and resin-P caused by initial DRW remained significant at S1 and S2 (Tables 3.6 and 3.7), although decreased overall from the initial concentrations. Initial DRW increased NaHCO<sub>3</sub>-P by just 8 % (by 0.5 mg kg<sup>-1</sup> to 7.5 mg kg<sup>-1</sup>) at low-P and 2 % (by 0.3 mg kg<sup>-1</sup> to 14.2 mg kg<sup>-1</sup>) at high-P, and resin-P by 7 % (by 0.3 mg kg<sup>-1</sup> to 5.0 mg kg<sup>-1</sup>) at high-P only (averaged across irrigation regimes and sampling times and depths). Therefore the higher NaHCO<sub>3</sub>-P and resin-P concentrations in initially DRW compared to FM soil were most pronounced when measured at pre-irrigation treatment. In contrast to pre-irrigation effects, water-extractable P concentrations were all affected and the greater increases caused by DRW occurred at low-P for TP and MRP (by 16 % and 60 % respectively, both 0.2 mg kg<sup>-1</sup>), whereas high-P for MUP (by 9 %, 0.1 mg kg<sup>-1</sup>) (averaged across the irrigation regimes and sampling times and depths). Thus the increases in all P forms (except MBP) caused by initial DRW depended on the P level.

The interaction between the initial water treatment and irrigation regime was significant for all P forms at both sampling times, except for NaHCO<sub>3</sub>-P (S1 and S2) and resin-P (S1) (Table 3.6). Whereas under CS irrigation TP and MUP were increased by initial DRW (by 28 %, 0.8 mg kg<sup>-1</sup> and 23 %, 0.5 mg kg<sup>-1</sup> respectively), under CF they were decreased (by 16 % and 21 % respectively, both 0.5 mg kg<sup>-1</sup>), compared to concentrations in initially FM soil (averaged across P levels and sample times and depths). Therefore increased P availability caused by initial DRW

depended on the irrigation regime, with different effects according to the P form. Overall, the most prominent effects of initial DRW were increased  $\text{NaHCO}_3\text{-P}$  and decreased MBP, at both P levels, before the irrigation regimes were established.

### **Effects of irrigation regime on soil moisture and phosphorus availability**

#### **Gravimetric water content and soil water potential**

Whilst the control soils remained continuously saturated (CS) or flooded (CF) throughout the experiment, the GWC and SWP of dried and re-wet (DRW) and re-flooded (AWD) soils decreased gradually until sampling. In all cases, GWC and SWP were affected by the irrigation treatment according to the P level and initial water treatment (Table 3.8). Between the control treatments, mean pre- re-wetting GWC across sampling times and depths was 4.2 % higher for the CF compared to the CS treatment (Table 3.8), suggesting that the soil was not entirely saturated under CS. The mean pre- re-wetting GWC and SWP did not differ significantly between the DRW and AWD treatments, except for the lower SWP by -3.3 MPa (54 %) for the AWD treatment at sampling time S2 in the surface soil (Table 3.8). Thus apart from one exception, the soil was exposed to the same drying degree in both drying treatments across sample times and depths. Averaged across the P and initial water treatments for DRW and AWD, GWC and SWP were significantly lower ( $p < 0.001$ ) for the surface 2 cm than the bulk soil (by 4.4 % and -3.4 MPa respectively), and did not differ between the two sample times. Thus both drying treatments (DRW, AWD) imposed similar soil water status, which was lower at the soil surface. Overall, the bulk soil remained above the SWP threshold (for increased  $\text{NaHCO}_3\text{-P}$ ) for this soil (-2.3 MPa; Table 2.6) whereas the surface soil was wetter (Table 3.8).

Table 3.8: Pre- re-wetting gravimetric water content (GWC) and soil water potential (SWP) according to P level (P), initial water treatment (W) and irrigation regime (I), and between two sampling times and depths. Irrigation regimes are: continuously saturated, CS; dried and re-wet from and to saturation, DRW; continuously flooded to 1 cm surface water, CF; or dried and re-wet from and to flooding to 1 cm surface water. Data are means ( $\pm$  SE); n = 4. Within all columns, GWC and SWP differed significantly according to I and the P\*I and W\*I interactions according to 3-way ANCOVA ( $p < 0.001$ ) with the initial GWC as a covariate. Different letters indicate significant differences between the I treatments within each column, averaged across P and W (only DRW and AWD for SWP;  $p \leq 0.05$ ).

I	P	W	GWC (%)				SWP (MPa)			
			Sampling 1		Sampling 2		Sampling 1		Sampling 2	
			Surface	Bulk	Surface	Bulk	Surface	Bulk	Surface	Bulk
CS	L	FM	38.4 (0.30)	37.1 (0.55)	35.2 (0.45)	35.8 (0.47)	0.0	0.0	0.0	0.0
		DRW	37.7 (0.94)	34.7 (0.78)	33.0 (1.07)	35.1 (0.48)	0.0	0.0	0.0	0.0
	H	FM	40.7 (0.41)	38.2 (0.26)	35.9 (0.70)	37.2 (0.17)	0.0	0.0	0.0	0.0
		DRW	39.2 (0.67)	36.4 (0.65)	34.8 (0.48)	36.3 (0.56)	0.0	0.0	0.0	0.0
	Overall		<b>39.0 b</b> <b>(0.40)</b>	<b>36.6 b</b> <b>(0.42)</b>	<b>34.7 b</b> <b>(0.43)</b>	<b>36.1 b</b> <b>(0.28)</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>

[Continues]

I	P	W	GWC (%)				SWP (MPa)			
			Sampling 1		Sampling 2		Sampling 1		Sampling 2	
			Surface	Bulk	Surface	Bulk	Surface	Bulk	Surface	Bulk
DRW	L	FM	11.5 (2.65)	15.1 (1.44)	12.9 (1.42)	17.3 (0.88)	-3.5 (0.74)	-1.8 (0.28)	-2.8 (0.73)	-1.3 (0.26)
		DRW	7.7 (0.44)	11.0 (0.58)	9.8 (1.42)	13.9 (1.49)	-6.6 (0.90)	-2.9 (0.34)	-4.7 (0.97)	-1.9 (0.38)
	H	FM	13.6 (0.69)	16.3 (0.60)	8.3 (1.03)	13.9 (1.27)	-2.2 (0.26)	-1.4 (0.15)	-6.1 (1.38)	-2.4 (0.44)
		DRW	8.5 (0.61)	12.3 (0.46)	7.7 (1.07)	13.3 (1.02)	-5.8 (0.96)	-2.4 (0.23)	-4.3 (1.67)	-2.0 (0.35)
	Overall		<b>10.3 c</b> <b>(0.88)</b>	<b>13.7 c</b> <b>(0.67)</b>	<b>9.7 c</b> <b>(0.76)</b>	<b>14.6 c</b> <b>(0.67)</b>	<b>-4.5 a</b> <b>(0.57)</b>	<b>-2.1 a</b> <b>(0.18)</b>	<b>-4.5 a</b> <b>(0.63)</b>	<b>-1.9 a</b> <b>(0.19)</b>

[Continues]

I	P	W	GWC (%)				SWP (MPa)			
			Sampling 1		Sampling 2		Sampling 1		Sampling 2	
			Surface	Bulk	Surface	Bulk	Surface	Bulk	Surface	Bulk
CF	L	FM	42.3 (0.60)	39.8 (0.85)	42.2 (0.49)	39.5 (0.86)	0.0	0.0	0.0	0.0
		DRW	41.2 (0.39)	38.5 (0.60)	40.5 (0.35)	39.6 (0.33)	0.0	0.0	0.0	0.0
	H	FM	41.6 (0.84)	39.4 (0.50)	43.1 (0.27)	41.8 (0.19)	0.0	0.0	0.0	0.0
		DRW	41.2 (0.75)	37.8 (0.59)	42.7 (0.58)	41.4 (0.46)	0.0	0.0	0.0	0.0
	Overall		<b>41.6 a</b> <b>(0.32)</b>	<b>38.9 a</b> <b>(0.36)</b>	<b>42.1 a</b> <b>(0.33)</b>	<b>40.6 a</b> <b>(0.36)</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>

[Continues]

I	P	W	GWC (%)				SWP (MPa)			
			Sampling 1		Sampling 2		Sampling 1		Sampling 2	
			Surface	Bulk	Surface	Bulk	Surface	Bulk	Surface	Bulk
AWD	L	FM	9.5 (1.01)	12.8 (0.87)	8.4 (1.05)	12.7 (1.08)	-5.3 (1.59)	-2.3 (0.18)	-7.0 (1.89)	-2.4 (0.30)
		DRW	7.3 (0.96)	11.1 (1.12)	7.3 (0.73)	14.0 (0.58)	-7.7 (1.74)	-3.3 (0.71)	-7.3 (1.32)	-1.7 (0.16)
	H	FM	13.4 (0.67)	16.3 (0.33)	8.3 (2.33)	13.2 (2.32)	-2.2 (0.18)	-1.3 (0.09)	-8.9 (2.47)	-2.5 (0.83)
		DRW	8.8 (0.53)	13.7 (0.45)	6.4 (0.57)	12.4 (0.61)	-5.2 (0.88)	-1.8 (0.16)	-8.0 (1.27)	-2.1 (0.16)
	Overall		<b>9.7 c</b> <b>(0.69)</b>	<b>13.5 c</b> <b>(0.60)</b>	<b>7.6 c</b> <b>(0.64)</b>	<b>13.1 c</b> <b>(0.62)</b>	<b>-5.1 a</b> <b>(0.76)</b>	<b>-2.2 a</b> <b>(0.25)</b>	<b>-7.8 b</b> <b>(0.83)</b>	<b>-2.2 a</b> <b>(0.22)</b>

**Effects of irrigation regime according to phosphorus level and initial water treatment**

Compared to the pre-irrigation treatment P concentrations (Table 3.7), the irrigation regimes had different effects on the different P forms according to the P level and initial water treatment (Table 3.6). Soil P concentrations changed from pre-irrigation regime values under all irrigation regimes, with notable reductions in  $\text{NaHCO}_3\text{-P}$ , resin-P and MBP (Tables 3.7 and 3.9). Irrigation regime effects on P concentrations were significantly affected by the initial water treatment, except for  $\text{NaHCO}_3\text{-P}$  and resin-P at S1 (Table 3.6). The most consistent treatment response was the significant interaction between the P level and irrigation regime across all P forms and sampling times and depths (Table 3.6). Averaged across the initial water treatment and the sampling times and depths, at the low-P level  $\text{NaHCO}_3\text{-P}$  was decreased by DRW compared to CS (by  $1.06 \text{ mg kg}^{-1}$ , 14 %) and by AWD compared to CF (by  $2.13 \text{ mg kg}^{-1}$ , 25 %), whereas in the high-P treatment differences between the control and drying irrigation treatments were not significant (Table 3.9). In contrast, resin-P significantly increased under DRW compared to CS at both P levels (by  $0.49 \text{ mg kg}^{-1}$ , 26 % at low-P and  $2.48 \text{ mg kg}^{-1}$ , 62 % at high-P), although concentrations did not differ between CF and AWD at either P level (Table 3.9). Between the drying treatments,  $\text{NaHCO}_3\text{-P}$  and resin-P were both significantly higher under DRW compared to AWD (by 11 % and 25 % respectively) under high P conditions only (Table 3.9). Furthermore, the drying treatments reduced TP compared to the controls at both P levels (by 52-91 %). Whereas MRP and MUP were significantly lower under DRW compared to CS at both low-P/ high-P (MRP by 77 / 86 %; MUP by 78 / 93 %), these P forms were lower under AWD compared to CF at high-P only (MRP by 74 %; MUP by 85 %). The decrease in MBP from pre-irrigation concentrations was greatest under CS and CF, suggesting the drying regimes were more favourable to the soil microbial biomass. Therefore P concentrations were generally higher under the control (CS and CF) compared to the drying (DRW and AWD) irrigation regimes, except resin-P (highest under DRW) and MBP (highest under DRW and AWD).

Table 3.9: Phosphorus concentrations by P level and irrigation regime across the different sampling times and depths and across the initial water treatment. The irrigation regimes are continuously saturated (CS), dried (from saturated) and re-wet (to saturation) (DRW), continuously flooded (CF) and dried (from flooded) and re-wet (to flooded with the water level 1 cm above the soil surface) (AWD). Different letters represent significant differences between the irrigation treatments within each row according to two-way ANOVA with bootstrap analysis ( $n = 1000$ ;  $p \leq 0.05$ ). Data are means ( $\pm$  SE) (TP, MUP:  $n = 103$ ; MRP,  $\text{NaHCO}_3\text{-P}$ :  $n = 128$ ; resin-P, MBP:  $n = 64$ ).

P form	P level	Irrigation regime			
		CS	DRW	CF	AWD
TP	Low	2.00 (0.32) ab	0.41 (0.05) c	2.05 (0.26) a	0.98 (0.16) bc
	High	4.43 (0.65) a	0.41 (0.03) b	4.03 (0.42) a	0.73 (0.09) b
MRP	Low	0.48 (0.09) a	0.11 (0.01) b	0.49 (0.05) a	0.26 (0.03) ab
	High	1.40 (0.20) a	0.20 (0.02) b	1.40 (0.14) a	0.37 (0.03) b
MUP	Low	1.37 (0.23) a	0.30 (0.05) b	1.50 (0.22) a	0.72 (0.15) ab
	High	2.94 (0.44) a	0.20 (0.01) b	2.86 (0.29) a	0.44 (0.06) b
$\text{NaHCO}_3\text{-P}$	Low	7.45 (0.29) b	6.39 (0.09) c	8.62 (0.25) a	6.46 (0.12) bc
	High	14.16 (0.36) ab	15.00 (0.24) a	13.76 (0.32) ab	13.30 (0.16) b
Resin-P	Low	1.91 (0.050) b	2.40 (0.07) a	1.80 (0.05) b	2.16 (0.07) ab
	High	4.02 (0.19) b	6.50 (0.17) a	3.87 (0.16) b	4.91 (0.13) b
MBP	Low	7.25 (0.72) b	20.67 (0.83) a	4.06 (0.55) b	18.12 (0.71) a
	High	9.04 (0.97) b	24.59 (0.63) a	5.52 (0.67) b	22.12 (0.54) a

### Multiple drying cycles: phosphorus availability at different sampling times

The different treatments significantly affected P concentrations across the two sampling times, S1 and S2, which each occurred 1.5 hours after a drying and re-wetting or re-flooding cycle (Table 3.6). Where  $\text{NaHCO}_3\text{-P}$  significantly differed, concentrations were higher at S1 than S2 in all cases. There was no consistent response according to the P level, initial water treatment or irrigation regime, including between the control and drying treatments. The opposite effect was observed for MRP, which had higher concentrations at S2 compared to S1 in all cases in which the differences were significant. Similarly to  $\text{NaHCO}_3\text{-P}$ , there was no consistent effect of the P level, initial water treatment and irrigation regime. Resin-P was significantly higher at S2 than S1 in one case, with no other significant differences between sampling times. Similarly to  $\text{NaHCO}_3\text{-P}$ , MBP was significantly higher at S1 compared to S2 in all cases in which the difference was significant, suggesting the microbial biomass declined during the experiment. There were no consistent treatment effects, except that the higher MBP concentration at S1 was significant at all P levels and initial water treatment combinations under CF (by 3.37 - 8.06  $\text{mg kg}^{-1}$ ), suggesting that the adverse effect of CF irrigation on MBP over time dominated the treatment effects. Amongst the treatment combinations with significantly different P concentrations at S1 and S2, four were common for the  $\text{NaHCO}_3\text{-P}$ , MRP and MBP forms, but without any unifying treatment levels.

Mean  $\text{NaHCO}_3\text{-P}$  concentration in the Madagascar soil, which was maintained at low-P under initial DRW and AWD, was also significantly higher at S1 (1.21  $\text{mg kg}^{-1}$ , SE = 0.04) than at S2 (0.99  $\text{mg kg}^{-1}$ , SE = 0.04) ( $p = 0.028$ ). MRP was not significantly different between the sample times, and sampling time did not appear to affect mean resin-P (0.31  $\text{mg kg}^{-1} \pm 0.03$  and 0.02 respectively for S1 and S2) or mean MBP (4.64  $\text{mg kg}^{-1} \pm 0.43$  at S1 and 5.30  $\text{mg kg}^{-1} \pm 0.50$  at S2). Thus, in the main experiment changes in soil P concentrations between S1 and S2 occurred in both the control and the drying treatments, implying changes occurred with time not necessarily related to soil drying and re-wetting.

**Soil moisture gradient: phosphorus availability at different sample depths**

Averaged across the initial water treatment and sample times, but according to each P level and irrigation regime, soil P concentrations were significantly higher in the bulk compared to the surface soil in most cases, including the control as well as the drying treatments (Figure 3.3). Exceptions to this dominant trend were the lack of difference between surface and bulk P concentrations for: TP and MUP under CS, DRW and AWD at low-P;  $\text{NaHCO}_3\text{-P}$  and MUP under DRW at high-P; TP under CF at high-P; and  $\text{NaHCO}_3\text{-P}$  under AWD at high-P (Figure 3.3). These exceptions for TP and MUP were likely due to lower sample numbers and comparatively high standard errors, but suggested different soil responses affecting  $\text{NaHCO}_3\text{-P}$  in both of the drying treatments under high P. Thus, P concentrations were not higher at lower pre- re-wetting SWPs, unless undetected because P was released at the surface but readily transferred vertically within the soil profile.

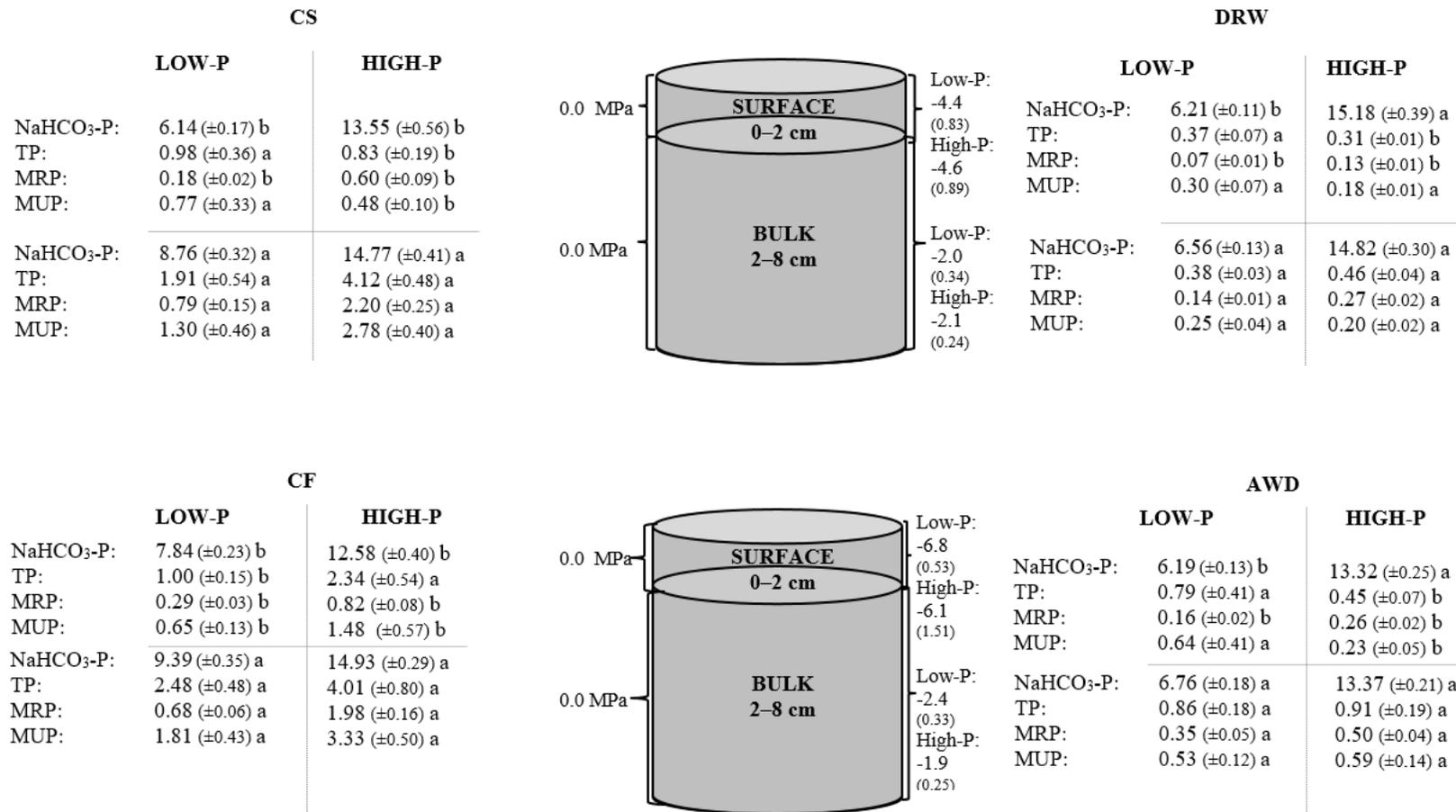


Figure 3.3: Phosphorus concentrations in the surface and bulk soils for each irrigation regime and P level. CS is continuously saturated, DRW is dried and re-wet (from and to saturation), CF is continuously flooded to 1 cm of surface water and AWD is dried and re-flooded (from and to flooding). P concentration data are means (mg kg<sup>-1</sup>) ± SE; n = 6-16 and SWP data for DRW and AWD (MPa) are means (± SE); n = 16. Different letters indicate significant differences between the surface and bulk soils for each P form within a P\*I treatment according to paired samples t-tests (p ≤ 0.05).

**Summary of treatment effects on phosphorus availability at the whole-pot scale**

Plant-available  $\text{NaHCO}_3\text{-P}$  concentrations varied significantly according to the P level, initial water treatment and irrigation regime. Figure 3.4 illustrates the differences at Sampling 1 averaged across the surface and bulk soil, to show the maximum P potentially available to plants during the experiment at the whole-pot scale. At low-P, all irrigation regimes increased  $\text{NaHCO}_3\text{-P}$  above the pre-irrigation treatment level, and the greatest increase (by  $6.84 \text{ mg kg}^{-1}$ , 247 %) occurred in soil following initial DRW and maintained under continuous flooding. At high-P,  $\text{NaHCO}_3\text{-P}$  decreased after the irrigation regimes were established, especially under AWD (by up to  $6.23 \text{ mg kg}^{-1}$ , 32 %), and did not significantly increase under any combination of the initial water treatment or irrigation regime (Figure 3.4). Thus, initial DRW and continuous flooding slightly increased  $\text{NaHCO}_3\text{-P}$  at low-P but the treatments did not increase  $\text{NaHCO}_3\text{-P}$  at high-P; and P application was the dominant treatment effect.

