SOIL NUTRIENTS MOBILIZATION AS INFLUENCED BY CLIMATE-CHANGE DRIVEN SOIL DRYING-REWETTING OR DRYING-FLOODING

Soil nutrients mobilization as influenced by climate-change driven soil drying-rewetting or drying-flooding

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List of Abbreviations

C	Cycles
DF	Degrees of Freedom
DRP	Dissolved Reactive Phosphorus
DRW	Drying-Rewetting
DUP	Dissolved Unreactive Phosphorus
DWE	Dry Weight Equivalent
FT	Freeze-Thaw
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
kg	Kilogram
LOD	Limit of Detection
M.C	Moisture Content
mg	Milligram
µg	Microgram
OM	Organic matter
P	Phosphorus
PP	Particulate Phosphorus
SS	Suspended Solids
TDP	Total Dissolved Phosphorus
TP	Total Phosphorus
USDA	United States Department of Agriculture
WHC	Water Holding Capacity

Abstract

As phosphorus and micronutrients (e.g. Fe, Mn, Zn, Cu, Co and Ni) are essential elements for all living organisms including plants, their availability in soils is important for sustaining a healthy ecosystem. Climate change driven soil drying-rewetting or drying-flooding processes may promote increased mobilization and potential loss, and thus scarcity of these elements to plants, with potential implications for soil fertility and surface water quality. A series of controlled laboratory experiments were carried out to better understand the influence of soil drying-rewetting on DRP leaching and drying-flooding on mobilisation of phosphorus and micronutrient metals following rewetting/flooding of dried soils.

Contribution of microbial biomass phosphorus to DRP leaching: The aim of this experiment was to determine microbial biomass phosphorus contribution to the dissolved reactive phosphorus (DRP) leaching under the influence of drying-rewetting processes. The results showed that soil (Hallsworth-I) drying (30°C or 40°C for 2-days or 14-days) induced reduction in microbial biomass phosphorus could partly be the reason that the DRP concentration in the leachates increased relative to the control. The results suggest that soil drying at higher intensity (40°C) and for prolonged duration (14-days) affect the microbial biomass phosphorus to a greater extent than low intensity-short duration drying (30°C for 2-days), and subsequently causes greater DRP leaching following rewetting of dried soil as observed from the drying-rewetting leaching experiment.

Influence of drying-rewetting cycles on DRP leaching: The aim of this experiment was to examine how the intensity and duration of soil drying, rate of soil rewetting and frequency of rewetting cycles influence leaching of soil-borne phosphorus. The results showed that the soil (Hallsworth-I) drying at 25ºC, 30ºC, 35ºC or 40ºC for 2-days or 14-days followed by the first rapid or slow rewetting cycle leached significantly greater DRP concentrations relative to the control moist counterparts. The largest percentage increase in the DRP concentration occurred for the extended drying period (14-days) at 40°C. However, relatively smaller increase in leachate DRP concentration was observed for the shorter drying duration (2-days) at 25°C. The rate at which the dried soil was rewetted also affected DRP leaching, as the leachate concentrations tended to be higher where the soil was rewetted at the slow rate compared to the rapid rewetting counterparts. The frequency of rewetting cycle also influenced DRP leaching, though without a clear trend as the concentrations showed increasing, decreasing or no change in the second rewetting cycle relative to the first rewetting. However, the DRP leachate concentrations remained higher than the control moist counterparts. In the third rewetting cycle, DRP leachate concentrations tended to decline in most of the treatments, particularly those where the soil was dried for the longer duration (14-days), possibly due to depletion in the easily leachable phosphorus

Influence of drying-rewetting cycles on micronutrients leaching: The aim of this experiment was to examine how the intensity and duration of soil drying influence leachate dissolved concentrations of micronutrients (Fe, Mn, Cu, Co, Ni and Zn) following rewetting of dried soils. The results showed that the soil (Hallsworth-I) drying at 30°C or 40°C followed by the first rewetting cycle leached greater concentrations of micronutrients relative to its control moist counterpart. Also, drying soil at 40°C resulted in considerably greater micronutrient leachate concentrations as compared to soil dried at 30°C. The soil dried for 14-days (either at 30ºC or 40ºC) followed by the first rewetting cycle, leached significantly greater dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn relative to their 2-days drying counterparts. The

frequency of rewetting cycles also influenced leachate dissolved metal concentrations, with the concentrations showing varied trends (increased, remained similar or decreased) in the second and third rewetting cycles for those treatments where the soil was dried for 2-days. However, in the treatments where the soil was dried for 14-days, the dissolved leachate concentrations of Fe, Mn, Co, Ni, Cu and Zn significantly decreased in the second and third rewetting cycle relative to the first rewetting counterparts, as observed for DRP, possibly because soluble forms of micronutrients would have been leached.

Influence of drying-flooding on mobilisation of phosphorus and micronutrients: The aim of this study was to examine how soil drying followed by extended flooding might influence solubilisation of phosphorus and micronutrients (e.g. Cu, Co, Zn and Ni). The onset of flooding increased dissolved concentrations of phosphorus. The increase in total dissolved phosphorus (TDP) and micronutrients (e.g. Co and Ni) concentrations in the water column coincided with a reduction in redox potential, suggesting reductive dissolution of Fe/Mn oxy/hydroxides minerals. This was further supported by a strong positive correlation between Fe and Mn with TDP or with Co and Ni. The significant positive correlation between total dissolved concentrations of Al and P indicates that non-reductive dissolution of Al-organic matter-P complexes may have also been partly responsible for phosphorus release to the water column. Flooding of soils which were previously dried generally caused greater solubilisation of dissolved concentrations of phosphorus (total dissolved phosphorus, dissolved reactive phosphorus and dissolved unreactive phosphorus) and metals (Mn, Co, Ni and Cu) in water column relative to their moist-flooded counterparts. Crediton dry-flooded soil released higher concentrations of DRP than Hallsworth-II dry-flooded soil. However, most of the phosphorus in the water column of dry-flooded soils was unreactive, with the Hallsworth-II dry-flooded soil releasing higher concentrations of dissolved unreactive phosphorus (DUP). Hallsworth-II dry-flooded soil generally released greater total dissolved metal concentrations of Fe, Mn, Co and Ni in most of the sampling days relative to all other treatments (Hallsworth-II moist-flooded, Crediton moist-flooded and Crediton dried-flooded), possibly due to its greater organic matter (OM) and easily reducible ammonium oxalate extractable Fe-oxide content.

The results suggest that soil drying followed by rewetting or flooding have the potential to promote greater mobilisation of soil macro- (e.g. P) and micro-nutrients (e.g. Mn, Co, Ni and Cu) compared to rewetting/flooding of moist soils. Thus, have implications for soil fertility and surface water quality, especially under changing-climate with predicted increase in the intensity and frequency of these climate-extremities (drought/floods) for many regions in the UK. Nevertheless, in this study dissolved organic phosphorus (DOP) and dissolved organic carbon (DOC) were not measured. Measuring DOP and DOC would have provided further insights into the processes, particularly the role of microbial biomass-P and transformation between inorganic and organic P. Furthermore, these findings cannot be precisely replicated under natural-field conditions where nutrients mobilised in runoff may be retained in the sub-soil and/or elsewhere in the landscape along the pathway of runoff before they reach catchment waters.

Chapter 1- Impacts of climate change on soil nutrients mobilisation

1.1 Introduction

The warming trend of climate is unambiguous since the mid-20th century; this is evident from the recent observed increase in atmospheric and water temperatures, melting of glaciers, and rising sea levels (Stocker et al., 2013; IPCC, 2014). Kundzewicz et al. (2007) projected an increase of 3°C to 4°C temperature by the end of 21st century. Summers are projected to have more increase in the temperature than winters except for arctic latitudes (Kundzewicz et al., 2007). Since the hydrological cycle is intimately linked with atmospheric temperature, global warming is likely to alter the hydrological cycle, leading to increased frequency and intensity of tropical storms, floods and droughts (Huntington, 2006; Bates et al., 2008).