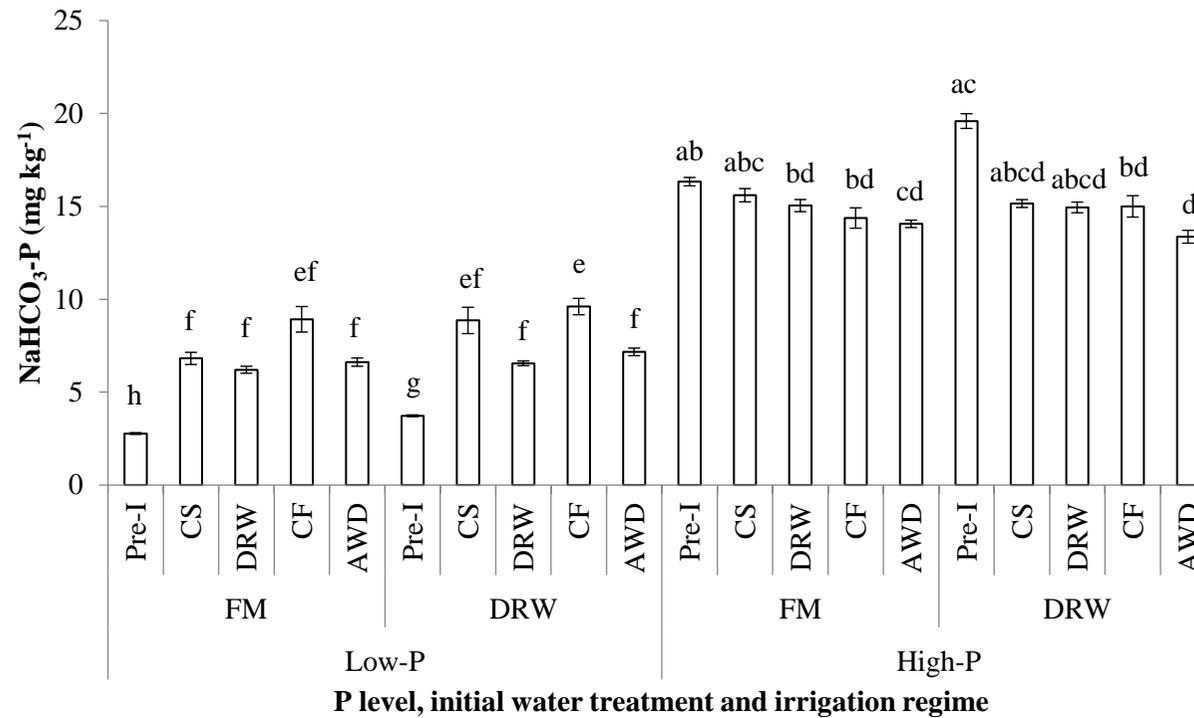


Figure 3.4: NaHCO<sub>3</sub>-P concentrations according to P level (low and high), the initial water treatment (maintained continuously field-moist, FM; or air-dried and re-wet, DRW) and irrigation regime (pre-irrigation treatment, pre-I; continuously saturated, CS; dried and re-wet from and to saturation, DRW; continuously flooded to 1 cm surface water, CF; or dried and re-wet from and to flooding to 1 cm surface water), averaged across the sampling depths at Sampling 1. Data are means ( $\pm$  SE) ( $n = 8$  except pre-I:  $n = 2-3$ ). Different letters indicate significant differences between the treatments according to Welch's ANOVA and Games-Howell pairwise test ( $p \leq 0.05$ ).

In summary, initial soil air-drying (to mean  $-57.4$  MPa) and re-wetting significantly reduced MBP whilst increasing (plant-available)  $\text{NaHCO}_3\text{-P}$  and resin-P concentrations at the start of the experiment (Table 3.7), suggesting that microbial cells may have been the source of released P initially. Despite lower GWCs and SWPs in the drying compared to control irrigation regimes (Table 3.8), P concentrations were generally higher under CS and CF than DRW and AWD apart from MBP, suggesting drying did not cause P release (Table 3.9). Changes in soil P concentrations between S1 and S2 occurred in both the control and the drying treatments, implying changes occurred with time not necessarily related to soil drying and re-wetting. The higher  $\text{NaHCO}_3\text{-P}$  after one compared to two drying and re-wetting cycles was unaffected by initial DRW, and was also observed in the Madagascar soil. Despite significantly lower SWPs in the surface than the bulk soil at maximal soil drying (by  $-3.4$  MPa averaged across P levels and initial water treatments; Table 3.8) at both sample times, soil P concentrations were significantly higher in the bulk compared to the surface soil in most cases, including the control irrigation regimes, suggesting that P availability was not increased due to lower SWPs with surface drying (Figure 3.3). All irrigation regimes significantly increased initial  $\text{NaHCO}_3\text{-P}$  concentrations at low-P, particularly in initially DRW soils under CF, whilst the irrigation regimes tended to decrease  $\text{NaHCO}_3\text{-P}$  at high-P, particularly AWD (Figure 3.4). Although P level dominated the treatment effects, initial DRW and CF may be the most promising water management treatments for increasing soil P availability.

## **Experiment 2**

The major soil properties for the Whiddon Down and Madagascar soils are outlined in Table 3.10.

Table 3.10: Major soil physical and chemical properties for the soil used in Experiment 2. Analyses were carried out by NRM Laboratories, UK and Rothamsted Research, UK.

Soil property	Results	Units	Method
	Rowden		
Texture class	Clay		Particle size distribution via laser diffraction
Sand	13	% w/w	
Silt	37	% w/w	
Clay	50	% w/w	
Organic Matter	11.5	% w/w	Loss on ignition (LOI)
pH	4.9		In water (1:2.5)
Total C	4.79	% w/w	Combustion catalytic oxidation
Total N	0.48	% w/w	
C:N Ratio	10.0:1		
Total P	674	mg kg <sup>-1</sup>	Aqua-regia soluble elements : HCl and HNO <sub>3</sub> digestion with analysis via ICP-OES
Total K	1572	mg kg <sup>-1</sup>	
Total Mg	730	mg kg <sup>-1</sup>	
Total Mn	986	mg kg <sup>-1</sup>	
Total Fe	39336	mg kg <sup>-1</sup>	
Available Fe	171	mg L <sup>-1</sup>	DTPA extraction, or dithionite/ ammonium oxalate extraction (Tadham Moor)

### Effects of initial air-drying and re-wetting on soil phosphorus concentrations

At the start of the experiment, following the initial water treatment but before P application, initial air-drying (to -37.6 MPa) and re-wetting (DRW) increased NaHCO<sub>3</sub>-P by 1.39 mg kg<sup>-1</sup> (39 %) compared to the continuously moist (FM) soil (p = 0.050) but decreased TP (p = 0.050) and MRP (p = 0.046) by 0.48 and 0.16 mg kg<sup>-1</sup> (52 % and 73 %) respectively (Table 3.11). This indicated that whilst concentrations of water-extractable P forms decreased, the greatest magnitude of absolute change caused by DRW was the increased (plant-available) NaHCO<sub>3</sub>-P.

Table 3.11: Soil P concentrations at the start of the experiment ( $\text{mg kg}^{-1}$ ). The initial water treatment comprised field-moist (FM) and air-dried and re-wet (DRW) soils. Different letters indicate significant differences within columns according to Kruskal-Wallis independent samples tests ( $p \leq 0.05$ ). Data are means ( $\pm$  SE) ( $n = 3$ ).

Initial water treatment	TP	MRP	MUP	NaHCO <sub>3</sub> -P
FM	0.92 (0.13) <b>a</b>	0.22 (0.01) <b>a</b>	0.70 (0.13) <b>a</b>	3.56 (0.07) <b>b</b>
DRW	0.44 (0.08) <b>b</b>	0.06 (0.01) <b>b</b>	0.38 (0.09) <b>a</b>	4.95 (0.25) <b>a</b>

### Soil moisture gradient: phosphorus availability at different sample depths

Adding dissolved  $\text{KH}_2\text{PO}_4$  to the soil surface significantly increased plant-available  $\text{NaHCO}_3\text{-P}$  in the surface and bulk soil, by 2.6 and 0.3  $\text{mg kg}^{-1}$  respectively averaged across the initial water (W) and irrigation (I) treatments, measured at harvest.  $\text{NaHCO}_3\text{-P}$  concentrations increased from the start of the experiment to harvest (Table 3.11 and Figure 3.5). In the surface soil, the interactions between P level and W and I were significant (Table 3.12). In the low-P soil,  $\text{NaHCO}_3\text{-P}$  was significantly lower in the DRW compared to FM soil under both irrigation regimes (by 1.5  $\text{mg kg}^{-1}$ , 23 %), whilst under high-P,  $\text{NaHCO}_3\text{-P}$  was only lower in the DRW soil when the irrigation frequencies were low (by 1.3  $\text{mg kg}^{-1}$ , 14 %) (Figure 3.5). Thus, low irrigation frequency significantly increased  $\text{NaHCO}_3\text{-P}$  (by 0.7  $\text{mg kg}^{-1}$ , 11 %), but only in low-P soil that was initially maintained FM. In contrast, in the bulk soil initial DRW increased  $\text{NaHCO}_3\text{-P}$  above the level in FM soil, although only in the high-P treatment with low irrigation frequency (by 0.8  $\text{mg kg}^{-1}$ , 14 %) (Figure 3.5). Notable treatment effects on other P forms were that the P addition increased TP and MRP only in the surface soil (by 0.08  $\text{mg kg}^{-1}$ , 33 % and 0.12  $\text{mg kg}^{-1}$ , 71 %); and W affected all P forms except for MUP, whilst I only affected MRP in the surface soil (Table 3.12). Therefore the effects of the initial water treatment and irrigation frequency on P concentrations depended on the P level and soil depth, and overall, initial DRW only reduced plant-available  $\text{NaHCO}_3\text{-P}$  at harvest in the soil surface.

Table 3.12: Main effects of P supply level (P), initial water treatment (W) and irrigation regime (I) and all 2- and 3-way interactions on soil P concentrations according to three-way ANOVA (with log-transformed data or bootstrap analysis, n = 1000). Significant differences are: not significant (ns),  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*) and  $p \leq 0.001$ . Data are for all harvested treatments and replications (n = 69).

Model	Significance of effect of model term on soil P concentration						
	TP		MRP		MUP	NaHCO <sub>3</sub> -P	
	S	B	S	B	B	S	B
<b>P</b>	***	ns	***	ns	ns	***	**
<b>W</b>	*	*	***	***	ns	*	***
<b>I</b>	ns	ns	**	ns	ns	ns	ns
<b>P*W</b>	**	ns	ns	***	ns	***	ns
<b>P*I</b>	ns	ns	ns	ns	ns	***	*
<b>W*I</b>	*	ns	ns	***	ns	*	*
<b>P*W*I</b>	ns	ns	ns	ns	ns	***	ns

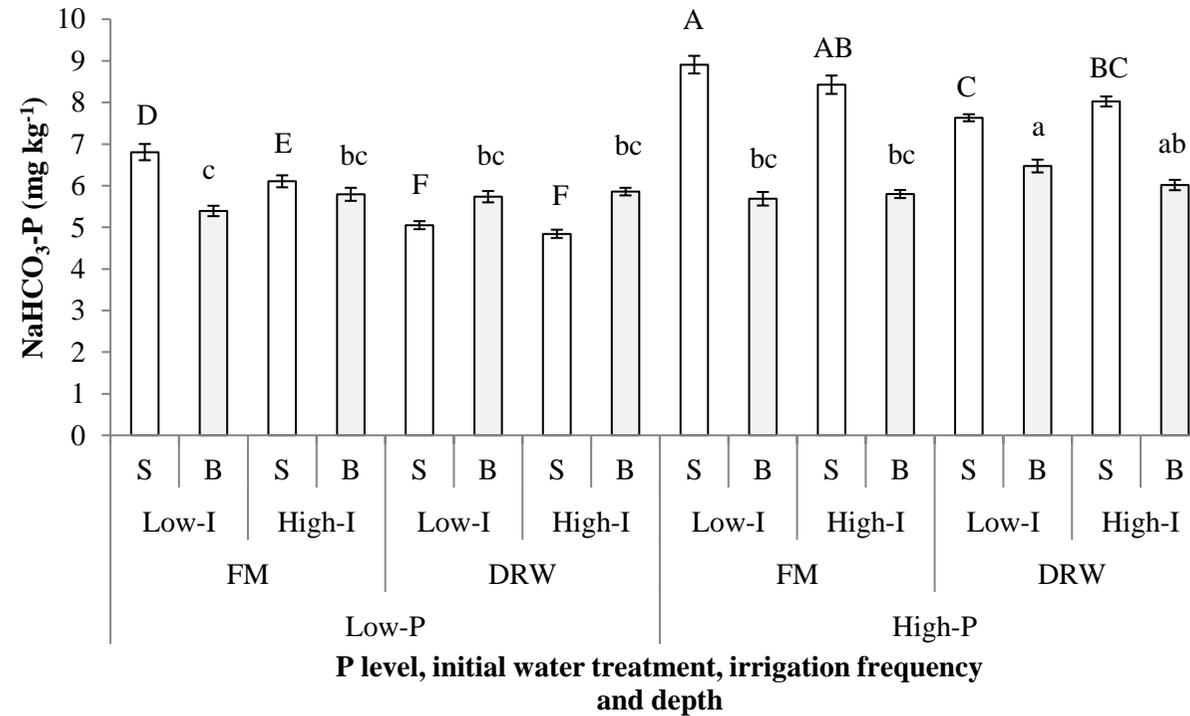


Figure 3.5: Effects of the initial water treatment (W) and irrigation frequency (I) at the low-P and high-P levels on plant-available NaHCO<sub>3</sub>-P concentrations, at the surface (S; white columns) and bulk (B; grey columns) soils, measured at harvest. Data are means ( $\pm$  SE) ( $n = 7-10$ ). Different letters represent significant differences between the treatments according to one-way ANOVA with Tukey's pairwise tests ( $p \leq 0.05$ ) within the surface (upper-case letters) and bulk (lower-case letters) soils.

### Soil drying and re-wetting effects on plant yields and phosphorus uptake

Grain yields and plant biomass were significantly affected by the initial water treatment and its interactions with P level and irrigation frequency (Table 3.13). Averaged across the irrigation treatments, grain yield increased with DRW by 230 % and 206 % at low-P and high-P respectively (Figure 3.6). Total biomass (root and shoot) was similarly 251 % and 222 % higher when soil was initially DRW compared to maintained FM in the low-P and high-P treatments respectively. The tendency for the initial water treatment to affect HI ( $p = 0.051$ ) resulted in a significant P\*W interaction (Table 3.13). Therefore initial soil DRW more than doubled grain yields and total biomass production compared to plants grown in continuously FM soil.

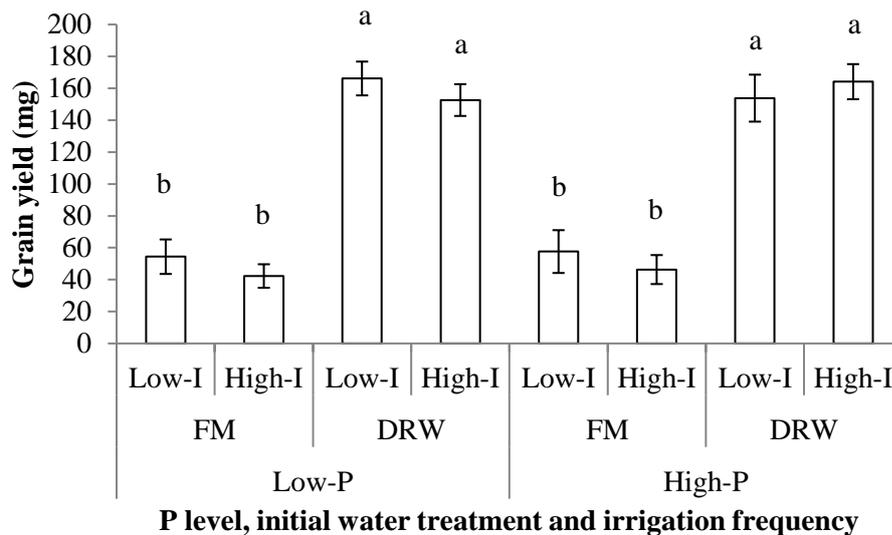


Figure 3.6: Effects of the initial water treatment (W) and irrigation frequency (I) at the low-P and high-P levels on grain yield. Data are means ( $\pm$  SE) ( $n = 7-10$ ). Different letters represent significant differences between the treatments according to one-way ANOVA with Tukey's pairwise test ( $p \leq 0.05$ ).

Table 3.13: Main effects of P level (P), initial water treatment (W) and irrigation regime (I) and all 2- and 3-way interactions on plant biomass (dry weights), root to shoot ratio, harvest index (grain yield / total biomass), shoot P concentration (conc.), total shoot P uptake (shoot P content = shoot P concentration \* shoot biomass), and PUE (grain yield / total P uptake, mg mg<sup>-1</sup>) (results from three-way ANOVA with bootstrap analysis, n = 1000). Shoot was total above-ground biomass and straw was total above-ground biomass minus the grain. Significant differences are: not significant (ns),  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*) and  $p \leq 0.001$  (\*\*\*). Data are for all harvested treatments and replications (n = 69).

Model	Significance of effect of model term on plant yields and P concentration and content									
	Total shoot biomass (mg)	Grain (mg)	Straw (mg)	Root (mg)	Shoot (mg)	Root/ Shoot ratio	Harvest Index	Shoot P conc. (mg kg <sup>-1</sup> )	Total shoot P uptake (mg)	PUE for grain yield
<b>P</b>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<b>W</b>	***	***	***	***	***	ns	ns	***	***	***
<b>I</b>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<b>P*W</b>	***	***	***	***	***	ns	*	**	***	***
<b>P*I</b>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<b>W*I</b>	***	***	***	***	***	ns	ns	**	***	***
<b>P*W*I</b>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Total P uptake showed similar treatment effects as grain yields since both variables were only affected by the initial water treatment and interactions (Table 3.13; Figures 3.6 and 3.7), and they were significantly positively correlated ( $R = 0.930$ ,  $n = 67$ ,  $p < 0.001$ ). This indicated that the increases in grain yields (and similarly total biomass) and P uptake caused by initial DRW were proportional. Averaged across the irrigation treatments, initial DRW increased total P uptake by 302 % at low-P and 316 % at high-P (Figure 3.7). Therefore there was little variation in shoot TP concentrations between the treatments (Figure 3.8). Shoot TP concentrations were significantly higher (by 48 %) at high-P in initially DRW soil regardless of irrigation frequency, compared to FM soil under high irrigation frequency, but did not vary otherwise (Figure 3.8). The lack of variation in shoot TP concentration at low-P—suggested that the significantly increased grain yields in initially DRW soil at both P levels (Figure 3.6) resulted from factors other than a direct increase in P uptake. Initial DRW significantly reduced PUE (Table 3.13), by 38 % averaged across the P level and irrigation frequency treatments, because DRW similarly increased total P uptake as well as grain yields (Figures 3.6 and 3.7). Therefore the higher yields under DRW were not associated with more efficient P use.

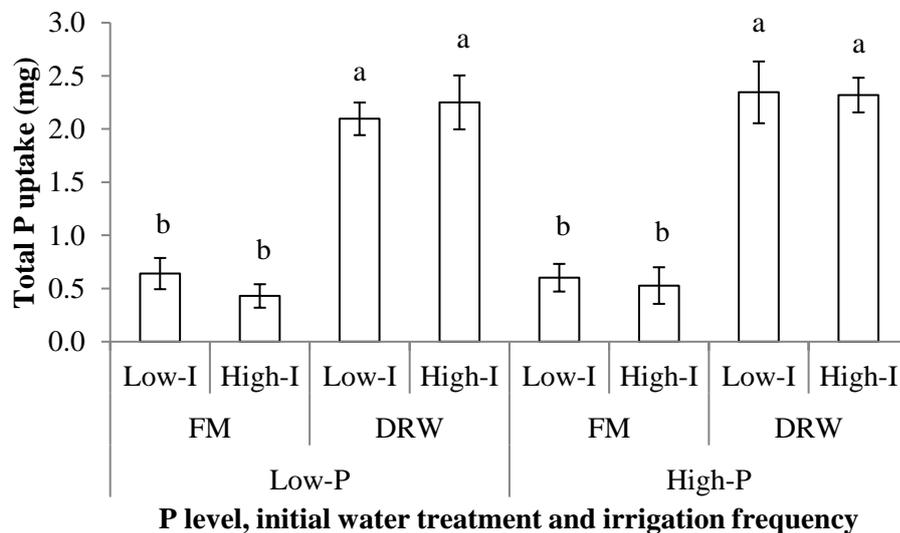


Figure 3.7: Effects of the initial water treatment (W) and irrigation frequency (I) at the low-P and high-P levels on total P uptake. Data are means ( $\pm$  SE) ( $n = 5-10$ ). Different letters represent significant differences between the treatments according to one-way ANOVA with Tukey's pairwise test ( $p \leq 0.05$ ).

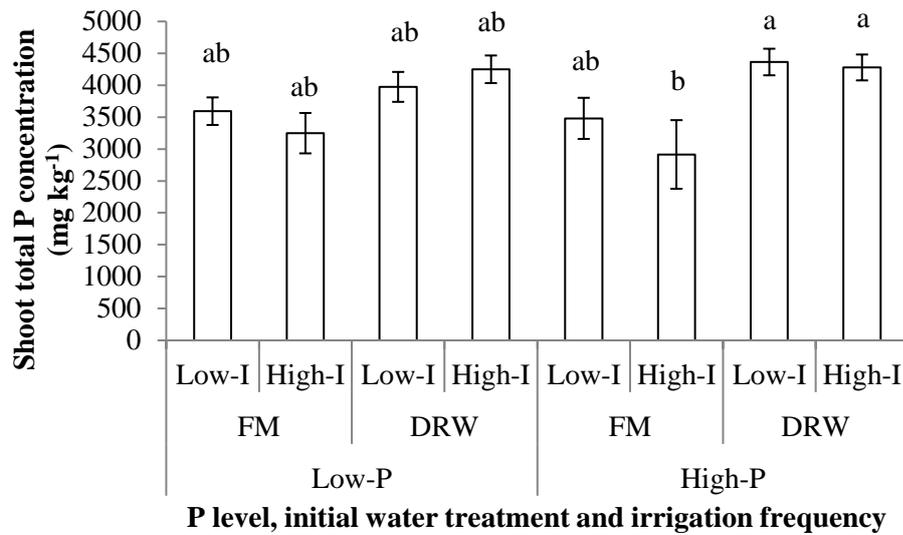


Figure 3.8: Effects of the initial water treatment (W) and irrigation frequency (I) at the low-P and high-P levels on shoot total P concentration. Data are means ( $\pm$  SE) ( $n = 5-10$ ). Different letters represent significant differences between the treatments according to one-way ANOVA with Tukey's pairwise test ( $p \leq 0.05$ ).