Some parts of the world may receive significantly reduced precipitation or may have major alterations in the timing of wet and dry seasons. The UK is likely to be affected by most of the expected global impacts of climate change. Winter precipitation in the UK could increase by 5-15% for a low emission scenario and > 30% for a high emission scenario by 2080 (Hulme et al., 2002). This is supported by the increased incidence and extent of flooding in the UK seen in recent years (Marsh, 2007), and climate change predictions suggest that this trend is likely to continue as evident by increased winter river flows (Fowler and Kilsby, 2007). Similarly, summer precipitation could decrease by > 20% for the low emission scenario and > 40% for the high emission scenario, leaving much of the UK drier (Hulme et al., 2002).

In nature, soils are continuously under the influence of physical stresses, such as drying-rewetting (DRW), flooding, compaction and freezing-thawing (FT) (Blackwell et al., 2010). These physical stresses have potential to influence soil organic matter (SOM) mineralisation rates and can mobilise soil macro (e.g. P, N, C) and micro (e.g. Fe, Mn, Zn, Co, Cu, and Ni) nutrients (Blackwell et al., 2010), which can potentially be transported to catchment waters through leaching and runoff. The frequency and intensity of these physical stresses is likely to increase due to changing pattern of climate (Stocker et al., 2013; IPCC, 2014). Longer periods of drying followed by intense rainfall events, as projected, can increase mineralisation of soil organic matter, with consequential release of macro (e.g. N, P and C) and micro (e.g. Cu, Zn) nutrients in runoff (Wilby et al., 2006). This clearly increases the

risk of excess nutrient inputs to catchment waters, with potential implications on water quality and soil nutrients supply.

For countries in the temperate zone, climate change is likely to decrease the number of rainy days but increase the average volume of individual rainfall events (Kundzewicz et al., 2007; Bates et al., 2008). That is, more intense episodic rains, as predicted (IPCC, 2014) can cause soil saturation or flash floods, especially in low-lying landscapes. Extended periods of flooding can impair quality of catchment waters by mobilising and transporting nutrients from land to catchment waters (Van vliet and Zwolsman, 2008; Shaheen et al., 2014a, b; Amarawansha et al., 2015).

Recent climate change projections have shown that nutrients loading from land to catchment waters will increase because of changing climate (Hagg et al., 2014; Huttunen et al., 2015). For instance, Huttunen et al. (2015) predicted that phosphorus and nitrogen loading to the Baltic sea may increase in future under changing climate. These predictions are consistent with recent laboratory experiments and field studies on grasslands, forests, paddy soils, wetlands and flood plains linking enhanced nutrients loading into surface water with elevated runoff and flash floods (Loeb et al., 2008; Chen et al., 2013; Caetano et al., 2012; Lee et al., 2013; Li et al., 2015;Yan et al., 2015).

In recent years numerous studies have tested the effects of soil DRW cycles on nutrients release. There is evidence in literature that soil DRW cycles can increase extractability of soil-nutrients. For instance, Bunemann et al. (2013)**,** Styles and Coxon (2006) and Koopmans et al. (2006) reported increased extractability of phosphorus as a result of soil drying. Turner and Haygarth (2003) observed that air-drying increased bicarbonate extractable inorganic and organic phosphorus in 29 pasture soils from England and Wales. Gordon et al. (2008) reported DRW induced significant increase in dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN) in the leachates from two UK grassland soils. Likewise, in a flooding study increased solubility of iron (Fe) and manganese (Mn) due to flooding induced reduction in redox potential was reported (Shaheen et al., 2014a).

These findings support the notion that the changing pattern of climate has the potential to alter soil nutrient dynamics. Phosphorus is an essential element for all living organisms including crops and livestock production. However excessive loading of phosphorus to aquatic environment from non-point pollution sources is the most common cause of

eutrophication (Carpenter, 2008; Lewis et al., 2011; Kolahchi and Jalali., 2013; Dodds and Smith, 2016). Here phosphorus is particularly important, as it is a non-renewable, depleting resource (Vance et al., 2003). Trace elements (e.g. Fe, Mn, Cu, Zn, Ni etc.) are important micronutrients for all living organisms, including crop health and food production, their availability in soils is important for sustaining a healthy ecosystem. However increased input of these micronutrients in catchment waters can pose detrimental environmental concerns with potential consequences for aquatic life and human health (Zhao et al., 2012; El-Moselhy et al., 2014), and deplete their supply to crop plants.

Thus, higher water temperatures and variations in rain-fall pattern with consequential floods and droughts can potentially undermine water quality and possibly could affect soil nutrient supply, crop productivity and human health (Bates et al., 2008).

The effects of soil drying and subsequent rewetting or flooding on nutrients mobilisation and losses are poorly understood. Especially the effects on the phosphorus and micronutrients (e.g. Fe, Mn, Cu, Zn, and Ni) are not well studied, particularly in terms of their leaching losses. Drying and rewetting or drying and flooding under controlled laboratory conditions is a useful alternative to actual field situations to examine the influence of climate change on soil nutrient mobilisation and loss. Although the influence of all these factors in natural environments is complex, the outcome from a controlled laboratory condition is likely to provide a mechanistic understanding of the extent nutrients can be mobilised and released under the imposed conditions.

The main aim of this research is to understand naturally occurring soil processes (drying-rewetting and drying-flooding) under controlled laboratory conditions, with the following research questions: (1) How intense precipitation events interspersed with drier periods are likely to alter soil nutrient dynamics. The novelty of this research lies in how nutrient leaching is affected by increasing the intensity of soil drying. (2) How variability in precipitation pattern with projected reduced mean summer precipitation and more frequent heavy winter precipitation events would affect soils phosphorus dynamics – to examine this, two different rewetting rates were chosen - slow rewetting mimicking slow rainfall and rapid rewetting mimicking heavy rainfall. (3) Will increase in the number of consecutive dry days as projected in lower mid-latitudes have any significant effects on soil nutrient dynamics? – For this purpose, two drying durations (2-days or 14-days) were imposed. (3) Will succeeding rainfall events likely to have any effects on the quantities of nutrients mobilised

– for this purpose soils were subjected to three rewetting cycles. (4) How prolonged periods of drought followed by intense rainfall events with increased risk of winter floods and flash floods are likely to mobilise nutrients. To investigate the above questions, the objectives of this doctoral thesis were:

1.2 Research objectives

1. To examine the influence of soil drying and rewetting cycles on the leaching of DRP.
2. To examine the influence of soil drying and rewetting cycles on the leaching of micronutrients (Fe, Mn, Cu, Zn, Co and Ni).
3. To evaluate the role of soil microbial biomass-P to the leaching of DRP following drying and rewetting.
4. To examine if soil drying followed by extended period of flooding increases mobilisation of phosphorus and micronutrients (Fe, Mn, Cu, Zn, Co and Ni) in the overlying water column under the influence of flooding induced variations in redox potential.

Chapter 2 - Impact of climate change driven soil processes on nutrients mobilisation: A literature review

2.1 Introduction

In nature top-soils are continuously under the influence of physical stresses e.g. drying-rewetting, flooding, freezing-thawing (Blackwell et al., 2010). These physical stresses increase mineralisation of soil organic matter (SOM) and mobilise nutrients from soil solid phase into solution phase (Blackwell et al., 2010). The frequency and intensity of these physical stresses is likely to increase due to changing pattern of climate (Stocker et al., 2013; IPCC, 2014) with increase ambient temperature and variations in rain-fall pattern, and thus may have implications for soil fertility and surface water quality.

Climate change mediated intense episodic rain-fall events have been suggested to increase soil erosion (Kostaschuk et al., 2003; Zhang and Nearing, 2005). Zhang and Nearing (2005) predicted increase in run-off and soil erosion at central Oklahoma for the period of 2070-2099 due to increased frequency of large storms. Previously, Kostaschuk et al. (2003) measured high suspended solid concentrations during major flood periods caused by high intensity tropical cyclones, TC Joni and TC Kina, in the River Rewa, Fiji.