#### Treatment effects on plant water use

Total cumulative water use (irrigation volume) was significantly higher for plants with high compared to low irrigation frequency ( $p < 0.001$ ), but was unaffected by P level and the initial water treatment. However, water use efficiency (WUE) and water productivity (WP) were significantly affected by the initial water treatment and its separate interactions with P level and irrigation frequency ( $p < 0.001$ ). Plants grown in soil that was initially DRW had 254 % higher WUE compared to continuously FM soil in the low-P treatment, and 227 % higher WUE in the high-P treatment. The WUE was 215 % and 279 % higher for plants grown in initially DRW compared to FM soil when irrigation frequency was low and high, respectively. These changes in WUE and WP reflected significantly higher biomass production and grain yields when soil was initially DRW with no change in cumulative water use (total evapotranspiration) compared to continuously FM soil.

**Summary**

In summary, initially air-drying (to  $-37.6$  MPa) and re-wetting the soil prior to planting significantly increased plant-available  $\text{NaHCO}_3\text{-P}$  compared to continuously moist soil (Table 3.11). By harvest the  $\text{NaHCO}_3\text{-P}$  concentration was lower in soil that had been initially DRW compared to continuously FM, only in the surface soil under low P conditions (Figure 3.5). Initial DRW increased grain yields (by up to 230 %; Figure 3.6), biomass (by up to 251 %) and total P uptake (by up to 316 %; Figure 3.7) regardless of the P level and irrigation regime. However, across all treatments shoot P concentrations showed little variation (Figure 3.8), suggesting that factors other than P likely caused the increased yields. These results also indicated that increased shoot P uptake caused by initial DRW did not entirely explain the decreased soil  $\text{NaHCO}_3\text{-P}$  concentrations. Grain yields were not limited in the low P soil compared to the high P soil (Figure 3.6). Although the P level was the dominant treatment affecting soil P concentrations (Figure 3.5), similarly to Experiment 1 (Figure 3.4), the initial water treatment dominated the effects on grain yields and total P uptake (Figures 3.6 and 3.7). Initial DRW decreased PUE for grain yield (by 38 %) although increased the WUE (by up to 279 %), indicating greater efficiency of water but not P during the crop cycle. Key results from Experiments 1 and 2 are summarised in Figure 3.9.

Figure 3.9: Summary of key results in relation to the hypotheses, expressed as percent change caused by soil drying and re-wetting as described.

Percent change	Increase	Decrease
100 +		
67 - 99		
34 - 66		
1 - 33		
No significant difference		
Most important result shown elsewhere within column		

### 1.

#### Hypothesis:

Soil P availability will be higher in initially air-dried and re-wet compared to continuously moist soil.

#### Consistent with the hypothesis:

Measured at the start of the experiments, initial DRW increased immediately available P ( $\text{NaHCO}_3\text{-P}$ , resin-P). After initiating other treatments, initial DRW increased immediately ( $\text{NaHCO}_3\text{-P}$ , resin-P, MRP) and potentially (TP, MUP) available P.

#### Contrary to the hypothesis:

Measured at the start of the experiments, initial DRW decreased immediately (MRP) and potentially (TP, MBP) available P. After initiating other treatments, initial DRW decreased immediately ( $\text{NaHCO}_3\text{-P}$ ) and potentially (TP, MUP, MBP) available P under certain treatment combinations.

Percent changes caused by initial soil drying and re-wetting, Experiments 1 and 2.

Treatment		TP		MRP		MUP		NaHCO <sub>3</sub> -P		Resin-P		MBP	
Experiment		1	2	1	2	1	2	1	2	1	2	1	2
<b>INITIAL</b>													
<b>W</b>		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<b>W*P</b>	<b>LP</b>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	<b>HP</b>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<b>EXPERIMENTAL (Sampling 1 and 2)</b>													
<b>W</b>		NA		NA		NA		NA		NA	NA	NA	NA
<b>W*P</b>	<b>LP</b>	NA		NA				NA	Surface	NA	NA		NA
	<b>HP</b>					NA		NA	Surface	Bulk			
<b>W*I</b>	<b>CS</b>	NA				NA					NA		NA
	<b>CF</b>	NA				NA					NA		NA

## 2.

**Hypothesis:**

Soil P availability will be higher in soils exposed to drying and re-wetting or re-flooding compared to soils maintained under continuously saturated or flooded conditions.

**Consistent with the hypothesis:**

DRW increased immediately available P (resin-P), and both DRW and AWD increased potentially available P (MBP).

**Contrary to the hypothesis:**

DRW and AWD decreased immediately ( $\text{NaHCO}_3\text{-P}$ , MRP) and potentially (TP, MUP) available P.

**Soil P availability in drying compared to saturated or flooded treatments, Experiment 1.**

Treatment		TP	MRP	MUP	$\text{NaHCO}_3\text{-P}$	Resin-P	MBP
Irrigation	P level						
DRW vs CS	Low						
	High						
AWD vs CF	Low						
	High						

## 3.

**Hypothesis:**

Soil P availability will be increased by DRW, with multiple DRW cycles causing a greater increase than a single cycle.

**Consistent with the hypothesis:**

Immediately available P (resin-P) did not change with time in the continuously saturated or flooded soils. Immediately available P was increased after two cycles compared to one cycle following DRW (MRP) and AWD (MRP and resin-P).

**Contrary to the hypothesis:**

Immediately and potentially available P increased (MRP) or decreased ( $\text{NaHCO}_3\text{-P}$ , MBP) with time in the continuously flooded and saturated soils. Immediately ( $\text{NaHCO}_3\text{-P}$ ) and potentially (MBP) available P was lower after two cycles compared to one AWD cycle, and did not change under DRW ( $\text{NaHCO}_3\text{-P}$ , resin-P, MBP).

**Maximum significant differences at S2 (two cycles) compared to S1 (one cycle), Experiment 1.**

<b>Irrigation treatment</b>	<b>MRP</b>	<b><math>\text{NaHCO}_3\text{-P}</math></b>	<b>Resin-P</b>	<b>MBP</b>
<b>CS</b>				
<b>DRW</b>				
<b>CF</b>				
<b>AWD</b>				

## 4.

**Hypothesis:**

Soil P availability will be higher in the surface compared to the bulk soil within drying treatments.

**Consistent with the hypothesis:**

Immediately and potentially available P was not different between the surface and the bulk soil in the control treatments (CS: TP, MUP at low-P; CF: TP at high-P).

**Contrary to the hypothesis:**

Immediately and potentially available P was otherwise lower in the surface compared to the bulk soil within drying treatments and also control treatments.

**Differences at surface compared to bulk soil, Experiment 1.**

Irrigation	P level	TP	MRP	MUP	NaHCO <sub>3</sub> -P
CS	Low	Grey	Dark Blue	Grey	Light Blue
	High	Dark Blue	Dark Blue	Dark Blue	Light Blue
DRW	Low	Grey	Light Blue	Grey	Light Blue
	High	Light Blue	Light Blue	Grey	Grey
CF	Low	Light Blue	Light Blue	Light Blue	Light Blue
	High	Grey	Light Blue	Light Blue	Light Blue
AWD	Low	Grey	Light Blue	Grey	Light Blue
	High	Light Blue	Light Blue	Light Blue	Grey

## 5.

**Hypothesis:**

Increases in soil P availability at the whole-pot level caused by DRW will be sufficient to increase plant P uptake, biomass and yields.

**Consistent with the hypothesis:**

In soils that were initially DRW, shoot P concentrations (at high-P) and total P uptake, biomass and grain yields were significantly increased.

**Contrary to the hypothesis:**

Initial DRW or reduced irrigation frequency did not affect shoot P concentrations at low-P.

**Effects of initial DRW compared to initially maintained FM, Experiment 2.**

Treatment		Total biomass (g)	Grain (g)	Shoot P concentration (mg kg <sup>-1</sup> )	Total shoot P uptake (mg)
W*P	Low-P				
	High-P				
W*I	Low-I				
	High-I				

## Discussion

### Effects of initial soil drying and re-wetting on phosphorus availability

Air-drying the Whiddon Down (Experiment 1) and Rowden (Experiment 2) soils to -57.4 MPa and -37.6 MPa respectively, prior to re-wetting, was expected to increase  $\text{NaHCO}_3\text{-P}$  by 3.70 and up to 7.09  $\text{mg kg}^{-1}$  respectively, based on Chapter 2 (Tables 2.6 and 2.5, respectively). In Experiment 1, initial DRW increased  $\text{NaHCO}_3\text{-P}$  in low-P treatments by 0.96  $\text{mg kg}^{-1}$  (35 %) and in high-P treatments by 3.26  $\text{mg kg}^{-1}$  (20 %). The increase was less compared to in Chapter 2, for which the soil was also at low-P, possibly due to increased storage time causing reduced MBP concentrations (Turner and Romero, 2010). In contrast to Chapter 2, MRP significantly reduced, although by a low magnitude (0.02  $\text{mg kg}^{-1}$  at low-P), perhaps indicating another effect of increased storage time. The reduction in MBP (by 15.4  $\text{mg kg}^{-1}$  at low-P, 34 %; Table 3.7) compared to continuously FM soil suggested that the microbial biomass may have been a partial source of released P. Similarly, MBP concentrations reduced by 31 % in soil dried to 2 % GWC and then re-wet (measured after 1 hour) compared to soil maintained at 40 % GWC (Bünemann et al., 2013). Whether re-wetting results in complete microbial recovery depends on the soil drying intensity (Sun et al., 2017 a,b; Figure 1.2) and the microbial community composition (Fierer et al., 2003). In Experiment 2, initial DRW also increased  $\text{NaHCO}_3\text{-P}$ , by 1.39  $\text{mg kg}^{-1}$  (39 %) whilst decreasing TP by 0.48  $\text{mg kg}^{-1}$  (52 %) and MRP by 0.16  $\text{mg kg}^{-1}$  (73 %) (Table 3.11), in contrast to Chapter 2 where MRP increased in the Rowden soil (Figure 2.3). An important distinction is that the soil was mixed (3:1) with sand to create a substrate more suitable for *Brachypodium* growth; thus the concentrations could be expected to be approximately 25 % higher in soil without sand. The greatest magnitude of absolute change caused by initial DRW was decreased MBP (Experiment 1) and increased  $\text{NaHCO}_3\text{-P}$  (Experiments 1 and 2), suggesting benefits for P availability to plants.

Another important distinction for Experiment 2 was the inclusion of plants, to determine whether P uptake, biomass and yields were affected by initial DRW. Despite increased P

availability immediately following initial DRW, at harvest the  $\text{NaHCO}_3\text{-P}$  concentration was lower in soil that had been initially DRW compared to continuously FM under low P conditions (Figure 3.5). This suggested that released P was taken up by the plants and because of the greater biomass production and grain yields in DRW soil, P demand (and uptake) were higher. Initial DRW was the dominant cause of increased biomass and grain yields, which were not affected by P level or irrigation frequency within the initial water treatment (Figure 3.6). Similarly, DRW increased biomass at low-P compared to plants grown in FM soil at high-P (by 218 %). Increased plant biomass caused by initially air-drying soil (to 4 % GWC) prior to planting was previously reported, with approximately 70 % and 20 % increase in maize production when the FM soils had received 5 or 10 mg P kg<sup>-1</sup> fertiliser respectively (Bünemann et al., 2013). In contrast to Brachypodium, increased maize biomass corresponded to increased shoot P concentrations by approximately 90 % and 30 % respectively for each fertiliser rate. Since other nutrients were well-supplied, the increased P uptake and biomass were attributed to increased P availability caused by soil DRW (Bünemann et al., 2013). The maize plants were harvested after just 22 days so whether the benefit to biomass production also increased yields, as for Brachypodium in Experiment 2, is unknown. Furthermore, initial DRW increased rice P contents by 250 % compared to plants grown under continuously flooded conditions, although P concentrations reduced because biomass increased in response to higher available N (Tsujimoto et al., 2010), which was not measured in the soil or plant tissue for Brachypodium. The greater effect at high-P compared to unfertilised soils was likely because P was applied as part of a NPK fertiliser treatment, indicating the importance of combined nutritional effects (Tsujimoto et al., 2010). Therefore initial DRW significantly increased biomass production and yields in Brachypodium, but plants at low-P were unlikely P-limited (Figure 3.6) and it remains unclear whether the response was caused by increased soil P availability.

**Effects of subsequent soil drying and re-wetting on phosphorus availability**

It was hypothesised that since soil P availability increased as SWP decreased (Chapter 2), soils exposed to drying with lower SWPs would have higher P availability compared to soils maintained saturated or flooded in Experiment 1. Despite significantly lower SWPs under drying irrigation regimes (DRW and AWD) (Table 3.8), P availability was generally higher under the control (CS and CF) regimes (Table 3.9). Similarly, reduced irrigation frequency in Experiment 2 (imposing partial soil drying at the soil surface) had no distinct effects on soil P availability that were independent of the other treatments: low irrigation frequency significantly increased  $\text{NaHCO}_3\text{-P}$  (by  $0.7 \text{ mg kg}^{-1}$ ), but only in low-P soil that was initially maintained FM (Figure 3.5). These results contrasted with other studies reporting increased P availability following soil drying and re-wetting under both aerobic and anaerobic conditions. For example, DRW significantly increased resin-P, MRP and MUP compared to continuously moist soils due to the destruction of aggregates (slaking) and microbial cell lysis (Bünemann et al., 2013; Figure 1.2). Furthermore, soluble MRP concentrations increased under pulsed compared to continuously reducing conditions, indicating that alternately flooded and drying conditions caused P release (Scalenghe et al., 2012). This response was attributed to the repeated reduction-oxidation of Mn- and Fe-oxides, releasing adsorbed/ occluded P, whilst substantial changes to organic P concentrations likely involved initial oxidation of organic matter and subsequent mineralisation and release of P, indicating coupled biological and physical sources. In Experiment 1, MBP was higher under soil DRW and AWD (Table 3.9), suggesting that exposure to lower SWPs did not cause cell lysis and P release from the microbial biomass. Since the MBP concentrations averaged across S1 and S2 were lower than before the irrigation regimes were established, regardless of the initial water treatment (Tables 3.7 and 3.9), the drying treatments did not stimulate the microbial biomass but caused a lesser decrease than the CS and CF treatments. Possibly the CS and CF treatments reduced the microbial populations thereby releasing P to the soil, explaining the higher MRP, MUP and  $\text{NaHCO}_3\text{-P}$  concentrations. Alternatively, the lower P availability under DRW and AWD may have resulted from the higher

MBP concentration, because soil microbes assimilated available P in the absence of plant competition. Isotope studies could be used to test these hypotheses, to determine the P cycling dynamics in different pools including the microbial biomass. Studying the oxygen isotope composition of phosphate in soils showed available P was sourced from the microbial biomass (Tamburini et al., 2012), and tracing  $^{32}\text{P}$  in soil pools and plants showed different sensitivities of soil microbes and plants to P uptake under drought conditions (Dijkstra et al., 2015). Such studies could be carried out across a range of SWPs.

The responses to the irrigation regimes varied according to the P level. The initial P concentrations in Experiment 1 confirmed that all P forms were increased by the addition of P in solution; and that the low-P and high-P soils would be classified as indices 0 and 2 respectively based on the  $\text{NaHCO}_3\text{-P}$  concentrations (DEFRA, 2017). All irrigation regimes significantly increased the pre-irrigation  $\text{NaHCO}_3\text{-P}$  concentrations at low-P, particularly initially DRW soils under CF, whilst  $\text{NaHCO}_3\text{-P}$  was decreased by the irrigation regimes at high P supply, especially AWD (Figure 3.4). Therefore the P level dominated the treatment effects. Several studies have also reported different responses to DRW according to initial soil P concentrations. For example, DRW caused a higher magnitude of increased extractable P (Bray-P) in P-fertilised compared to unfertilised soils, suggesting that higher adsorption of the applied P during soil drying meant that newly mineralised P following DRW was less readily adsorbed, thus more available (Chepkwony et al., 2001). In Experiment 1,  $\text{NaHCO}_3\text{-P}$  was decreased rather than increased by DRW (by  $1.06 \text{ mg kg}^{-1}$ , 14 %) and AWD (by  $2.13 \text{ mg kg}^{-1}$ , 25 %) at low-P but was unaffected at high-P. This suggested that P was adsorbed during drying but not released or mineralised following re-wetting, so at low-P the  $\text{NaHCO}_3\text{-P}$  decreased whilst at high-P the adsorption sites were saturated and so  $\text{NaHCO}_3\text{-P}$  did not change. Similarly, relative increases in soluble TP following DRW increased with  $\text{NaHCO}_3\text{-P}$  concentration, and also with the degree of sorption saturation (Styles and Coxon, 2006). This indicated that the magnitude of P increase was dependent on (and thus could be limited by) how saturated the P sorption sites were, emphasising the relevance of soil P concentration to release following DRW. The 12 soils

studied by Scalenghe et al. (2012) received at least double the recommended P fertiliser rates, so the greater magnitude of increased MRP reported under AWD compared to CF compared to the Whiddon Down soil in Experiment 1 may have been caused by greater saturation of sorption sites. Measuring the P sorption capacity of the soil, and degree of saturation of P sorption sites according to treatment, is needed to support this idea. This is particularly relevant since DRW can stimulate the P sorption capacity of some soils, decreasing P availability under drying but which may recover to initial levels following re-wetting (Haynes and Swift, 1985); and sorption is also affected by alternately flooded and dried soil conditions (Phillips and Greenway, 1998). Thus, the P level was important and increased P availability following DRW is likely greater in soils with low P sorption capacities, or with saturated sorption sites.

The soil P concentration also affects microbial activity, since P mineralisation was increased in fertilised compared to unfertilised soils following DRW (Grierson et al., 1998). However, MBP was higher under DRW and AWD compared to the controls at both P levels in Experiment 1, suggesting the effects of P level were not microbial. Stability of available P concentrations following DRW or AWD compared to CS or CF is suggested to result from a methodological reason (Butterly et al. 2011b). Since soil P was measured following DRW at their re-wetted moisture contents, which was the same for the CS and CF soils, any increases in P would likely be lower compared to measurement of air-dried soils (for which the measurement procedures impose re-wetting). Whereas some studies have measured air-dried soils (e.g. Turner et al., 2002; 2003), which is useful to standardise water contents which differ between samples (Experiment 2), measures of P availability are more realistic (and more comparable to continuously moist soils) when DRW effects are measured at re-wetted water contents (Styles and Coxon, 2006). In summary, soil drying and re-wetting from and to saturated or flooded conditions (Experiment 1) or by reducing irrigation frequency (Experiment 2) did not clearly increase soil P availability, partially due to interactions with P level. The greater effects of site management, such as fertiliser application, than DRW on soil P availability (Butterly et al.,

2011b) indicated that environmental conditions unrelated to water management also determine whether DRW increases soil P availability.

### **Effects of multiple cycles of soil drying and re-wetting on phosphorus availability**

It was hypothesised that soil P availability would be increased by DRW, with multiple DRW cycles causing a greater increase than a single cycle (without plant uptake) due to additive effects of P release caused by microbial cell lysis. However, changes in soil P concentrations between S1 and S2 occurred in both the control and the drying treatments, with no consistent effects of the initial water treatment or P level as well as irrigation regime. Therefore an initial and more intense DRW cycle (-57.4 MPa rather than -1.9 to -7.8 MPa) also did not affect P release during subsequent DRW cycles. The GWCs and SWPs were reduced in the drying regimes and did not significantly differ between S1 and S2 (with just one exception; Table 3.8), so the results were comparable. This implied that changes occurred with time not necessarily related to soil drying and re-wetting, contrasting with reports of recurrent DRW or AWD cycles increasing P availability (Jarvis et al., 2007; Scalenghe et al., 2012; Chen et al., 2016). Interpreting the effects of soil DRW on P release from the microbial biomass needs to consider the timescale of measurement. Whereas soil samples were taken to measure MBP concentrations 1.5 hours after re-wetting the soil in Experiment 1, MBP recovered to *c.* pre-DRW levels within seven days after re-wetting (Chen et al., 2016). Thus, sampling at different timescales after re-wetting would better indicate microbial survival and recovery, such as before as well as after a second DRW cycle.

Similarly to the responses to irrigation regime, the comparative P concentrations between the two sampling times depended on the P form. Where  $\text{NaHCO}_3\text{-P}$  and MBP significantly differed, concentrations were higher at S1 than S2 in all cases, whereas the opposite effect was observed for MRP. These results suggested that the microbial biomass declined during the experiment, which particularly occurred under CF, likely because the soil was collected from a grassland site and the microbial community was not adapted to flooded conditions. This was consistent

with a previous report of decreased MBP caused by DRW cycles during a longer incubation period (90 days) at lower temperature (25 °C) compared to Experiment 1 (Chen et al., 2016), but contrasted with reportedly increased MBP with soil DRW over a shorter incubation period (26 days) at a similar temperature (38 °C) (Grierson et al., 1998). The result that both MBP and  $\text{NaHCO}_3\text{-P}$  were higher at S1 than S2 was consistent with reports that changes in  $\text{NaHCO}_3\text{-P}$  did not relate to MBP (Chen et al., 2016), and fluctuations in MBP did not correlate with P mineralisation (Grierson et al., 1998). Thus it appeared that decreased MBP caused by DRW did not contribute to increased  $\text{NaHCO}_3\text{-P}$  following mineralisation, although it is possible that decreased MBP contributed to the increased MRP at S2 compared to S1. Consistent with Experiment 1, MRP concentrations also increased with multiple AWD cycles in 12 soils, with the maximum increase occurring after four redox cycles (Scalenghe et al., 2012). As well as due to higher P concentrations as discussed, the higher magnitude of increase in MRP compared to Experiment 1 may have been artefactual because Scalenghe et al. (2012) dried soil more intensely, by freeze-drying, with rapid drying causing greater effects (Figure 1.2) and freezing and thawing soils having similar effects on P release as DRW (Blackwell et al., 2012). Nevertheless, the much larger increase in MRP reported for a paddy rice soil (Scalenghe et al., 2012) was not observed in the Madagascar soil in Experiment 1 (data not shown), although higher  $\text{NaHCO}_3\text{-P}$  at S1 compared to S2 was observed (as for the Whiddon Down soil), indicating that plant-available P declined even in a soil frequently exposed to alternate drying and flooding. Thus, the varying effects of multiple cycles of DRW on P availability according to P form reflected varying reported responses, suggesting the relevance of soil properties and management.