Nutrients loading from land to surface waters have been predicted to increase (Hagg et al., 2014; Huttunen et al., 2015). Huttunen et al. (2015) predicted that phosphorus and nitrogen loading to the Baltic sea may increase in future under changing climate. These projections are consistent with recent laboratory experiments and field studies on paddy soils, wetlands and flood plains linking enhanced P loading into surface waters with elevated runoff and flash floods (Loeb et al., 2008; Caetano et al., 2012; Chen et al., 2013; Lee et al., 2013; Li et al., 2015;Yan et al., 2015). For example, Chen et al. (2013) reported enhanced particulate-P loading into rivers of Taiwan due to increase in rainstorm triggered soil erosion. In a field study, Drewry et al. (2009) recorded elevated levels of suspended solids and TP in Tuross River during storm events. Likewise, Lee et al. (2013) recorded high levels of total P **(**> 5 mg L^{-1}) in drainage and leaching from a paddy soil. Figure 2.1 outlines an overview of the potential impacts of climate change on surface waters quality due to enhanced mobilisation of nutrients.

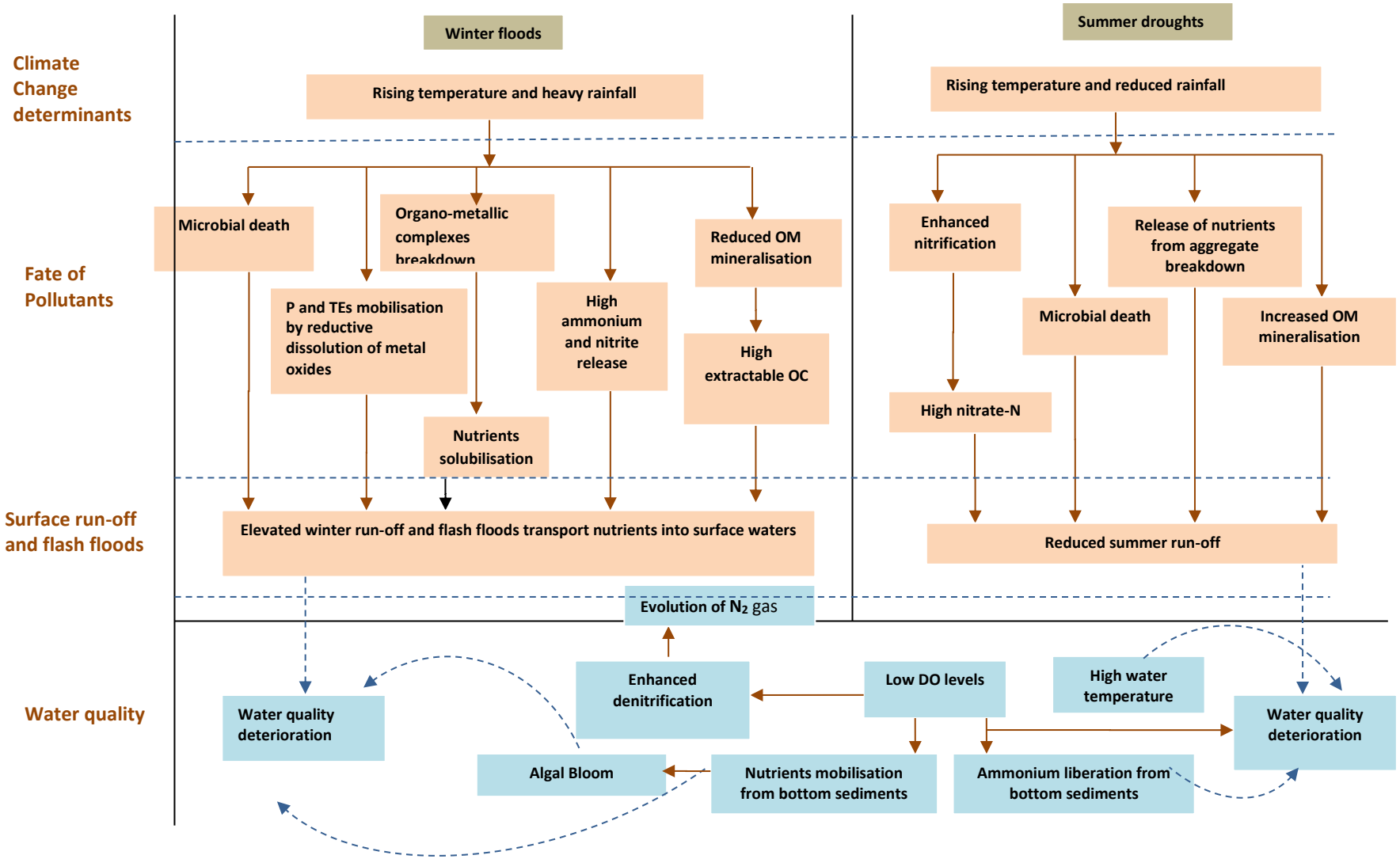

Figure 2.1: A schematic diagram outlining the potential impacts of climate change on surface water quality due to enhanced mobilisation of nutrients.

Drying-rewetting (DRW) and freeze-thaw (FT) provoked increased mobilisation of nutrients have often been attributed to disruptive effects of these processes on microbial biomass (Turner et al., 2003; Gordon et al., 2008; Achat et al., 2010; Turner and Romero, 2010; Bunemann et al., 2013), aggregates stability (Fierer and Schimel, 2002; Xiang et al., 2008; Xu et al., 2011; Bunemann et al., 2013), and stability of organic matter and organo-metallic complexes (Turner and Haygarth, 2003; Peltovuori and Soinne, 2005; Styles and Coxon, 2006; Butterly et al., 2009, 2011; Soinne et al., 2010). Studies have shown DRW and FT induced increased mobilisation of dissolve organic carbon (DOC) (Grogen et al., 2004; Koopmans and Groenenberg, 2011), phosphorus, (Nguyen and Marschner, 2005; Blackwell et al., 2010; Bunemann et al., 2013), nitrogen (Gordon et al., 2008) and micro-nutrients (Peltovuori and Soinne, 2005; Koopmans and Groenenberg, 2011). The