In Experiment 2, the lack of significant effect of reduced irrigation frequency on soil P concentrations suggested that multiple cycles of DRW did not increase P availability compared to the initial, more intense (-37.6 MPa) soil DRW. This contrasted with the idea that deficit irrigation regimes via controlled soil drying managed throughout different stages of crop phenological development can stimulate nutrient uptake (Wang et al., 2012; 2017). However,

the SWP was not measured and it is possible that insufficient amounts of soil reached low SWPs (-3.0 MPa for the Rowden soil; Table 2.5) to increase P availability. Overall, there was no consistent effect of the number of drying cycles on soil P availability according to the P level, initial water treatment and irrigation regime, suggesting that these treatments had stronger effects.

### **Differential soil drying: relevance of soil depth to phosphorus availability**

It was hypothesised that soil P availability would be higher in surface compared to bulk soil within the drying treatments, because SWPs would be lower in the surface soil due to evaporation. In Experiment 1, despite significantly lower SWPs in the surface than the bulk soil at maximal soil drying (by -3.4 MPa averaged across P levels and initial water treatments) at both sample times, soil P concentrations were significantly higher in the bulk compared to the surface soil in most cases, suggesting that P availability was not increased due to lower SWP. Furthermore, P concentrations were also higher in bulk than surface soils in the control (CS and CF) irrigation regimes, suggesting that the effect was independent of surface soil drying. This result was unexpected since  $\text{NaHCO}_3\text{-P}$  significantly increased by  $0.56 \text{ mg kg}^{-1}$  (31 %) when the Whiddon Down soil was dried to -2.3 MP (Table 2.6). Similarly to the different results between sampling times, the exceptions to higher P concentrations in bulk compared to surface soils occurred for different P forms and were inconsistent across all treatments (Figure 3.3), suggesting that the effect was not dominated by any single treatment. In contrast, the P level dominated the differences between P concentrations in surface and bulk soils in Experiment 2 because P fertiliser was applied to the soil surface. Thus, P concentrations were not higher at lower pre-re-wetting SWPs, unless undetected because P was released at the surface but readily transferred vertically in the soil profile in Experiment 1. Vertical leaching of P is widely documented; for example through lysimeters as demonstrated by TP, MRP and MUP concentrations measured in leachates from four UK grassland soils (Turner and Haygarth, 1999). Therefore further study of the effects of vertical soil drying via reduced irrigation frequency, such as AWD irrigation, is needed (Chapter 4).

### **Effects of soil drying and re-wetting on plant phosphorus uptake, biomass production and yields**

It was hypothesised that increases in soil P availability caused by soil drying and re-wetting would be sufficient to increase plant P uptake, biomass and yields. In Experiment 1, drying from and re-wetting to either saturated or flooded conditions did not significantly increase P availability above continuously flooded or saturated levels (Figure 3.4). From the pre-irrigation concentration at low-P (2.77 mg kg<sup>-1</sup> FM; 3.73 mg kg<sup>-1</sup> DRW), NaHCO<sub>3</sub>-P was increased sufficiently to be classified above Index 0 (0-9 mg kg<sup>-1</sup>) at Index 1 (10-15 mg kg<sup>-1</sup>) according to RB209 (DEFRA, 2018b) only in soil that was initially DRW and maintained under continuous flooding (to 9.61 mg kg<sup>-1</sup>; the classification is integer-based). This suggested benefits of that treatment combination compared to others; yet recommended P fertiliser rates for arable, grassland and forage crops aim to increase concentrations to within the range for Index 2 (16–25 mg kg<sup>-1</sup>). At high-P, all treatments reduced NaHCO<sub>3</sub>-P from the pre-irrigation levels within Index 2 to concentrations within Index 1, except for soil that was initially maintained FM and was continuously saturated (Figure 3.4). However, RB209 recommendations use air-dried soil whereas determining the effects of DRW on P availability requires that soils are measured at their re-wetted moisture contents (Styles and Coxon, 2006; Butterly et al., 2011b), as in Experiment 1. Therefore RB209 cannot compare soils at different water contents. The changes in P concentrations could be also calculated at the field scale from results of laboratory experiments. In 12 soils, increased MRP concentrations caused by AWD represented increases by 15-36 kg P ha<sup>-1</sup> within the surface 10 cm of soil (Scalenghe et al., 2012); whilst for forest soils, Dinh et al. (2016) calculated that 2-3 kg P ha<sup>-1</sup> could be released following a DRW cycle. However, they cautioned that although low SWPs (-100 MPa) may be reached in surface soils during the summer, conditions in the laboratory were likely unrepresentative of field conditions due to the rapid and complete re-wetting, whereas at the field scale preferential flow pathways exist. Although this concern related to forest soils and preferential flow may be slightly less relevant to arable soils, the cautioning that their

calculations represented the theoretical maximum values hold true; therefore further studies at the field scale are needed (Chapter 4).

Since it is difficult to accurately compare  $\text{NaHCO}_3\text{-P}$  concentrations at plant-relevant SWPs to guide P fertiliser requirements, direct measurements of plant nutrient contents are needed to appraise the relevance of DRW. In Experiment 2, initially air-drying (to -37.6 MPa) and re-wetting the soil prior to planting significantly increased  $\text{NaHCO}_3\text{-P}$  compared to continuously moist soil. Although by the harvest the  $\text{NaHCO}_3\text{-P}$  concentration was lower in soil that had been initially DRW compared to continuously FM under low P conditions, this was not clearly related to differences in P uptake (Figure 3.5). Across all treatments the main effect on grain yields (and biomass) was the significant increase caused by initial soil DRW (Figure 3.6). One explanation may be that the increased soil P concentrations caused higher biomass production and yields as a direct result of increased P nutrition. However, shoot TP concentrations showed little variation across treatments (Figure 3.8). Furthermore, based on statistically similar yields between the equivalent initial water treatments, yields were not limited in the low-P soil compared to the high-P soil (Figure 3.6), further suggesting that differences in P availability and uptake could not explain the variation in biomass and yields. If DRW caused higher plant biomass for alternative reasons (discussed below), plant P demand would be increased; therefore increased P uptake (allowing for the similar shoot TP concentrations between treatments; Figures 3.7 and 3.8) would have caused  $\text{NaHCO}_3\text{-P}$  to be lower under DRW when supply was low, as observed (Figure 3.5). Furthermore, with significantly higher root biomass when grown in soil that was initially DRW (by 329 %, averaged across the P level and irrigation frequency treatments), plants were able to access more soil P (as well as other nutrients). Therefore increased P nutrition caused by soil DRW unlikely explained the significantly increased biomass and > doubled grain yields.

Soil physical changes caused by DRW affect nutrient release and plant growth. For example, soil DRW can disrupt aggregate structure, decreasing mean aggregate diameter in dry compared to wet soils (increasing P availability), whilst re-wet soils had intermediate diameter aggregates

suggesting partial recovery (Bünemann et al., 2013). In a silt loam, aggregates became stable and resistant to slaking after two DRW cycles when soils were air-dried to 1-2 % GWC (Deneff et al., 2001). This suggested that initial DRW had more profound effects on aggregate size and related processes (including decomposition of SOM and P release) than subsequent DRW; therefore these processes related to the number of preceding DRW cycles and not only SWP. In contrast,  $\text{NaHCO}_3\text{-P}$  concentrations and total P uptake by lettuce and soybean was higher from soils with larger aggregate size because of reduced P fixation in three highly weathered soils, with reduced exposure of P sorption sites (Wang et al., 2001). However, shoot biomass was also increased in these species when grown in soil with larger aggregates so it was unclear whether greater P uptake could be isolated as the cause. Soil DRW can also physically shrink soil volume, which affects soil water release characteristics and availability to plants (Gregory et al., 2010). In Experiment 2, mean soil evaporation (calculated from unplanted pots) tended to be higher in initially DRW compared to FM soil (data not shown), which could be further explored with higher replication ( $> n = 3$ ) to determine whether this was caused by altered aggregate size and resulted in reduced plant transpiration, whilst increasing yields. Therefore changes in aggregate size distribution caused by DRW, affecting P sorption and water retention, may have contributed to the greater root growth, biomass production and yields in *Brachypodium*. Since initial DRW increased biomass and yields without requiring higher water use, initial DRW also increased the WUE and WP. However, overall more water was used to replace the water lost via evaporation during initial air-drying and re-wetting. Nevertheless, at the field scale, where soil drying occurs without continuous irrigation, allowing soil to dry prior to planting a crop may not require more irrigation water to re-wet the soil compared to maintaining constant irrigation, but this would need to consider hysteresis (Whitmore and Whalley, 2009) and be tested for specific scenarios.

## Conclusions

This chapter reported two experiments aiming to determine whether soil DRW would increase P availability with realistic drying regimes. Initial soil DRW significantly increased P availability, although contrary to expectations, soil P availability was not increased over time by surface soil drying under irrigation treatments imposing drying and re-wetting from and to saturation or flooding. Under low-P conditions in Experiment 1, the greatest increase in soil P availability from pre-irrigation levels, thus highest  $\text{NaHCO}_3\text{-P}$  concentration, occurred in soil that was initially DRW then maintained under continuous flooding. Managing crops under continuous flooding is relevant to lowland irrigated (paddy) rice cultivation. Although air-drying the full root zone may not be practical at the field scale, partial (surface) soil drying occurs under AWD, and the greatest challenges to soil P availability occur in soils which are highly P-fixing (Chapter 4). Growing crop plants makes it difficult to disentangle effects of different irrigation regimes on soil P availability caused by soil dynamics and differences in plant P uptake (Yang et al., 2011), and different water use as biomass production varies. However, determining the agronomic effects of soil DRW by measuring plant biomass and yields, as well as P uptake, is also essential. Soil DRW significantly increased biomass and more than doubled grain yields in the model cereal crop *Brachypodium*, although this was likely not a direct effect of increased soil P availability and uptake since shoot TP concentrations hardly varied between the treatments. Changes in the availability of other nutrients, combined with changes in soil aggregate size, were likely important. Temporal changes in P concentrations in unplanted pots (data not shown) within control as well as drying treatments, particularly the decrease in  $\text{NaHCO}_3\text{-P}$ , emphasised the importance of the limited window in which P is available for uptake by plants (Chepwonky et al., 2001), which are in competition with other biogeochemical processes (microbial immobilisation and P sorption) (Grierson et al., 1998; Bünemann et al., 2013; Chen et al., 2016). Variation in SWP, via initial and subsequent drying and re-wetting or re-flooding cycles, affected soil P availability in two soil types and increased biomass and yields in *Brachypodium*; therefore managing P availability via irrigation

in accordance with crop demand and uptake targets may increase P efficiency and yields (Chapter 4).

## **Chapter 4: Effects of alternate wetting and drying irrigation on phosphorus availability, uptake and partitioning, and biomass and grain yields, in lowland irrigated rice (*Oryza sativa* L.)**

### **Abstract**

**Context:** Whether alternate wetting and drying (AWD) irrigation improves phosphorus (P) use efficiency of rice at the field scale, compared to continuous flooding (CF), remains uncertain.

**Hypotheses:** A field trial aimed to determine: whether AWD increased rice yields compared to CF due to increased soil P availability; and whether post-anthesis soil drying reduced luxury P uptake.

**Strategic approach:** A factorial design with three irrigation treatments (conventional continuous flooding, CF; continuous flooding until anthesis and drying thereafter, CFA; and AWD until anthesis and drying thereafter, AWD) and three P supply levels (0, 10, 25 kg P ha<sup>-1</sup>) was established on a farmer's paddy field in central Madagascar.

**Conclusions:** AWD tended to increase P uptake (by up to 60 %) whilst P fertiliser application increased rice yields (by up to 202 %).

### **Introduction**

Rice is amongst the crops providing the majority of calories globally (Fischer et al., 2014) whilst its production uses the most water after wheat, together consuming 45 % of freshwater used by 126 crops globally due to the large cropping areas as well as water footprints (Mekonnen and Hoekstra, 2010). Another estimate suggests that irrigated paddy rice, grown under flooded conditions, uses approximately 25 - 33 % of global total freshwater resources (Bouman, 2009). Additionally, rice uses the third highest share (14 %) of nitrogen (N), potassium (K) and phosphorus (P) fertilisers amongst global crops (Heffer, 2013). Therefore reducing water and fertiliser inputs in these systems without compromising yields, by alternately flooding and

drying soil to limit irrigation water input whilst stimulating P release (Chapters 2 and 3), could greatly increase water and P use efficiencies.

### **Alternate wetting and drying irrigation: principles and context**

Where soil P limits rice production and fertiliser is inaccessible, agronomic practices which increase its availability are necessary. The System of Rice Intensification (SRI) was originally developed in Madagascar to increase the productivity of agricultural systems characterised by poor soil fertility and low access to resources (Stoop et al., 2002). The SRI approach emphasises the importance of field-based research to determine how the interaction of multiple factors may be managed to maximise rice yields. In SRI, various non-conventional practices are applied in flexible combinations including: transplanting younger seedlings at lower density, applying organic fertilisers (compost and manure), and employing more frequent mechanical weeding, as well as intermittent irrigation (Stoop et al., 2002). Contrary to the SRI approach, studies aiming to improve integrated nutrient management in lowland irrigated rice production largely exclude the effects of irrigation on nutrient, including P, availability (e.g. Dobermann et al., 1998). Controlling nutrient availability by managing irrigation, meeting P and water requirements of the crop to maximise yields, is a knowledge-intensive, integrated management approach consistent with SRI. Whereas lowland irrigated rice is traditionally cultivated under continuously flooded (CF) conditions, the International Rice Research Institute (IRRI) has developed an alternative irrigation system termed alternate wetting and drying (AWD) which involves periods of intermittent flooding followed by periods of soil drying. IRRI's guidelines for applying "safe" AWD (to minimise yield loss) suggest that the water table should drop no lower than 15 cm below the soil surface (Bouman and Lampayan, 2009). The AWD technique is a main component of SRI (Stoop et al., 2002; Uphoff et al., 2011) and these water demanding rice systems require further research to determine optimal irrigation regimes for P and water use efficiencies as well as yields.

**Alternate wetting and drying effects on rice biomass and yields**

The effects of AWD on biomass production and grain yields vary widely according to when it is implemented during crop phenological development and the extent to which soil dries (Price et al., 2013; Carrijo et al., 2017). For example, compared to daily irrigation to maintain continuously flooded conditions, rice grain yields were not affected when irrigation was applied every 3 days, whereas decreased by 12 % when irrigated every 5 days (Hazra and Chandra, 2014). A meta-analysis of 56 studies, involving 528 comparisons of adjacent CF and AWD treatments, indicated that the effects of AWD on yield depended on the extent of the water deficit (Carrijo et al., 2017). Applying “safe” AWD guidelines (Bouman and Lampayan, 2009) did not limit yield while reducing water use by 23.4 %, implying improved water use efficiency. However, more severe drying (SWP < -0.02 MPa) reduced yields by 22.6 % compared to CF. Whereas AWD is often applied during the entire crop cycle (Yang and Zhang, 2010; Carrijo et al., 2017), it is sometimes applied from approximately two weeks after transplanting to the onset of flowering (Price et al., 2013; Carrijo et al., 2017). Prolonging AWD throughout the crop life cycle (spanning vegetative and reproductive stages, rather than solely during either stage) exacerbated yield losses, implying that AWD cycles had a cumulative negative impact (Carrijo et al., 2017). Nevertheless, post-anthesis soil drying can stimulate stem-to-grain carbohydrate remobilisation, improving grain-filling and harvest index (Yang and Zhang, 2006; 2010; Li et al., 2016). Thus, careful scheduling of AWD is important to maintaining optimal yields. Further study is required to determine whether increased P availability under AWD also contributes to the variable effects on grain yields.

**Alternate wetting and drying irrigation and soil phosphorus availability**

Phosphorus transformations under soil drying and re-wetting are distinctive under flooded, anaerobic conditions compared to aerobic conditions (Figure 1.3), and distinct chemical P transformations occur in alternately flooded and dried soils. Flooding decreases microbial turnover and mineralisation rates, limiting the rate and extent of P mineralisation (Turner,

2006). Intermittent irrigation allows the soil to dry out and become aerobic, increasing mineralisation and therefore P availability. It was reasoned that this soil DRW effect, accelerating turnover of organic P, may improve P uptake of rice grown under SRI compared to conventional cultivation (Turner, 2006). However, under aerobic conditions, despite higher rates of microbial activity and turnover increasing mineralisation and P availability (Turner, 2006), P fixation by oxidised iron, Fe(III), is also higher thereby lowering P availability. In contrast, flooding can increase soil pH and reduce Fe(III), releasing adsorbed P thus making it more available (Amery and Smolders, 2012). For example, in a paddy soil, flooding increased resin-P by 0.8 mg kg<sup>-1</sup> (from 2.66 to 3.45 mg kg<sup>-1</sup>, 30 %) compared to the concentration in non-flooded (aerobic) soils (Rakotoson et al., 2014). Another pot study with a different unfertilised clay soil from a lowland rice field reported 1.8-fold higher resin-P under flooded compared to saturated conditions (Huguenin-Elie et al., 2009). Thus, anaerobic conditions limit microbial P release and mineralisation whilst increasing the availability of already mineralised P, whereas aerobic conditions accelerate microbial turnover and mineralisation but fix mineralised P. By intermittently imposing both conditions, AWD may increase P availability compared to conventional, continuously flooded conditions. Although the potential contribution of large pools of organic P from soil microbial biomass in response to alternate flooding and drying was specifically mentioned as an important effect of SRI (Stoop et al., 2002), the effects of AWD in isolation from other management practices need to be determined (*cf.* Pan et al., 2015). More broadly, whilst the effects of soil DRW on P availability have been widely documented, there are few reports from lowland irrigated rice systems.

#### **Alternate wetting and drying effects on phosphorus uptake and partitioning**

Optimising rice yields in soils with low P availability is a major challenge, since increased P uptake clearly enhances rice yields (Dobermann and Fairhurst, 2000). However, luxury P uptake (above the critical concentrations that increase yields) at grain-filling represents a loss of P from the agricultural system that should be avoided (Rose et al., 2013). With sufficient available soil

P, uptake adjusts with crop phenological development according to its changing demand, with source-to-sink translocation *in planta* becoming important after flowering. At maturity, P in grain is largely stored as phytate, the role of which in the human diet is unclear, with both positive and negative effects reported (Hailelassie et al., 2016). For example, phytate can reportedly benefit human health by decreasing blood lipids and cholesterol levels and positively affecting the immune system (Shi et al., 2004), whilst phytate also binds zinc and iron, sometimes contributing to human micronutrient deficiency (Gemede, 2014). Since P supplied in food is not limiting to humans and is largely wasted, achieving high P concentrations in rice grains (grown for human consumption) without increasing yields is considered unnecessary and reduces P available for subsequent crops (Rose et al., 2013). Therefore low grain P concentration is an attractive and important plant breeding target in addressing agricultural P limitation (Rose et al., 2013). Furthermore, rice P uptake and yields strongly depend on environmental conditions (including P and other nutrient availability), thereby regulating grain P concentrations (Vandamme et al., 2016), indicating the role of agronomic management in meeting this target.

Applying AWD to increase P availability and uptake during establishment and throughout vegetative growth, whilst withholding irrigation during grain-filling to avoid luxury P uptake, may increase P use efficiency. In rice maintained under fully flooded field conditions, total P was largely sourced from P acquired from soil post-anthesis (Julia et al., 2016). The high contribution of P taken up post-anthesis to total plant P at maturity could be specific to flooded rice, at 70 % in two separate field studies (Ye et al., 2014; Julia et al., 2016) compared to 40% in upland (aerobic) rice (Rose et al., 2010). This discrepancy suggested that drying topsoil during grain-filling likely enhanced the contribution of remobilised P (taken up before and during anthesis) to grain P (Julia et al., 2016). Thus avoiding flooding during grain-filling might reduce the post-anthesis P uptake, thereby improving P use efficiency for grain yield ( $PUE = \text{grain yield} / P \text{ uptake}$ ). The P balance is the difference between P fertiliser inputs and P outputs in plant material, and is a useful indicator of P efficiency in rice production where negative

values indicate depletion of soil P reserves (Andriamananjara et al., 2016), emphasising the benefit of reducing P uptake whilst maintaining grain yields.

The effects of soil flooding on rice P concentrations are inconsistent. For example, increased resin-P concentrations in flooded compared to non-flooded soils did not increase shoot P concentrations, likely because the increase was only marginal (Rakotoson et al., 2014), whereas greater increases in P availability have occurred under pulsed redox cycles rather than continuous flooding (Scalenghe et al., 2012). Alternatively, flooding increased shoot P uptake by 2.5 to 3-fold (Huguenin-Elie et al., 2009), possibly due to the greater relative increase in resin-P, and/ or greater extraction of less soluble P forms by roots in flooded soils. Flooding also increases diffusion of P to the root surface (Dobermann and Fairhurst, 2000) by increasing the volume of soil solution. However, the SWP of -0.02 MPa which decreases rice yields (Carrizo et al. 2017) is unlikely to release significant amounts of P upon re-wetting (Chapters 2 and 3). Thus, further study of the impacts of “safe” AWD on soil P availability, uptake and rice yields is needed.

### **Objectives and Hypotheses**

Applying AWD irrigation potentially increases P availability and uptake, benefiting yields. Under high P supply allowing optimal growth and yields, withholding irrigation during grain-filling may potentially avoid luxury P uptake. Key uncertainties are: whether AWD increases soil P availability and plant P uptake in lowland irrigated rice compared to continuously flooded (CF) conditions; and whether post-anthesis soil drying during grain-filling reduces grain P concentrations whilst maintaining yields, increasing P use efficiency at the field scale. A field trial aimed to address three hypotheses:

- 1. Soil P availability will be higher under AWD compared to CF.** Microbial biomass (and thus MBP concentrations) will be stimulated by drying and re-wetting via AWD

irrigation, with P release from cell lysis and mineralisation following AWD cycles increasing soil P availability.

**2. Biomass production and grain yields will be higher under AWD compared to CF.**

This response will be due to increased soil P availability and uptake; therefore effects will be most pronounced under low P supply.

**3. Soil drying during grain-filling will decrease grain P concentrations compared to CF, without affecting yield.**

Withholding irrigation during grain-filling (via AWD and via continuous flooding only until anthesis, CFA) will reduce P availability and stimulate stem-to-grain translocation of P, thereby avoiding luxury P uptake.

## **Materials and methods**

### **Site and conditions**

A field trial was established near to the town of Behenjy, in the Andramasina region, within the central highlands of Madagascar (19°10'46.5"S, 47°29'49.4"E; 1361 masl), between October 2015 and April 2016. During this period, the region has a mean temperature of 19.3 °C (minimum 10.9 °C and maximum 25.4 °C) and mean total rainfall of 1283 mm (climate-data.org, 2017). The trial was conducted on land rented from a farmer, on which rice was the previous crop and zebu cattle had grazed subsequently. The land was prepared traditionally, by ploughing aided by a zebu. The soil is a clay soil of the Gleysol group, characterised by saturation for extended periods resulting in reducing conditions (IUSS Working Group WRB, 2015). A bulked soil sample was taken from the field site and air-dried prior to further analysis of soil physical and chemical characteristics. The main properties were a clay content of 40 %, total carbon (C) and nitrogen (N) contents of 2.10 % and 0.16 % respectively, total P content of 285 mg kg<sup>-1</sup>, and pH 4.8 (see Table 4.4 for further soil properties). Before transplanting, a soil sample was taken at 0-15 cm depth in the middle of each sub-plot and bulked into one composite sample per main block (replication), resulting in four composite soil samples which were

analysed by research staff at the Laboratoire des Radioisotopes (LRI), Antananarivo. Mean soil organic carbon (SOC) was 29.95 g kg<sup>-1</sup> and cation exchange capacity (CEC) was 2.27 cmol kg<sup>-1</sup> (therefore classified as low; Dobermann and Fairhurst, 2000). The P sorption index (PSI) is the amount of P adsorbed to the solid phase at a given soil solution P concentration (0.2 mg P L<sup>-1</sup>) (Six et al., 2013). The soil at the Behenja site had a PSI of 367 mg P kg<sup>-1</sup> (T. Rakotoson, unpublished), and was therefore considered a high P sorbing soil (Sanchez and Goro, 1980).

## **Experimental design**

### **Treatments and layout**

A split-plot design was used, with water supply (managed with irrigation and drainage) comprising the main plots and P supply as the sub-plot treatment. Sub-plots were 3 x 4 m, with 0.8 m spacing between plots, and there were four replications. After ploughing the field, plots were marked out and the bunds and irrigation and drainage channels created. Nitrogen (N) and potassium (K) were applied to attain optimal levels, as urea (CH<sub>4</sub>N<sub>2</sub>O) and potassium chloride (KCl) respectively, as follows: at transplanting 30 kg N ha<sup>-1</sup> and 25 kg K ha<sup>-1</sup>; at maximum tillering (2-3 weeks before panicle initiation) 25 kg N ha<sup>-1</sup>; and at panicle initiation 25 kg N ha<sup>-1</sup> and 25 kg K ha<sup>-1</sup>. The local rice genotype X265 was selected for its tolerance of a wide range of soil moisture contents (Rabeharisoa et al., 2012) including flooded and non-flooded conditions (Rakotoson et al., 2015) and promotion in the region by agricultural support programmes (FAO/ WFP, 2016). Seeds were sown on the 22<sup>nd</sup> of October in a seedbed adjacent to the plots with the same soil type amended with organic manure, then transplanted on the 20<sup>th</sup> of November at two seedlings per hill (planting space within a row) at the two-leaf stage at 20 cm spacing between hills. These are common local practices to promote tillering and are also part of SRI (Stoop et al., 2002), except for the extended period between sowing and transplanting (29 days after sowing - DAS) due to slow growth caused by cold weather in the trial. Weeds were manually removed in the early growth stages with uniform intensity. The experimental layout is illustrated in Figure 4.1.

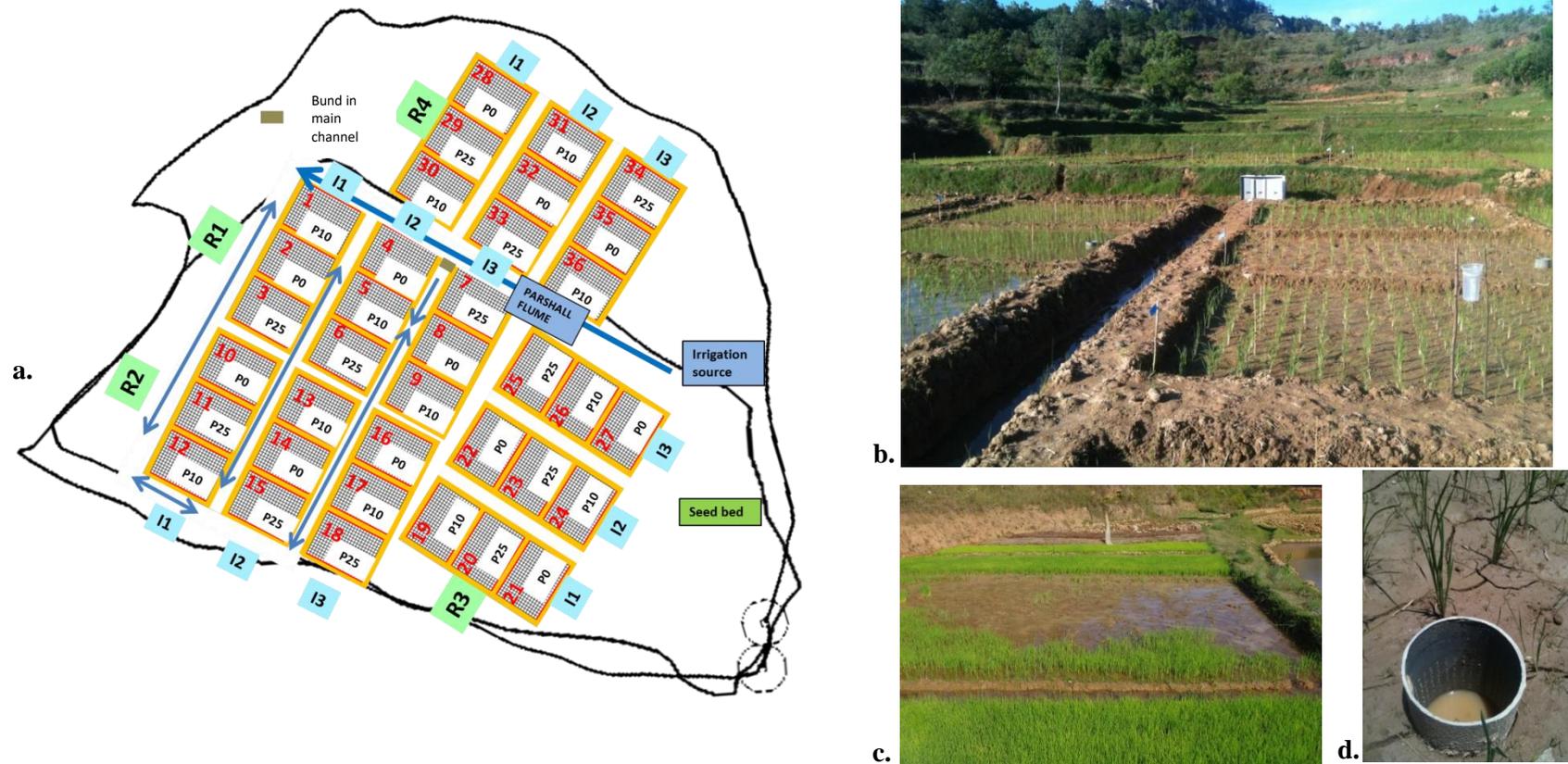


Figure 4.1: Experimental layout and photographs. (a) Illustrates the layout and key features (adapted from Dr. Arisoa Rajaona, unpublished), where R1-4 are the four replications, I1-3 are the continuous flooding (CF), continuous flooding until anthesis (CFA) and alternate wetting and drying (AWD) irrigation regimes, respectively, and P 0, 10 and 25 are the P fertiliser application rates ( $\text{kg ha}^{-1}$ ); (b) is a photograph showing part of the layout and an irrigation/ drainage channel (note CF vs AWD plots); (c) is a photograph of the seed bed taken during transplanting; and (d) is a photograph of one of the perforated tubes installed in an AWD plot to measure the water table height.

## **Irrigation**

Three irrigation treatments were applied: continuous flooding throughout the crop cycle (CF), continuous flooding until the end of anthesis with no irrigation thereafter (CFA), and alternate wetting and drying from establishment (43 DAS; 14 days after transplanting, DAT) until the end of anthesis with no irrigation thereafter (AWD) (Figure 4.2). Maintaining continuously flooded conditions initially, from 0 to 14 DAT, also allowed uniform diffusion of the fertiliser granules within the irrigation treatments.

Principal Growth Stage	0		1		2		3		4		5		6		7		8		9	
	Germination		Leaf development		Tillering		Stem elongation		Booting		Inflorescence emergence, heading		Anthesis		Fruit development		Ripening		Senescence	
CF	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Open	Open
CFA	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Shaded	Open	Open	Open	Open	Open	Open	Open	Open
AWD	Shaded	Shaded	Open	Open	Shaded	Open	Shaded	Open	Shaded	Open	Shaded	Open	Shaded	Shaded	Open	Open	Open	Open	Open	Open

Figure 4.2: Irrigation treatments. Based on the phenological growth stages and Biologische Bundesantalt, Bundessortenamt and Chemische Industrie (BBCH)-identification keys of rice (*Oryza sativa* L.); Lancashire et al., 1991.

*Note: Flooded and drained periods are represented by shaded and open cells, respectively. AWD treatment within a growth period is represented by occurrence of a shaded and open cell. Column width does not represent relative time of growth stage.*

Within the AWD periods, IRRI's recommendations for drainage and re-flooding to manage "safe" AWD were followed (Bouman and Lampayan, 2009), with wet (anaerobic) periods maintaining a standing water depth of approximately 5 cm, and dry (aerobic) periods allowed to dry, or intentionally drained (by opening the bund), to a soil water depth of 15 cm below the soil surface. Perforated plastic tubes (diameter 140 mm) were installed in one plot for the CF and CFA water treatments and each plot for the AWD treatment, and water level recorded daily (Figure 4.1d). Water treatments were managed at the sub-plot level via irrigation and drainage channels. To prevent lateral water seepage from the CF and CFA to AWD plots, the main plots were separated by a subsurface layer of plastic placed vertically at the edges to approximately 50 cm depth. The irrigation application procedures are further described in Table 4.1.

Table 4.1: Irrigation application procedures.

Treatment	Directions
CF	CF with a water layer of 5 cm maintained by daily irrigation as needed.
CFA	CF with a water layer of 5 cm maintained by daily irrigation as needed until one week after 50 % flowering. The plots were then drained daily until maturity, and allowed to drain naturally even if the depth dropped below 15 cm.
AWD	From establishment onwards up to the onset of flowering (first appearance of flowers), 2 weeks of CF was alternated with a period of drainage when plots were drained continuously (via opening bunds to drainage channels) until a water depth of 15 cm below the soil surface was reached; periods of drainage did not exceed 2 weeks. From the onset of flowering until 1 week after 50 % flowering, CF with a water layer of 5 cm was maintained by irrigation as needed to avoid adverse impacts of drying on yields. From 1 week after 50 % flowering onwards, the plots were drained daily until maturity.

### Phosphorus supply

Initial soil tests were carried out by T. Rakotoson in May 2015 (unpublished) in order to select a suitable site for the trial. An initial soil test showed that the field site had Olsen P of 3.0 mg kg<sup>-1</sup> (SE = 0.3; n = 2) and resin-P of 6.0 mg kg<sup>-1</sup> (SE = 0.1; n = 2); therefore rice would highly likely respond to increased available P according to the IRRI nutrition guide (Fairhurst et al., 2007). Previous field experiments on P deficient paddy soils in Madagascar showed that under CF, good yields and optimal P balance were achieved when 20 kg P ha<sup>-1</sup> was supplied, whilst the P balance was inefficient at higher rates (40 and 80 kg P ha<sup>-1</sup>) (Andriamananjara et al., 2016). To determine whether potential increases in soil P availability caused by AWD were sufficient to mitigate P deficiency, three rates of inorganic P fertiliser (0, 10 and 25 kg ha<sup>-1</sup>) were supplied

at transplanting, as triple superphosphate (TSP) (supplied by SEPCM, Antananarivo) (Table 4.2).

Table 4.2: Phosphorus treatments and descriptions.

<b>ID</b>	<b>P application rate (kg P ha<sup>-1</sup>)</b>	<b>Description</b>
P-0	0	Low P:  P supplied from soil only.
P-10	10	Intermediate P:  “Maintenance” P application to offset P removal with grains, based on anticipated grain yield of 4-5 t ha <sup>-1</sup> and P removal of 2-3 kg ha <sup>-1</sup> per ton of grain yield (Dobermann and Fairhurst, 2000).
P-25	25	Optimal P:  Sufficient for optimal growth and yields.

### **Sampling procedures**

The outer two rows of border plants were avoided during sampling. Therefore the net plot size was 2.2 x 3.2 m with a maximum of 176 hills per plot.

### **Soil sampling**

Soil samples for P analyses were taken twice from each plot: before any nutrient or water amendments (referred to as before flooding, BF); and at flowering (FL), which occurred at different times according to the P supply. Samples were taken at 0-15 cm depth by bulking sub-samples from five points (along an imagined “X” design; Abawi and Gugino, 2007) within the

plot. Samples were sealed in plastic bags to maintain field moisture contents and stored at approximately 4 °C until preparation and analysis.

### **Plant sampling**

Plant sampling, preparation and analytical procedures were conducted by staff and students at LRI. The highest P fertiliser rate (P-25) accelerated phenological development compared to the other P rates across irrigation treatments, so these plants were sampled at the first of two final harvest times. Within each sampling event (covering all P treatments), two sub-samples allowed detailed measurements of yield components; then the remaining net plot was also sampled. Table 4.3 summarises the parameters measured for each sub-sample.

Table 4.3: Parameters measured in different sub-samples taken at the final harvest.

Sub-sample	Number of hills	Parameters measured
1	10 Randomly selected.	Growth components: <ul style="list-style-type: none"> <li>- Plant height;</li> </ul> Yield components: <ul style="list-style-type: none"> <li>- Number of panicles per hill;</li> <li>- Number of grains per hill (filled and empty);</li> <li>- Oven-dry weight of 1000 grains.</li> </ul>
2	6 x 6 In a 1.2 x 1.2 m area with no missing hills.	Yield components: <ul style="list-style-type: none"> <li>- Grain P concentration;</li> <li>- Straw P concentration;</li> <li>- Air-dried total grain weight;</li> <li>- Air-dried filled grain weight after winnowing;</li> <li>- Grain moisture content (oven-dried);</li> <li>- Oven-dried straw weight;</li> <li>- Harvest index.</li> </ul>
Remaining net plot	130 Maximum.	Yield components: <ul style="list-style-type: none"> <li>- Number of missing hills;</li> <li>- Air-dried total grain weight.</li> </ul>

### **Plant sampling for phosphorus concentration**

At flowering, plants of two neighbouring hills at two random locations in the plot (resulting in four harvested hills, representative of the whole plot assuming a similar mean tiller number for the respective treatment) were cut and stored together in a sampling bag. The plants were dried, weighed, ground and shoot P concentration determined. At maturity, the straw and grains from sub-sample 2 were separately prepared for P analysis.

### **Plant sampling for yield parameters**

At maturity, the numbers of missing and harvested hills were recorded. To harvest, 10 hills were randomly selected and plants were cut at the soil level. The panicles were separated from the remaining biomass and the numbers of panicles in the sub-samples were recorded. Subsequently, the spikelets were separated (from the peduncle and rachis) and stored in a paper bag. The peduncles and the rachis were added to the plant stem and leaves, and the total (above-ground) biomass other than grains (hereafter referred to as 'straw') was stored in a paper bag. For all remaining panicles, spikelets were separated from the peduncle and rachis. Unfilled grains were removed from the grains by winnowing. Moisture contents were determined with a grain moisture meter.

### **Sample analyses**

#### **Soil sample preparation**

Soil sample preparation and analytical procedures were conducted at the Lancaster Environment Centre. Soil samples were passed through a 2 mm sieve, with stones and plant roots removed. Gravimetric water contents were determined by drying two replicates per sample to constant weights in an oven at 35 °C for 24 hours and then 105 °C for 24 hours, for air-dried and oven-dried water contents respectively, and calculating the replicate mean. This procedure was repeated if the difference between the replicates was greater than 10 % of the water content

(occurring on two occasions). All analyses were conducted on soils at their sampled GWCs (20.4 to 39.2 %) in triplicate and are expressed on a dry weight equivalent (DWE) basis.

### **Soil phosphorus concentrations**

#### **Microbial biomass phosphorus (MBP)**

Microbial biomass P (MBP) was measured via hexanol fumigation and extraction with anion exchange resin membranes, as described in Chapter 2. The 16 soil samples comprising the most extreme treatments (CF and AWD; P-0 and P-25;  $n = 4$ ) from both sampling times (BF and FL) were analysed.

#### **Water-extractable total phosphorus (TP), molybdate reactive phosphorus (MRP) and molybdate unreactive phosphorus (MUP)**

Water-extractable TP, MRP and MUP concentrations were determined as described in Chapter 2, although for MRP using malachite green reagent and a plate reader at 625 nm absorbance (as Chapter 3). The following modifications were made: due to higher moisture contents, samples were shaken at 200 rpm for greater agitation, and the samples taken at flowering (FL) were centrifuged for 15 minutes.

#### **Sodium bicarbonate extractable phosphorus ( $\text{NaHCO}_3\text{-P}$ )**

$\text{NaHCO}_3\text{-P}$  was measured as described in Chapter 2, using malachite green reagent and a plate reader at 625 nm absorbance.

#### **Measurement using Diffusive Gradient in Thin Films (DGT-P)**

DGT-P concentrations were determined in soil samples following the established protocol (Zhang, 2010). Soil samples were prepared by weighing 25 g DWE (BF samples,  $n = 1$ ) and 60 g DWE (FL samples,  $n = 3$ ) of field-moist soils into pots and brought to 80 - 100% of water holding capacity with Milli-Q water. Samples were left sealed in this condition (as “slurries”)

for 24 hours to equilibrate. Slurries were then transferred to petri-dishes and spread evenly to ensure uniform depth and a smooth surface. Assembled DGT devices (DGT® Research, UK) each comprising a membrane filter covering a 0.08 cm diffusive gel overlying a 0.06 cm Fe-oxide binding gel within a plastic housing, were deployed on the samples. These DGT devices were rinsed with Milli-Q water before a small amount of the sample was spread on the surface (filter membrane), to ensure contact when deployed gently using a twisting action. The room temperature was recorded. Deployed DGTs were placed on moistened blue roll and covered with clean plastic sheets and a plastic box, to maintain moisture. After 26 hours, DGTs were eluted by rinsing the surface to remove soil particles, breaking open the plastic casing, discarding the filter paper and diffusive membrane, and carefully placing each Fe-oxide membrane (using clean plastic tweezers) into a separate Eppendorf tube containing 1 mL of 0.25 M H<sub>2</sub>SO<sub>4</sub>. These samples were left at least overnight, then the eluates were analysed using malachite green reagent and a plate reader (Multiskan™ GO Microplate Spectrophotometer, ThermoFisher Scientific, USA) at 625 nm absorbance. DGT-P concentrations were calculated according to the equations described by Zhang (2010), first determining the mass of P accumulated by the Fe-oxide gel (M):

$$M = C_e (V_{\text{acid}} + V_{\text{gel}}) / fe$$

Where:

$C_e$  is the concentration of P in the 0.25 M H<sub>2</sub>SO<sub>4</sub> elution solution;

$V_{\text{acid}}$  is the volume of 0.25 M H<sub>2</sub>SO<sub>4</sub> elution solution added to the Fe-oxide gel;

$V_{\text{gel}}$  is the volume of the Fe-oxide gel;

$fe$  is the elution factor for P (= 1).

The concentration of P measured by DGT ( $C_{\text{DGT}}$ ) was then calculated:

$$C_{\text{DGT}} = M \Delta g / (DtA)$$

Where:

$\Delta g$  is the thickness of the diffusive gel plus the thickness of the filter membrane (0.08 cm + 0.014 cm);

$D$  is the diffusion coefficient of phosphate in the gel (based on temperature);

$t$  is deployment time;

$A$  is exposure area (=3.14 cm<sup>2</sup>).

Additionally, since air-drying affects soil P availability, the effects of air-drying prior to deploying the DGT devices (as usually applied in the literature, e.g. Mason et al., 2010) was compared with deployment on field-moist (in some cases near-saturated) soil, since it was important to determine DGT-P concentrations for soils under field-moist conditions. Four soil samples were selected (20.4 % to 25.5 % GWC), representing each replication and based on greatest sample availability, and were analysed in triplicate. Samples were air-dried at 35 °C for 48 hours until constant weight, then brought to 50 - 60 % WHC with deionised water and left for 48 hours to equilibrate, as per the standard protocol. Replicates samples were analysed at field moisture contents. However, the DGT-P concentrations were below the detection limit.

## **Plant samples**

### **Plant tissue phosphorus concentrations**

The straw sub-samples were oven-dried at 65 °C for 3-5 days (until constant weight), and sub-sample straw dry weight was determined. The grain sub-sample was stored pending grain separation (filled, partially filled and unfilled grains). Total P concentrations were determined separately for grain and straw samples at LRI by acid digestion followed by P determination via spectroscopy, using a spectrophotometer.

**Biomass and yield**

Total grain weight was measured and grain moisture content was determined with a grain moisture meter. Grain yields at 14 % moisture content were calculated following convention, as a more realistic measure than oven-dried weights for the rice grains that are harvested, sold and bought (E. Vandamme, pers. comm., 25/10/2017).

**Calculations**

The following calculations were made as indicators of agronomic productivity and efficiency:

$$\text{Grain P content (kg ha}^{-1}\text{)} = \text{grain P concentration} * \text{grain yield at 14 \% moisture content}$$

$$\text{Harvest index (HI)} = \text{grain yield} / \text{total shoot biomass (grain + straw)}$$

$$\text{Total P uptake (kg ha}^{-1}\text{)} = \text{grain P content} + (\text{straw P concentration} * \text{straw biomass})$$

$$\text{P use efficiency (PUE) for grain yield (kg ha}^{-1}\text{)} = \text{grain yield at 14 \% moisture content} / \text{total P uptake}$$

$$\text{P balance (kg ha}^{-1}\text{)} = \text{P concentration applied} - \text{total P uptake}$$

## Data analysis

All data were analysed using SPSS version 23 and R Studio. In all cases, a confidence threshold of 0.05 was applied. Where relevant, all data transformation was log base 10 and for all bootstrap analysis,  $n = 1000$ . Two-way ANOVA determined the effects of the P and irrigation treatments and their interactions on soil and plant P concentrations and biomass and yields. For the initial P concentrations, transformation for MRP and DGT-P, and bootstrap analysis for  $\text{NaHCO}_3\text{-P}$  and MBP were performed. For the P concentrations at anthesis, transformation for  $\text{NaHCO}_3\text{-P}$  and MBP, and bootstrap analysis for TP, MRP, MUP and DGT-P were performed. Related-samples Wilcoxon signed rank tests determined whether P concentrations significantly differed between sampling times. For the plant P variables, transformation for grain P concentration and content and PUE for grain yield at maturity, and bootstrap analysis for the shoot P concentration at anthesis, straw P concentration at maturity and the P balance were performed. Linear regression determined the significance (or otherwise) of relationships between grain P content and total P uptake, and grain weight and grain P concentration. ANCOVA determined whether these relationships were significantly affected by P level or irrigation treatment. Logarithmic regression determined the significance (or otherwise) of relationships between soil  $\text{NaHCO}_3\text{-P}$  concentration and shoot P concentration at anthesis. Pearson's correlation was used for total shoot biomass and total P uptake. One-way ANOVA with Tukey's pairwise tests determined differences between the P\*I treatment combinations for grain yield, straw biomass, total P uptake and the P balance.

## Results

The major soil properties for the Madagascar soil are outlined in Table 4.4.

Table 4.4: Major soil physical and chemical properties for the field soil. Analyses were carried out by NRM Laboratories, UK and Rothamsted Research, UK.

Soil property	Result	Units	Method
	Madagascar		
<b>Texture class</b>	Clay		Particle size distribution via laser diffraction
<b>Sand</b>	27	% w/w	
<b>Silt</b>	33	% w/w	
<b>Clay</b>	40	% w/w	
<b>Organic Matter</b>	9.4	% w/w	Loss on ignition (LOI)
<b>pH</b>	4.8		In water (1:2.5)
<b>Total C</b>	2.10	% w/w	Combustion catalytic oxidation
<b>Total N</b>	0.16	% w/w	
<b>C:N Ratio</b>	13.1:1		
<b>Total P</b>	285	mg kg <sup>-1</sup>	Aqua-regia soluble elements : HCl and HNO <sub>3</sub> digestion with analysis via ICP-OES
<b>Total K</b>	175	mg kg <sup>-1</sup>	
<b>Total Mg</b>	392	mg kg <sup>-1</sup>	
<b>Total Mn</b>	65	mg kg <sup>-1</sup>	
<b>Total Fe</b>	33667	mg kg <sup>-1</sup>	
<b>Available Fe</b>	115	mg L <sup>-1</sup>	DTPA extraction, or dithionite/ ammonium oxalate extraction (Tadham Moor)

### Soil phosphorus concentrations

Pre-treatment P concentrations were first measured per plot, to determine there were no systematic differences across the field before the treatments were implemented. Soil P availability was very low and did not differ significantly according to the phosphorus level (P) or irrigation regime (I) treatments established after this initial soil sampling (Table 4.5). When sampled at anthesis, there were also no significant effects of P level, irrigation treatment and their interaction on soil P concentrations. Furthermore, the plant-available NaHCO<sub>3</sub>-P and resin-

P concentrations did not change between the sampling times. These results indicated that the applied P fertiliser had been assimilated by plants or soil microbes, or sorbed to soil minerals. Between the sampling times, MRP increased over two orders of magnitude whilst MBP decreased by one-third (Table 4.5), possibly indicating that the microbial biomass was partially a source of released MRP. The DGT-P concentrations were below the limit of detection (data not shown). Thus available P concentrations were very low and the greatest treatment effect measured at anthesis was increased MRP with decreased MBP across all P levels and irrigation regimes.

Table 4.5: Mean P concentrations of soil samples taken pre-treatment and at anthesis. For resin-P and MBP, only the P-0 and P-25 (P) and CF and AWD (I) levels were analysed. Data are means ( $\pm$  SE) ( $\text{NaHCO}_3\text{-P}$  and MRP:  $n = 36$ ; TP and MUP:  $n = 17$ ; resin-P and MBP:  $n = 16$ ). Different letters indicate significant differences between the sampling times for each P form according to related-samples Wilcoxon signed rank tests ( $p < 0.001$ ).

P form	P concentration ( $\text{mg kg}^{-1}$ )	
	Pre-treatment	Anthesis
TP	NA	1.50 (0.19)
MRP	0.04 (0.01) b	1.50 (0.24) a
MUP	NA	0.48 (0.09)
$\text{NaHCO}_3\text{-P}$	1.19 (0.05) a	1.36 (0.10) a
Resin-P	0.51 (0.03) a	0.54 (0.02) a
Microbial biomass P (MBP)	4.28 (0.37) a	2.85 (0.16) b

### Plant biomass and yields

At harvest, grain yield increased with P fertiliser application (Table 4.6), with a 202 % variation between the lowest (P-0, CF) and the highest (P-25, CFA) treatments (Figure 4.3). In contrast, supplemental P fertiliser did not increase the straw biomass, which varied by 144 % and 124 % between the lowest (P-10, CF) and the highest (P-0, CFA and P-25, AWD) treatments (Figure 4.4). Grain yield was not affected by irrigation (Table 4.6; Figure 4.3) whilst straw biomass was increased by AWD and CFA compared to CF but only across P levels (Table 4.6; Figure 4.4). The HI (grain yield / total shoot biomass) was not affected by irrigation but increased with supplemental P fertiliser by 83 % (P-10) and 70 % (P-25), compared to unamended soil (averaged across irrigation treatments) (Table 4.6). Higher HI at P-10 was likely because of the lower straw biomass at P-10 under CF compared to P-25 under AWD (Figure 4.4). Thus, grain yield was only enhanced by increasing P application whilst the highest HI occurred at the intermediate P level, without significant irrigation effects.

Table 4.6: Main effects of P level (P), irrigation regime (I) and their interaction on rice growth and yields according to two-way ANOVA (n = 36). Data are from oven-dry weights and grain yield was determined at 14 % moisture content. Treatment differences are: not significant (ns),  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*) and  $p \leq 0.001$  (\*\*\*).

Model	Significance of effect of model term on biomass and grain yield			
	Shoot biomass (kg ha <sup>-1</sup> )	Straw biomass (kg ha <sup>-1</sup> )	Grain yield (kg ha <sup>-1</sup> )	Harvest index
<b>P level</b>	***	**	***	***
<b>Irrigation</b>	**	**	ns	ns
<b>P*I</b>	ns	ns	ns	ns

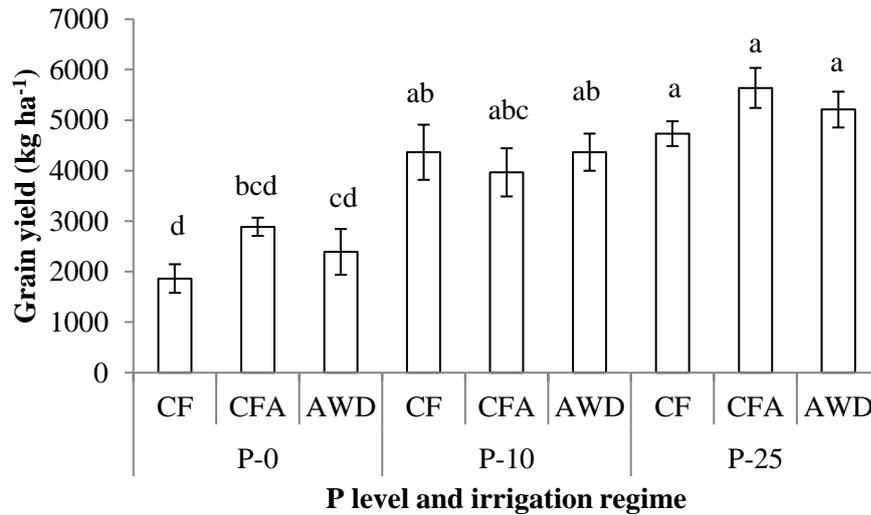


Figure 4.3: Effects of P level and irrigation regime on total grain yield. The P levels are application rates of 0, 10 and 25 kg P ha<sup>-1</sup> respectively; irrigation regimes are continuous flooding (CF), continuous flooding only to anthesis (CFA), and alternate wetting and drying to anthesis (AWD). Data are means ( $\pm$  SE) (n = 4). Different letters represent significant differences between the treatments according to one-way ANOVA with Tukey's pairwise test ( $p \leq 0.05$ ).

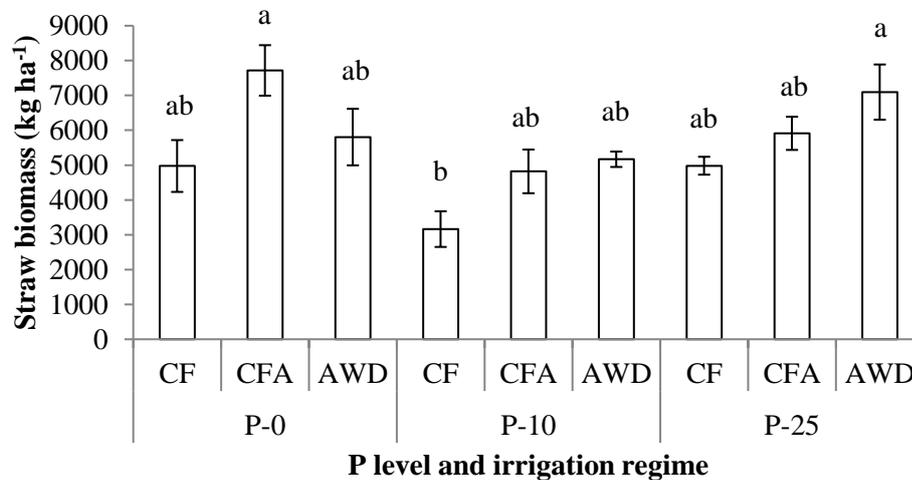


Figure 4.4: Effects of P level and irrigation regime on total straw biomass. The P levels are application rates of 0, 10 and 25 kg P ha<sup>-1</sup> respectively; irrigation regimes are continuous flooding (CF), continuous flooding only to anthesis (CFA), and alternate wetting and drying to anthesis (AWD). Data are means ( $\pm$  SE) (n = 4). Different letters represent significant differences between the treatments according to one-way ANOVA with Tukey's pairwise test ( $p \leq 0.05$ ).

**Phosphorus uptake by rice**

Grain yield and shoot biomass determined variation in P uptake, since P content but not concentration was affected by the P level (Table 4.7). Furthermore, grain P content was significantly correlated (positively) with grain yield ( $R = 0.842$ ,  $n = 35$ ,  $p < 0.001$ ) and the total shoot biomass ( $R = 0.563$ ,  $n = 35$ ,  $p < 0.001$ ). Grain P content increased with supplemental P fertiliser by 106 % (P-10) and 169 % (P-25) compared to unamended soil, averaged across irrigation treatments. Total P uptake was affected by irrigation as well as P (Table 4.7), tending to increase under AWD (and CFA) compared to CF, by up to 60 % (Figure 4.5). However, total P uptake was only significantly increased by P fertiliser at P-25 under AWD, compared to plants at P-0 under CF (by 214 %) and AWD (by 106 %), and at P-10 under CF (by 84 %) (Figure 4.5). This was also driven by the total shoot biomass (Figures 4.3 and 4.4 combined) as emphasised by the significant positive correlation ( $R = 0.671$ ,  $n = 34$ ,  $p < 0.001$ ). Therefore grain P content and total P uptake increased with P application, with irrigation only significantly affecting P uptake across P levels.

The P use efficiency (PUE) for grain yield was significantly affected by the P level (Table 4.7). In contrast to grain yield and P content, the highest PUE occurred in plants at the P-10 level, which was 37 % higher than for plants at P-0 and 2 % higher than plants at P-25. This was likely caused by the higher total P uptake at P-25 under AWD than at P-10 under CF (Figure 4.5), whilst grain yield did not significantly vary between these P levels when analysed between irrigation regimes (Figure 4.3). Thus, PUE was likely affected by differences in P uptake occurring between P levels and irrigation treatments, and was highest at the intermediate P level.

The P balance is the amount of applied P taken up by the plants, or in the case of P-0 application the amount of P depletion from soil P reserves. The mean P balance varied by 369 % from -6.7 to 17.9 kg ha<sup>-1</sup> and significantly increased with P application (Figure 4.6). The P balance tended to be highest under CF at all P levels, and lowest under CFA at P-0 whereas AWD at P-10 and P-25, explaining the significant interaction between P level and irrigation regime (Table 4.7;

Figure 4.6). Therefore the P balance increased with P application whilst was not affected by irrigation.

Table 4.7: Main effects of P level (P), irrigation regime (I) and their interaction on P uptake according to two-way ANOVA (n = 36) or with transformation (log base 10) or bootstrap analysis (n = 1000). P balance is (P applied - total P uptake), P use efficiency (PUE) for grain yield is (grain yield / total P uptake) and conc. is concentration. Treatment differences are: not significant (ns),  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*) and  $p \leq 0.001$ .

Model	Significance of effect of model term on P uptake						
	Anthesis	Harvest					
	Shoot P conc. (mg kg <sup>-1</sup> )	Straw P conc. (mg kg <sup>-1</sup> )	Grain P conc. (mg kg <sup>-1</sup> )	Grain P content (kg ha <sup>-1</sup> )	Total P uptake (kg ha <sup>-1</sup> )	PUE for grain yield (kg ha <sup>-1</sup> )	P balance (kg ha <sup>-1</sup> )
<b>P</b>	ns	ns	ns	***	**	*	***
<b>I</b>	ns	ns	ns	ns	*	ns	ns
<b>P*I</b>	ns	ns	ns	ns	ns	ns	***

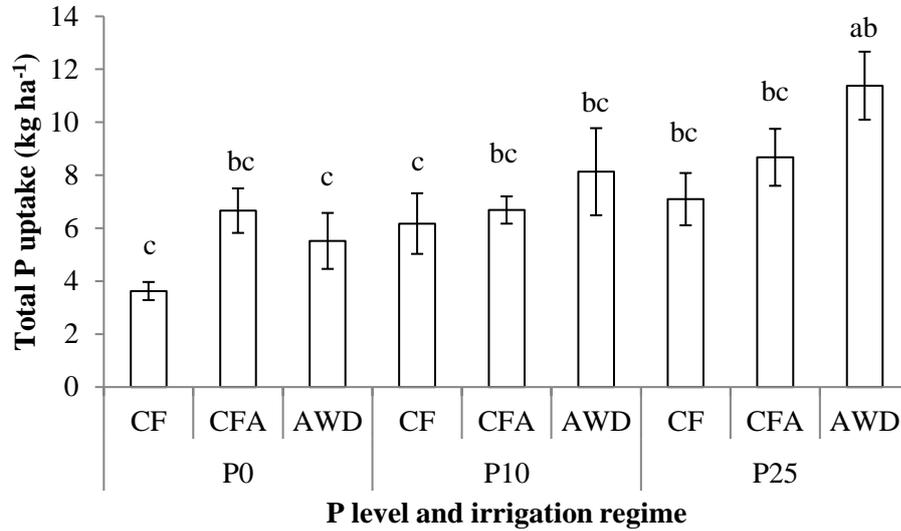


Figure 4.5: Effects of P level and irrigation regime on total P uptake (grain + straw P contents). The P levels are application rates of 0, 10 and 25 kg P ha<sup>-1</sup> respectively; irrigation regimes are continuous flooding (CF), continuous flooding only to anthesis (CFA), and alternate wetting and drying to anthesis (AWD). Data are means ( $\pm$  SE) (n = 4). Different letters represent significant differences between the treatments according to one-way ANOVA with Tukey's pairwise test ( $p \leq 0.05$ ).

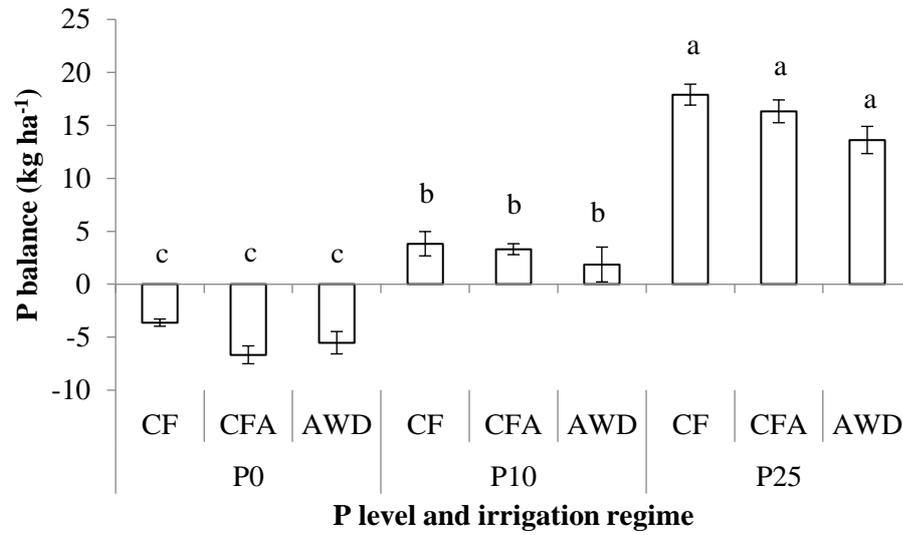


Figure 4.6: Effects of P level and irrigation regime on P balance (P applied – total P uptake). The P levels are application rates of 0, 10 and 25 kg P ha<sup>-1</sup> respectively; irrigation regimes are continuous flooding (CF), continuous flooding only to anthesis (CFA), and alternate wetting and drying to anthesis (AWD). Data are means ( $\pm$  SE) (n = 3-4). Different letters represent significant differences between the treatments according to one-way ANOVA with Tukey's pairwise test ( $p \leq 0.05$ ).

The significant positive linear regression between grain P content and total P uptake ( $R^2 = 0.822$ ,  $p < 0.001$ ) did not vary according to P level or irrigation regime, indicating that neither treatment significantly affected P partitioning to the grain. Furthermore, variation in grain weight according to grain P concentration did not differ according to the P level or irrigation regime. Therefore altering the P supply or irrigation regime did not decrease P concentrations whilst maintaining grain yields.

### **Soil phosphorus concentrations as predictors of rice phosphorus uptake**

At anthesis, the P application did not affect soil  $\text{NaHCO}_3\text{-P}$ , or shoot P concentrations (Table 4.7). Nevertheless, shoot P concentration at anthesis increased logarithmically as  $\text{NaHCO}_3\text{-P}$  decreased ( $R^2 = 0.338$ ,  $p < 0.001$ ), suggesting that this pool of available P contributed to total P uptake and the increased shoot biomass and P balance (Figures 4.3 - 4.6). Therefore  $\text{NaHCO}_3\text{-P}$  measured at anthesis was statistically equivalent to the pre-treatment concentration (Table 4.5), and at P-0 the P balance was negative (Figure 4.6), suggested that this pool was replenished from soil P reserves replacing P taken up by the plants.

In summary, soil P availability was very low in the field and P fertiliser application at the rates of 10 and 25  $\text{kg ha}^{-1}$  did not increase soil P concentrations measured at anthesis. This was likely due to sorption to soil minerals as well as assimilation by plants, because there was little variation in total P uptake between the P levels except the increase at P-25 under AWD (Figure 4.5). Grain yields were limited by P availability and were only enhanced by increased P fertiliser application (Figure 4.3), whilst the highest HI occurred at the intermediate P level (Table 4.6; Figures 4.3 and 4.4). Similarly, whilst grain P content increased with the P fertiliser rate, the highest PUE also occurred at the intermediate P level, without significant irrigation effects. Thus, applying P fertiliser had the greatest effect on grain yields, and AWD increased total P uptake compared to CF but only between P levels. Key results are summarised in Figure 4.7.

Figure 4.7: Summary of key effects of P level (P) and irrigation regime (I) on: (A) soil P availability, (B) biomass and yields, and (C) rice P uptake. Different colours represent the percent change as described. The corresponding text shows the treatment (P or I) or combination (P\*I) with maximum change from the lowest level (P-0 or CF) and all significant changes were increases.

Percent change	
100 +	
50 –99	
1 – 49	
No significant difference from P-0 or CF	

### 1.

#### Hypothesis:

Soil P availability will be higher under AWD compared to CF.

#### Contrary to the hypothesis:

Soil P availability and MBP concentrations did not differ according to the irrigation treatment.

(A) SOIL PHOSPHORUS CONCENTRATIONS (mg kg <sup>-1</sup> )						
Treatment	Water-extractable P			Plant-available P		Microbial P
	TP	MRP	MUP	NaHCO <sub>3</sub> -P	Resin-P	MBP
Phosphorus level						
Irrigation regime						

## 2.

**Hypothesis:**

Biomass production and grain yields will be higher under AWD compared to CF.

**Consistent with the hypothesis:**

Biomass production was higher under AWD compared to CF, with 25 kg ha<sup>-1</sup> P fertiliser supplied.

**Contrary to the hypothesis:**

Grain yields only increased with P fertiliser.

<b>(B) PLANT BIOMASS PRODUCTION AND YIELDS</b>			
<b>Treatment</b>	<b>Biomass</b>	<b>Yield</b>	<b>Efficiency</b>
	<b>Straw (kg ha<sup>-1</sup>)</b>	<b>Grain (kg ha<sup>-1</sup>)</b>	<b>Harvest Index</b>
<b>Phosphorus level</b>	<b>P0, CFA</b>	<b>P-25</b>	<b>P-10</b>
<b>Irrigation regime</b>	<b>and P-25, AWD</b>		

## 3.

**Hypothesis:**

Soil drying during grain-filling will decrease grain P concentrations compared to CF, without affecting yield.

**Contrary to the hypothesis:**

Grain P concentrations were not affected by the irrigation regime.

<b>(C) PHOSPHORUS UPTAKE BY RICE</b>							
<b>Treatment</b>	<b>Anthesis</b>		<b>Maturity</b>				
	<b>Concentrations (mg kg<sup>-1</sup>)</b>			<b>Content (kg ha<sup>-1</sup>)</b>	<b>P uptake and efficiency (kg ha<sup>-1</sup>)</b>		
	<b>Shoot P</b>	<b>Straw P</b>	<b>Grain P</b>	<b>Grain P</b>	<b>Total P uptake</b>	<b>P balance</b>	<b>PUE for grain yield</b>
<b>Phosphorus level</b>				P-25	P-25	P-25	P-10
<b>Irrigation regime</b>				AWD			

## Discussion

### **Alternate wetting and drying irrigation and soil phosphorus availability**

The field site was selected for low available P concentrations, which both the pre-treatment and the P-fertilised soil samples confirmed. Soil containing less than 5 mg kg<sup>-1</sup> Olsen P has been classified as infertile for rice production (Dobermann and Fairhurst, 2000), and although this classification is based NaHCO<sub>3</sub> extraction of air-dried samples it applies to the field soil, since the site was selected based on low Olsen P concentration (3 mg kg<sup>-1</sup>). A field survey of central and eastern Madagascar revealed that all six soils sampled (mostly Oxisols with low pH and low CEC) from rice-growing regions were P-deficient with Olsen P concentrations below 10 mg kg<sup>-1</sup>, and below levels of detection at the two sites nearest to the present study site at Behenjy (Turner, 2006). This emphasised widespread P depletion in the region consistent with the low concentrations measured, with DGT-P below the limit of detection.

It was hypothesised that AWD would increase soil P availability compared to CF, by accelerating mineralisation under aerobic conditions and releasing P from microbial cell lysis upon re-flooding. However, at anthesis there were no significant effects of the irrigation regime (or P level) on soil P concentrations. The lack of increase in the plant-available NaHCO<sub>3</sub>-P and resin-P concentrations between the sampling times, despite P fertiliser application, suggested that the applied P had been sorbed to soil minerals and/ or assimilated by plants. Similarly, the P fertiliser application did not increase water-extractable TP, MRP and MUP concentrations, as previously reported for MRP (Rakotoson et al., 2015), likely due to high P fixation. The lack of effect of irrigation on soil P concentrations, and the decrease in MBP between sampling times under AWD as well as CF, suggested that AWD did not increase mineralisation of organic P. Broadly, the ratio of MUP to TP indicates the proportion of potentially available P that is organic, and ranged from 3.7 % to 48.2 % (mean 32.0 %) whilst neither P form was affected by irrigation (Table 4.5). Similarly, across 13 rice fields in six locations in Madagascar the ratio of organic P to total P was 6.7 % to 28.5 %, amongst which there were no differences in the

amounts (or forms) of organic P according to whether the field was managed under conventional flooding or SRI (Turner, 2006), which often involves AWD. The low MBP concentrations, ranging from 1.12 to 8.37 mg kg<sup>-1</sup> (means in Table 4.5), likely reflected a decrease during storage since maintenance at 4 °C for longer than two weeks decreased MBP concentrations (Turner and Romero, 2010). Measuring MBP within two weeks of sampling, and sampling near-immediately after re-wetting (as Chapter 3) throughout the AWD period as well as at anthesis (and maturity), would provide further insight into the microbial P dynamics over multiple cycles. Nevertheless, MBP and other P forms were not affected by AWD over the time scale measured.

The Gleysol at the field site was also classified as infertile for rice based on low pH (< 6.5) and low CEC (< 10 cmol kg<sup>-1</sup>) (Dobermann and Fairhurst, 2000), indicating that these properties contributed to the low available P concentrations. In aerobic soils, pH had little influence on organic P forms and concentrations above pH 4 (Turner and Blackwell, 2013), whereas flooding can increase soil pH and result in the reductive dissolution of Fe(III) and Mn(IV) oxyhydroxides, thereby releasing sorbed P (Amery and Smolders, 2012). However, whether this mechanism increases P availability to plants depends on the soil having high CEC, so that cation sorption sites bind the Fe<sup>2+</sup> resulting from reductive dissolution of Fe(III) oxyhydroxides to decrease potential P sorbing sites (Amery and Smolders, 2012). The CEC of the field soil was very low (2.27 cmol kg<sup>-1</sup>), which likely contributed to low available P since the potential for P release was limited, and soil CEC was the major factor limiting P release from two other Gleysols (pH 4.5 and 4.6, CEC 2.7 and 5.4 cmol kg<sup>-1</sup>) and a Histosol (pH 4.4, CEC 4.1 cmol kg<sup>-1</sup>) from paddy rice fields in Madagascar (Amery and Smolders, 2012). Measuring the pH of soil samples paired with P concentrations would inform whether it decreased under AWD compared to CF, causing the lack of increase in soil P concentrations.

**Alternate wetting and drying effects on rice biomass and yields**

It was hypothesised that rice biomass production and grain yields would be higher under AWD compared to CF, particularly under low P supply. The straw biomass was highest under AWD and CFA, at P-25 and P-0 respectively (Figure 4.4), indicating that it did not increase with the P supply and the irrigation effects were only significant across P levels. Furthermore, only fertiliser application increased grain yields and HI, with maximum yields at the highest rate although maximum HI at the intermediate rate (Figure 4.3). Therefore AWD did not increase soil P concentrations thereby increasing grain yields. Similarly, P release caused by flooding was unlikely to ameliorate P deficiency in six different paddy soils in Madagascar (including two Gleysols) because of continued rice yield response to applied P (Rakotoson et al., 2014), and evidence that achieving 80 % of maximal yield required *c.* 19 mg kg<sup>-1</sup> resin-P (Six et al., 2013; Rakotoson et al., 2014). This concentration far exceeded the initial P concentrations in the present study, indicating AWD (and other management practices as in SRI) would need to greatly increase P concentrations to achieve optimal yields without mineral P fertiliser. The result was consistent with the principles of SRI, whereby combining various management practices can enhance long-term yields, although SRI advocates organic rather than inorganic nutrient fertilisers (Stoop et al., 2002). Adding organic matter (cattle manure and rice straw) to a Histosol from a Madagascan paddy field increased rice P uptake and yields, in flooded but not aerobic soils, likely because inorganic P was immobilised under aerobic conditions (Rakotoson et al., 2015). Nevertheless, the authors also concluded that increased biomass and yields depended on fertiliser application, because OM addition and flooding increased P availability only marginally (Rakotoson et al., 2015). Since rice farmers do not typically apply inorganic fertilisers in the region (pers. comm. with J. R. Raveloson, landowner and A. Rajaona, AfricaRice scientist, October 2015), further studies with unfertilised soils should determine whether the tendency towards increased grain yields and straw biomass under AWD, and particularly CFA, compared to CF was significant (Figures 4.3 and 4.4). Thus, AWD could not be considered an alternative to P fertiliser application, although the highest HI occurred at the

intermediate rate suggesting greater efficiency, which may be relevant where some P fertiliser can be accessed.

Although AWD did not increase yields, neither were they decreased compared to CF. Since soil drying below  $-0.02$  MPa significantly reduced rice grain yields by 22.6 % (Carrijo et al., 2017), this suggests that yield-limiting SWP thresholds were not reached in this trial. The irrigation was intentionally managed such that AWD and soil drying occurred only during vegetative growth and grain-filling respectively, whilst all treatments remained flooded during anthesis. Applying AWD from two weeks after transplanting until the onset of anthesis is a common approach (Price et al., 2013) that maintains yields (Carrijo et al., 2017). In contrast to previous reports of soil drying during grain-filling increasing yields and HI by stimulating stem-to-grain carbohydrate remobilisation (Yang et al., 2007; Yang and Zhang, 2006; 2010; Li et al., 2016), AWD and CFA did not increase grain yields or HI. Nevertheless, the maintenance of yields under AWD and CFA at the same levels achieved under CF indicated increased water use efficiency (WUE) and water productivity (WP) (although irrigation volume data were not available, discussed in Chapter 5). Conditions for achieving the multiple benefits of increased grain yields, HI and WUE in rice grown under controlled soil drying previously reported (Yang and Zhang, 2010) should be defined by determining optimal SWP ranges and scheduling for P release and yields for different soils.

#### **Alternate wetting and drying effects on phosphorus uptake and partitioning**

It was hypothesised that soil drying during grain-filling (AWD and CFA) would decrease grain P concentrations compared to CF. Although AWD can stimulate the translocation of photoassimilates from the stem to grain (Yang and Zhang, 2010; Li et al., 2016), aerobic conditions can fix soil P thereby decreasing P uptake. For example, AWD reduced tissue P concentrations compared to CF, likely via increased P sorption in drying soils (Ye et al., 2014). Contrary to the hypothesis, withholding irrigation during grain-filling via AWD and CFA did not affect grain and straw P concentrations (Table 4.7). Furthermore, the irrigation treatment

did not affect the relationship between grain P content and total P uptake, or between grain weight and grain P concentration, indicating that AWD or CFA did not reduce the partitioning of P to the grain or maintain grain yields with reduced P concentrations. However, determining whether straw-to-grain P translocation was affected would require more advanced methods than measuring tissue P concentrations (discussed in Chapter 5). Avoiding continued uptake from the soil after anthesis is a target to increase P efficiency (Julia et al., 2016); thus further study is required.

Greater efficiency occurred at the intermediate P fertiliser level. Similarly to biomass production and yields, the P fertiliser application affected grain P content, PUE for grain yield and the P balance whereas irrigation did not (Table 4.7). Increasing the P fertiliser rate increased grain P content, although it was not clear whether this indicated luxury P uptake since yields also increased. Nevertheless, the highest PUE occurred at the intermediate P supply. This suggested that reduced fertiliser input may be considered if maximal PUE (as well as HI) rather than yields was the target, for example if it was more financially economical or necessary to meet environmental standards (e.g. the EU Water Framework Directive; WFD, 2013). Although yields should also be maintained or enhanced, increased PUE is a key target for cereal production globally (Dhillon et al., 2017).

Determining optimal irrigation and P fertiliser strategies likely requires further measurements of the effects of AWD and P level on P uptake and partitioning. That the P balance remained below 10 and 25 kg ha<sup>-1</sup> at the respective P application rates suggested that averaged across the irrigation treatments, 7 and 9 kg ha<sup>-1</sup> of the applied fertiliser at P-10 and P-25 respectively was not utilised by the plant, despite yield increasing with fertiliser application. This was likely caused by high P fixation (discussed below), but also because the total P uptake did not account for the P concentrations in roots. A recent study showed that in rice grown hydroponically, 45 % of the <sup>33</sup>P tracer applied at 9 days after anthesis remained in the roots at maturity under deficient as well as optimal P levels (Julia et al., 2016). This suggested that providing roots remain unharvested under field conditions, P taken up during grain-filling largely remained

within the soil organic matter, providing a source of mineralisable organic P. Nevertheless, management practices are also important under field (rather than hydroponic) conditions. Whilst post-anthesis P accumulation contributed approximately 70% of total plant P at maturity in flooded rice in two separate field studies (Ye et al., 2014; Julia et al., 2016), the proportion was 40% in upland (aerobic) rice (Rose et al., 2010) and was decreased by topsoil drying during grain-filling (Julia et al., 2016). These results suggested that aerobic conditions decreased post-anthesis P uptake. The lack of significant treatment effects on shoot P concentrations at anthesis, as well as grain and straw P concentrations at harvest (Table 4.7), confirmed that P uptake was not affected by AWD, rather than that effects of AWD were ameliorated by flooding during anthesis and/ or drying during grain-filling. Further research should focus on the grain-filling period to reduce P uptake and straw-to-grain P translocation, via breeding (Rose et al., 2010; 2013; 2016; Rose and Wissuwa, 2012; Julia et al., 2016) and genotype by environment interactions (Vandamme et al., 2016), to determine optimal irrigation regimes (and complimentary management practices) for yields as well as PUE and HI.

#### **Were soil phosphorus concentrations explained by plant phosphorus uptake?**

The soil P concentrations were more likely explained by sorption to soil minerals than by plant P uptake. Statistically, there was little variation in total P uptake across the range of 0, 10 and 25 kg ha<sup>-1</sup> of applied mineral P fertiliser and different irrigation regimes (Figure 4.5), consistent with the lack of significant variation in soil P concentrations (Figure 4.7A). Nevertheless, the large (although not significant according to the conservative Tukey test applied throughout this thesis) irrigation effect on total P uptake, which increased by *c.* 50 % by rice under AWD compared to CF, warrants further study with higher replication to determine the significance and potential application of this response. Furthermore, the negative P balance at P-0 showed that up to a mean of 6.7 kg P ha<sup>-1</sup> was acquired from the soil P reserves, and a similar amount was also acquired by plants at P-10 and P-25 (Figure 4.6) whilst the differences likely contributed to the increased grain yields (Figure 4.3). Since NaHCO<sub>3</sub>-P measured at anthesis (at all P levels) was the same as the pre-treatment concentration, this pool was replenished from

soil P reserves replacing P taken up by the rice plants. This effect was previously reported from paddy rice fields (Gleysol and Histosol soils) in Madagascar, with the same genotype (X265) with organic amendment (farmyard manure) (Andriamananjara et al., 2016). These results suggested that plant P assimilation and soil P concentrations may have been in dynamic equilibrium, whereby sorbed P was released to replace P assimilated by plant roots. Another study showed a higher P content in rice tissue than could be explained by the available soil P concentration, suggesting that rice roots exuded P-solubilising agents (e.g. organic acids) and could therefore access otherwise inaccessible P (Huguenin-Elie et al., 2009). Therefore it appeared that the majority of applied P became sorbed and did not increase immediately available P, indicating the challenge of determining soil P availability when concentrations are very low and the soil is highly P-fixing, and it may be partially plant-regulated. Thus AWD may have increased soil P availability but it was rapidly sorbed, so was undetected within the measurement period. Further, better replicated studies should determine whether the tendency for higher P acquisition from soil reserves (higher P uptake and lower P balance) under CFA and AWD compared to CF was significant (Figures 4.5 and 4.6). As well as soil properties, further research should determine the effects of AWD on plant exudation of P-solubilising compounds and P-mineralising enzymes, root architecture, and P-mobilising mycorrhizal associations, which are important to P availability and uptake (Stutter et al., 2012).

## **Conclusions**

Soil P availability was very low and the lack of influence of P fertiliser application or irrigation on soil P concentrations suggested that soil physical properties (low pH and CEC, high PSI) strongly regulated P availability. However, the P balance indicated that applied P was not depleted, suggesting that although soil P concentrations were low, a dynamic equilibrium existed whereby sorbed P was released to replace P acquired by plants and that plants readily acquired newly available P. Nevertheless, grain yields were limited by P since they increased

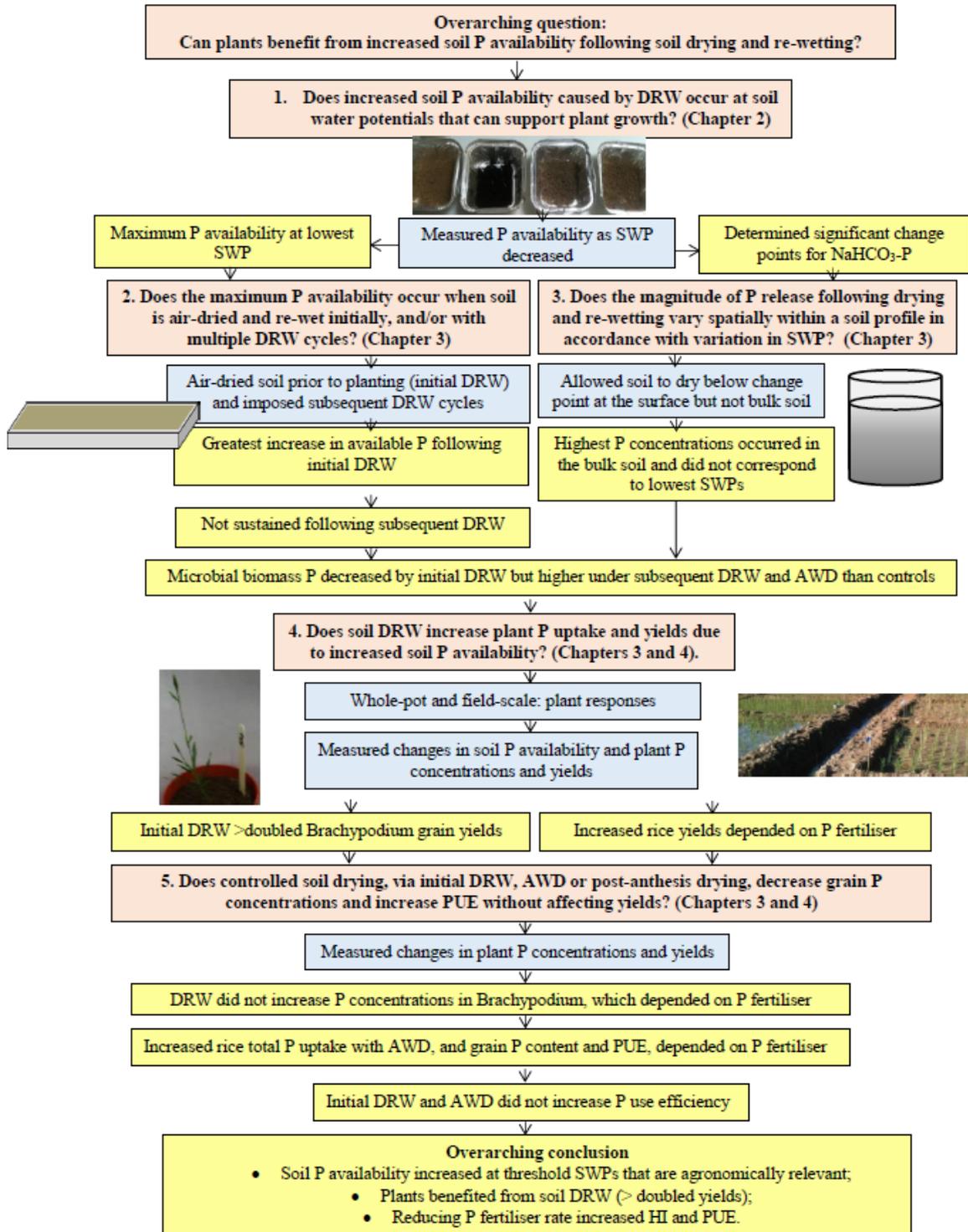
with P fertiliser application, averaged across the irrigation treatments which did not affect grain yields (Table 4.6). Although applying AWD and CFA did not decrease yields, contrary to the hypothesis they did not achieve equal grain yields at reduced P concentrations compared to CF (Table 4.7). This target should be studied further for a range of genotypes and environments. The highest HI and PUE occurred at the intermediate P application rate, suggesting that the P supply could be reduced to meet these efficiency targets, although limiting grain yields. Whether this is economically viable depends on P fertiliser prices, and future studies need to establish on-farm economic optimum fertiliser rates. However, when farmers cannot access P fertiliser, different management strategies are needed to optimise yields and P and water use efficiencies and secure long-term increases in rice productivity.

## Chapter 5: General Discussion

Water and phosphorus are essential resources for crop production, yet both are increasingly limited, threatening global food security (Wang et al., 2016; Cordell, et al., 2009). Thus, both resources need to be managed to optimise water and P use efficiencies, as well as enhance crop yields to meet increasing demand. The effects of different irrigation schedules on crop yields (Carrijo et al., 2017) and of soil drying and wetting on P availability (Dodd et al., 2015) are well-documented, although their integration remains limited as they are often studied by distinct science communities. Although soil DRW has reportedly increased P availability in numerous soils, many of these results have used much drier soils than plants would be able to tolerate. Furthermore, these “soil-exclusive” studies have often emphasised the magnitude of P release following DRW as proportional to field-moist reference conditions, which can exaggerate the relevance of the effect, especially in soils in which available P concentrations are already low.

In parallel, few studies have related crop responses to soil water deficits to the corresponding changes in soil nutrient availability. Therefore this research primarily determined the SWPs at which P availability increased in different soils; and whether the increases in concentrations resulting from DRW likely had agronomic relevance based on typical guidelines for crop nutrient management (DEFRA, 2017) and empirical effects on plant P uptake and yields. Five key questions were addressed by experiments carried out across a range of scales, from laboratory studies, through pot experiments, to a field trial, reported in Chapters 2, 3 and 4 respectively. The five main questions, different approaches, and key results are summarised as a flow chart in Figure 5.1. The following discussion draws upon the new knowledge that has been generated in relation to these five research questions, the limitations including questions that arose, and ideas for future studies.

Figure 5.1: Summary of knowledge generated by this. Boxes for research questions are pink, for approaches or processes are blue, and for key findings and research linkages are yellow.



## **Phosphorus availability increased as soil water potential decreased**

### **Key results and comparisons**

It was hypothesised that decreased SWP would increase P availability following DRW, and the SWP at which P availability increased would vary according to the soil type. This study showed that the SWP threshold at which  $\text{NaHCO}_3\text{-P}$  significantly increased was similar for the three soils investigated. Although it was hypothesised that greater increases in available P following DRW would occur in soils with higher MBP, no correlation was found. This contrasted with previous research and was likely due to the limited number of soil types (3 rather than 29) (Turner and Haygarth, 2001). The principal measure of plant-available P used throughout the study was  $\text{NaHCO}_3\text{-P}$ , and resin-P showed similar responses to DRW (Figures 3.9 and 4.7). An exception was the increased resin-P but not  $\text{NaHCO}_3\text{-P}$  under DRW compared to continuously saturated (CS) (Figure 3.9). This reinforced that although fertiliser recommendations are given based on both measures for grass and forage crops, the values are not equivalent (DEFRA, 2018a) because these tests measure different pools of soil P (Moody et al., 2013) and vary in their ability to predict plant yields (Six et al., 2013). Therefore this study used different soil P measures to indicate DRW effects on organic, adsorbed and solution P concentrations (Figure 1.1).

There was some discrepancy in the SWPs at which P availability increased following DRW, and the magnitude, within and between the experiments reported in Chapters 2 and 3. In the Rowden soil, the increase in  $\text{NaHCO}_3\text{-P}$  was greatest at T1 (by  $7.09 \text{ mg kg}^{-1}$ ), at nearly double the increase at T2 despite lower SWP (Chapter 2), and seven times the increase in Experiment 2 (Chapter 3) despite similar SWP. In the Whiddon Down soil, at similar SWP,  $\text{NaHCO}_3\text{-P}$  increased five times more following DRW in Chapter 2 than Chapter 3. Between Chapters 3 and 4, P concentrations in the Madagascar soil were similar. It is likely that increased soil storage time decreased MBP (Turner and Romero, 2010), thereby limiting the increases in P availability following DRW. Since the maximum P release occurred at SWPs far below the

plant PWP (-1.5 MPa), there are two soil management possibilities that may allow plants to utilise this resource:

- Air-drying and re-wetting the soil prior to planting (during fallow periods);
- Allowing only the surface soil to dry beyond the change points (via irrigation or by manipulating surface coverage by crop residue).

### **Limitations and further work**

The variable results between soils likely resulted from inherently different properties (Appendix 1), and between experiments likely resulted from changes in microbial communities due to differences in soil sampling depths and times and the pre-experiment storage time. Whereas the Tadham Moor, Little Burrows and Joseph's Carr soils were collected from 0-10 cm depth in May, the Rowden soil was collected in October and the Whiddon Down soil in February, both at 0-20 cm depth, and microbial biomass concentrations and mineralisation rates change markedly throughout the year (Yao et al., 2011). Soil was collected from 0-20 cm depth to acquire sufficient volumes for the pot experiments (Chapter 3), so it was important to also determine SWPs at which P availability increased (Chapter 2) at this depth. Microbial biomass P concentration is commonly measured in the surface 0-10 cm in grassland soils (Turner and Haygarth, 2001; Blackwell et al., 2009; 2012); therefore another sample of the Tadham Moor soil from 0-20 cm depth should have been used for Experiment 2. Future study would reduce pre-experiment storage time to less than two weeks (Turner and Romero, 2010), and increase sampling frequency during soil drying to allow greater precision in determining change points. Further experiments are also needed to determine the underlying mechanisms causing P release following DRW in different soils. Nevertheless, demonstrating that  $\text{NaHCO}_3\text{-P}$  increased logarithmically with decreasing SWP, with a significant change point at -2.9 MPa, suggests there may be times during the cropping cycle when similar SWPs are reached in surface soils.

## **Highest phosphorus availability was caused by initial soil drying and re-wetting**

### **Key results and comparisons**

It was hypothesised that soil P availability would be higher in initially air-dried and re-wet soils compared to continuously moist soil, because a lower SWP would be reached than for control soils or with subsequent DRW cycles (which aimed to maintain SWPs in the bulk soil above the change point). This was based on the conclusion that the maximum increase in  $\text{NaHCO}_3\text{-P}$  occurred at the lowest SWPs for these soils (Chapter 2). Consistently, initial DRW increased  $\text{NaHCO}_3\text{-P}$  in both experiments in Chapter 3, whereas subsequent DRW cycles (with less soil drying) did not (Experiment 1). Although change points in the SWP *versus* P availability relationship were exceeded during subsequent DRW, only initial DRW increased P availability. This result likely occurred because whereas in Chapter 2 soil re-wetting occurred as part of the soil P extraction process, in Chapter 3 soils were re-saturated or re-flooded 1.5 hours before sampling. This time may have allowed re-adsorption or microbial immobilisation, suggested by the higher MBP concentrations in the drying soils than the control soils (Table 3.9), thereby limiting P release to the soil.

It was also hypothesised that soil P availability would be higher after two subsequent DRW cycles compared to one cycle (without plant uptake), because the microbial biomass would decline after DRW (releasing P) but recover such that a subsequent cycle would have additive effects. Previous studies have reported inconsistent effects of multiple DRW cycles on P availability, due to differences in soil type, incubation period, and likely the degree of drying (Scalenghe et al., 2012; Chen et al., 2016; Dinh et al., 2016). This study showed that the initial increase in P availability was not sustained, possibly due to DRW increasing access to adsorption sites due to shrinkage and swelling of soil aggregates (Chepkwony et al., 2001; Blackwell et al., 2009), or because substrate availability reduced, limiting mineralisation (Yu et al., 2014; Shi and Marschner, 2017). Therefore consistent with the hypothesis, soil P availability was highest following initial DRW.

**Limitations and further work**

In contrast to Chapter 2, experiments in Chapter 3 imposed multiple cycles of soil DRW, although the effects of soil moisture release curve hysteresis (Whitmore and Whalley, 2009) were not considered. Hysteresis indicates that the relationship between SWP and GWC differs according to whether the soil is becoming progressively drier or wetter (Whitmore and Whalley, 2009), and effects are important over several DRW cycles. Re-wetting volumes were previously determined based on whole-pot GWC, because reproducing changes in SWP was considered less accurate due to hysteresis with repeated DRW (Lado-Monserrat et al., 2014). However, hysteresis possibly explained the slight differences in the relationships between GWC and SWP in the Whiddon Down soil between Chapter 2 with one DRW cycle (Table 2.6), and Chapter 3 with multiple DRW cycles (Table 3.8). Hysteresis affects soil water availability to plant roots, relating to physical soil properties such as porosity and shrink-swell characteristics, especially in clay soils such as those used here, which are affected by repeated DRW (Whitmore and Whalley, 2009). Therefore SWP should be measured directly following each drying (and re-wetting) event, since it cannot accurately be deduced from the GWC (Whitmore and Whalley, 2009). Although SWP can be continuously monitored using tensiometers within larger soil volumes over a restricted SWP range (Whalley et al., 2013), accounting for hysteresis would likely be necessary over more DRW cycles at a similar scale to Chapter 3, Experiment 1. This would have required larger soil volumes to sample more cores during the experiment. Measuring SWP within Experiment 2 was not possible due to soil and root disturbance within small soil volumes, further indicating the need for larger-scale studies of spatial variations in SWP and P availability. Furthermore, microbial competition with plants for P (and other nutrient) acquisition at low water potentials is another important field of research with regard to nutrient cycling (Dijkstra et al., 2015), particularly considering temporal dynamics to determine the potential crop acquisition of released soil P.

## **Spatial variation in phosphorus availability was not related to soil water potential**

### **Key results and comparisons**

Soil P availability increased with decreasing SWP, but fully air-drying soil is not always practical in the field, such as where fallow periods are not used or planting seasons occur during high rainfall. Therefore it was hypothesised that soil P availability would be higher in soils exposed to drying and re-wetting or re-flooding compared to soils maintained under continuously saturated or flooded conditions, particularly at the surface compared to the bulk soil. A pot experiment enabled the relationship between P availability and SWP to be investigated within the soil profile. Contrary to the hypothesis, P availability was not affected by irrigation and was instead higher in bulk than surface soil. This implied that surface soil drying did not increase  $\text{NaHCO}_3\text{-P}$ , although this contradicted Chapter 2's conclusions. More likely, P was transferred vertically in the soil profile, regardless of irrigation regime. Vertical P transfer may have occurred with water via leaching (Turner and Haygarth, 1999), as saturated flow in the control treatments (over days), whilst unsaturated (preferential) flow through fissures may have occurred in the drying treatments over a shorter timescale following re-wetting (minutes to hours) (Haygarth and Sharpley, 2000). In contrast, DRW increased resin-P, so the implications for plant-availability were contradictory. These results emphasised the importance of the measurement method (Moody et al., 2013), and the timescale for studies of soil P availability and transfer. Released P is only plant-available if the crop takes it up before it is acquired by microbes or fixed by soil minerals (Grierson et al., 1998; Bünemann et al., 2013). Returning soil moisture to pre-DRW levels provides a time-restricted window when plants compete with soil biogeochemical processes to access available P (Chepwonky et al., 2001; Chen et al., 2016). Thus, although decreased SWP increased soil P availability in laboratory experiments, other factors (such as leaching) are important at larger spatial (trays to pots) and temporal (24 hours in Chapter 2 to 59 days in Chapter 3) scales.

### **Limitations and further work**

Future studies, aiming to determine the soil factors regulating the effects of DRW on P availability, should also consider: the SWP, spatial variation in P availability and other properties within a soil profile, and temporal variation with multiple DRW cycles, ideally with soil samples taken during different seasons to determine whether the microbial contribution is greater during warmer seasons. Under soil flooding (Chapter 3, Experiment 1 and Chapter 4), studies should use redox/ oxygen sensors to determine whether anaerobic conditions can explain chemical changes affecting P availability. Additionally, measuring anaerobic soils under anaerobic conditions as far as possible (using a glove box) would more accurately determine soil P availability at specific sampling times. To determine the sources of released P, a comprehensive analysis of other (sometimes also changing) soil properties under DRW would be required (outlined in Appendix 2).

Along with SWP measurements, measuring root water potentials at different depths in the soil profile would determine whether plants may access water and nutrients. For example, SWP increased with depth in a soil under drip-irrigation supplied every three days, such that mild surface soil drying increased absolute water uptake rates from deeper layers (Li et al., 2002). Therefore if DRW increased soil surface P availability, it would be essential for root water potential near the soil surface to also increase (recover) with SWP upon re-wetting to acquire newly available P (unless vertical P transfer by leaching was substantial). Nevertheless, tracing isotopes is a more specific technique to determine the fate of soil P (discussed below).

### **Plant responses to soil drying and re-wetting were unlikely related to increased phosphorus availability**

#### **Key results and comparisons**

It was hypothesised that whole-pot increases in soil P availability caused by initial DRW would increase P uptake, biomass and yields in *Brachypodium*; and that AWD would increase rice

biomass production and grain yields compared to conventional continuous flooding. These responses were anticipated to be most pronounced under low P supply. The two studies reported in Chapters 3 (Experiment 2) and 4 contradicted this hypothesis. In *Brachypodium*, although initial DRW significantly increased biomass production and doubled yields, plant P concentrations and soil P availability were only increased by P fertiliser application (Figure 3.9) unlike previous studies (Tsujimoto et al., 2010; Bünemann et al., 2013), and reducing irrigation frequency had no effects. Similarly in rice, shoot P concentrations were not affected by irrigation whilst grain P contents were only increased by P fertiliser, and soil P concentrations were not affected by the treatments (Figure 4.7). Whereas soil P availability was expected to increase due to reduced irrigation frequency at a time of high P demand (anthesis) in *Brachypodium*, in rice the aim was to avoid luxury P uptake during grain-filling. Therefore soil was sampled at rice anthesis when all treatments were flooded, allowing treatments to be compared at their field moisture contents. In contrast, soil was sampled at maturity for *Brachypodium*, requiring the soil samples to be air-dried to be comparable between treatments. Regardless of these differences, DRW and AWD did not increase plant P uptake due to increased soil P availability. High P-fixing by the soils likely played a large role in determining the results, such that small increases in P availability following DRW and AWD were not detected by the methods used (Bünemann et al., 2004). This effect was particularly evident in the field soil which had a high P sorption index, since soil P concentrations and rice P uptake hardly varied between the P fertiliser rates (Figures 4.5 and 4.7A). Thus, the potential role of P release by DRW may have been undetected. Nevertheless, the doubling of yields following initial DRW demonstrated a major agronomic benefit, irrespective of whether the mechanisms were P-related.

### **Limitations and further work**

Further study is needed to identify the cause of increased yields following initial DRW and the role of P availability along with other regulatory factors. Firstly, directly relating plant P uptake to soil P concentrations in different pools would be valuable. Since changes in P concentrations,

especially small additions, can be undetected in weathered, highly P-sorbing soils, isotope labelling would be more sensitive in tracing applied P in different pools (Bünemann et al., 2004). Isotope labelling studies allowed applied  $^{32}\text{P}$  labelled inorganic P to be related to wheat P acquisition and total dry matter yields under DRW cycles (Chepkwony et al., 2001);  $^{32}\text{P}$  to be traced in soils, microbes and plants (Dijkstra et al., 2015); and  $^{33}\text{P}$  to be traced from the soil to grain in rice (Julia et al., 2016). Additionally, the concentrations of other nutrients should be determined for soils and plant tissue, since soil DRW increased soil N levels and increased N uptake in potato (Wang et al., 2009) and maize (Wang et al., 2012). Therefore determining why *Brachypodium* grain yields doubled when grown in soils that were initially exposed to DRW requires more precise and comprehensive measurement of nutrient availability and uptake.

### **Alternate wetting and drying increased biomass and phosphorus uptake only when phosphorus fertiliser was applied**

#### **Key results and comparisons**

It was hypothesised that soil drying during grain-filling (via AWD and via continuous flooding only until anthesis, CFA) would decrease grain P concentrations compared to CF, thereby avoiding luxury P uptake. The field trial reported in Chapter 4 was designed to test this hypothesis in a region where soil P availability is low (limiting rice yields) and farmers do not apply P fertiliser; therefore improving PUE is necessary for continued rice production. Contrary to the hypothesis, grain P concentrations were not affected by the irrigation regime, and total P uptake (shoot and grain P contents) was highest under AWD only in combination with the highest P fertiliser application rate. Therefore the P fertiliser dominated the treatment effect. Importantly, reducing the P fertiliser application rate increased the HI and PUE, which are key efficiency targets for crop production, indicating that benefits can be achieved where farmers can access a limited amount of P fertiliser ( $10 \text{ kg ha}^{-1}$  in this case). Increased HI and PUE are useful targets for long-term production, although the lower fertiliser rate reduced the grain yield by one-fifth in a single crop cycle (which was not improved by AWD). Therefore this study

importantly isolated AWD as a separate factor to other SRI (and other) practices such as organic matter amendment. Increased PUE with reduced fertiliser application is a key result to develop, likely combined with other practices to optimise yields.

### **Limitations and further work**

Field scale trials throughout a crop cycle are ultimately needed to determine the effects of DRW on soil P availability and plant P uptake, varying with depth in the soil profile and over time with multiple DRW cycles and crop development. Although field trials are the most relevant approach to determining the impacts of agronomic management strategies on site-specific water and P use efficiencies and yields, they are less controlled than laboratory and pot scale studies. The trial reported in Chapter 4 would have benefited from several experimental improvements to determine altered P concentrations and agronomic benefits. The very low P concentration of the soil meant that P fertiliser had the greatest effect on P availability, uptake and yields; and that DGT-P concentrations were below the limit of detection. Whilst DGT-P is sometimes better correlated with plant P concentrations than other soil P measures (Six et al., 2013), the relationship has not been reported for lowland irrigated rice but this could not be achieved from the field trial. Further studies should measure DGT-P in-situ over a longer (> 26 hour) deployment period, due to the high P-fixing properties of the soil. As well as available P, soil total P was 58 % and 55 % lower than the Rowden and Whiddon Down soils, respectively (Appendix 1), indicating that potentially available P (released by DRW) was more limited.

As well as determining P concentrations, measuring irrigation and rainfall volumes was planned (to determine irrigation water productivity as crop yield per unit of applied irrigation water; Sadras, 2009) but eventually not possible. These measures would be valuable since AWD and CFA irrigation dried the soil without decreasing grain yields or HI, perhaps representing an opportunity to increase WUE. Measuring SWP was also planned but not possible due to practical restraints but would have contributed important information, perhaps allowing SWPs in the field to be related to soil P concentrations (as in Figure 2.3). Future field trials studying

AWD should include tensiometers placed both above and below the water height limit for AWD of 15 cm (IRRI, 2009) (and/ or measure SWPs of soil cores). Furthermore, trials should consider irrigation scheduling based on P-releasing, and yield-maintaining SWPs (Carrizo et al., 2017) rather than water level, to more directly relate soil and plant processes regulating water and solute (nutrient) uptake. Since the highest P-releasing SWP in the soils measured (-2.3 MPa, Chapter 2) was not compatible with yield-maintaining SWPs (-0.02 MPa, Carrizo et al., 2017), determining the spatial variation in SWP and P availability at different depths within the soil profile (discussed above) should be a priority for future studies.

More complex challenges than practical considerations persist for field studies of crops, both academic and organisational. This trial importantly studied the effects of AWD at different P levels in isolation from other factors e.g. SRI practices; yet most likely a range of practices interacting with AWD have the greatest potential to increase P availability and yields where farmers cannot access P fertiliser (Stoop et al., 2002). It would be more relevant to exclude the P fertiliser application treatment and instead determine the effects of AWD and CFA on P availability and uptake combined with more locally-accessible organic fertiliser treatments, such as manure and rice straw from previous crops, to increase soil CEC and potential P release (Amery and Smolders, 2012; Rakotoson et al., 2014; 2015). To determine the most appropriate practices to include as experimental factors, designing on-farm collaborative research potentially has major benefits, since soils at research sites cannot accurately represent the soils of a particular localised agronomic system (Stoop, 2003). On-farm research is primarily how SRI developed (Dobermann, 2003) and continues to produce yield benefits amongst Malagasy communities (I. Rajaona, pers. comm., April 2016). As understood (Stoop et al., 2002; Glover, 2011) yet rarely implemented, future studies would undoubtedly benefit from involving local farmers, as well as researchers, in field trials from the outset, to ensure that practices enhancing efficiency and yields are feasible in the long-term.

## Conclusions

Through experiments at laboratory, pot and field scales, this research has shown:

- **Soil P availability increased as SWP decreased in three soils.** Soils need to be drier than plant PWP for P availability to increase upon re-wetting, but not as dry as often reported in soil-exclusive studies. To my knowledge, this is the only study characterising a logarithmic increase in plant-available  $\text{NaHCO}_3\text{-P}$  with decreasing SWP, and reporting significant change points.
- **Highest P availability was caused by initial soil air-drying and re-wetting.** This confirmed that more P was released at the lowest SWPs compared to subsequent cycles of DRW at the soil surface. Thus, there is the potential for DRW during a fallow period to produce a P pulse from which crops may benefit.
- **Spatial variation in P availability within the soil profile was not related to SWP.** This implied that the results derived from laboratory experiments could not be scaled up spatially since soil P availability increased with soil depth, even without changes in SWP. Since P-releasing and yield-maintaining SWPs are incompatible, P leaching within root zones is a key priority for future research.
- **Initial soil DRW more than doubled grain yields.** Proportional increases in grain yields and biomass production with total P uptake, whilst shoot P concentration hardly varied, suggested Brachypodium yields likely increased due to beneficial effects of soil DRW other than P availability. This significant beneficial DRW effect requires further research under P-limiting conditions.

- **Increased rice grain yields depended on P fertiliser, with optimal efficiency at an intermediate rate.** Thus, benefits of P release under controlled soil drying regimes were marginal compared to the effects of applying P fertiliser, in a highly P-fixing soil with low available P. Nevertheless, HI and PUE were highest at the intermediate P fertiliser rate, indicating an opportunity to improve resource efficiency which should be developed.

Thus, key progress was made with the knowledge generated:

- (1) Soil drying and re-wetting clearly increased P availability within a SWP range that is agronomically relevant, if carefully controlled since change points occurred below the plant PWP. More severe soil drying was needed to maximise soil P release upon re-wetting.
- (2) Pre-planting soil DRW doubled grain yields, demonstrating a significant benefit to plants which could be applied during a fallow period to increase crop yields.
- (3) The highest HI and PUE for grain yields occurred at an intermediate P fertiliser rate, indicating greater long-term efficiency of P fertiliser use in food production.

Finally, for crop plants to benefit from increased soil P availability following drying and re-wetting, integration with other locally-relevant agronomic practices which stimulate the response will be essential. Further research to determine optimal practices is urgently required to increase water and phosphorus use efficiencies and crop yields.

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## Appendix 1

Soil physical and chemical properties for the four principal soils used in these studies. Analyses were carried out by NRM Laboratories, UK and Rothamsted Research, UK.

Soil property	Result				Units	Method
	Tadham Moor	Rowden	Whiddon Down	Madagascar		
<b>Texture class</b>	Peat	Clay	Clay	Clay		Particle size distribution via laser diffraction
<b>Sand</b>	7	13	8	27	% w/w	
<b>Silt</b>	35	37	41	33	% w/w	
<b>Clay</b>	58	50	51	40	% w/w	
<b>Organic Matter</b>	No data	11.5	7.6	9.4	% w/w	Loss on ignition (LOI)
<b>pH</b>	5.9	4.9	4.9	4.8		In water (1:2.5)
<b>Total C</b>	28.58	4.79	2.97	2.10	% w/w	Combustion catalytic oxidation
<b>Total N</b>	1.99	0.48	0.32	0.16	% w/w	
<b>C:N Ratio</b>	14.36:1	10.0:1	9.3:1	13.1:1		
<b>Total P</b>	1026	674	640	285	mg kg <sup>-1</sup>	Aqua-regia soluble elements : HCl and HNO <sub>3</sub> digestion with analysis via ICP-OES
<b>Total K</b>	3064	1572	1450	175	mg kg <sup>-1</sup>	
<b>Total Mg</b>	4141	730	1570	392	mg kg <sup>-1</sup>	
<b>Total Mn</b>	647	986	239	65	mg kg <sup>-1</sup>	
<b>Total Fe</b>	19066	39336	37283	33667	mg kg <sup>-1</sup>	
<b>Available Fe</b>	7578/ 7970	171	181	115	mg L <sup>-1</sup>	DTPA extraction, or dithionite/ ammonium oxalate extraction (Tadham Moor)

## Appendix 2

For a comprehensive analysis of changes in P availability with other (sometimes also changing) soil properties under DRW to determine the sources of released P, the following properties should be analysed:

- Microbial biomass C and N (Gordon et al., 2008);
- Microbial activity (respiration and mineralisation) (Grierson et al., 1999; Gordon et al., 2008);
- Organic C content, which largely determines mineralisation rates (Sparling et al., 1985; Jarvis, 2007; Gordon et al., 2008; Blackwell et al., 2010);
- Microbial community structure and composition, e.g. PLFAs (Sun et al., 2017a);
- P sorption index (PSI) (Six et al., 2013);
- Degree of sorption saturation (Styles and Coxon, 2006);
- Elemental concentrations (e.g. Fe, Mn, Ca, Mg, Al, Zn) and Fe/ Al oxides;
- Aggregate stability (Bünemann et al., 2013);
- Organic P forms (Turner, 2006; Turner and Blackwell, 2013);
- Where plants are present, their effects on P cycling need to be considered, depending on species and genotype and associated root traits including P-solubilising exudates (Oberson et al., 2006; Stutter et al., 2012).

Additionally, under AWD:

- Soil pH, which can change with flooding (Amery and Smolders, 2012);
- Cation exchange capacity (CEC) (Amery and Smolders, 2012);
- Redox potential (Kirk et al., 1998);
- Organic matter content, an electron donor in redox reactions (Amery and Smolders, 2012; Rakotoson et al., 2014).

Analyses should consider:

- Effects of filtration (Soinne et al., 2010);
- Soil bulk density (DEFRA, 2017);
- Soil moisture regime history (Sparling et al., 1985; Evans et al., 2012);
- Drying and re-wetting rates (Blackwell et al., 2009; 2012);
- Timescale of measurement following re-wetting.