response to wetting or thawing in terms of quantities of nutrients mobilised varies with soil type (Zhao et al., 2010; Achat et al., 2012a,b), quantity of soil organic matter (Butterly et al., 2009, 2011; Soinne et al., 2010), soil hydrophobicity (Zhang et al., 2007), microbial tolerance to withstand soil stresses (Styles and Coxon, 2006; Gordon et al., 2008; Bunemann et al., 2013), frequency (Wu and Brookes, 2005; Freppaz et al., 2007; Hentschel et al., 2007; Xiang et al., 2008), rate (Blackwell et al., 2009; 2013), intensity (Sardans and Penuelas, 2007; Bunemann et al., 2013) and duration (Forber et al., 2017) of soil DRW and FT stresses.

In order to analyse the disruptive effects of these soil physical stresses (DRW, FT and flooding processes) both laboratory and field scale studies on soils from grasslands (Fierer and Schimel, 2002; Wu and Brookes, 2005; Gordon et al., 2008), forests (Turner and Romero, 2010; Achat et al., 2012a,b), pastures (Turner et al., 2003; Turner and Haygarth, 2003), arable lands (Yanai et al., 2004), wetlands (Bruland and DeMent, 2009; Berryman et al., 2009), flood plains (Loeb et al., 2008) and marshes (Lai and Lam, 2008) and have been considered. The review has been structured to first highlighting the sources of nutrients (macro and micro) mobilisation under the effect of DRW and FT, which include: (1) microbial biomass destruction, (2) aggregate breakdown, (3) disruption of organic matter and organo-metallic complexes. This discussion is followed by factors determining quantities of nutrients into leachates. These factors include: (1) soil type, (2) soil hydrophobicity, (3) microbial adaptations to withstand stresses, (4) intensity (5) duration, (6) frequency, and (7) rate of soil DRW and FT stresses. This section is then proceeded with impacts of drying and flooding of soils on nutrient dynamics. This section critically highlights both biotic and abiotic factors affecting nutrients mobilisation and release in flooded soils. These factors include: (1) pH, (2) redox potential, (3)

metal oxides, (4) organic matter, (5) sulphates, (6) Ca/Mg – P system, and (7) microbial biomass.

2.2 Sources of nutrients mobilisation

2.3 Microbial Sources

2.3.1 Microbial responses to soil drying-rewetting

Mechanisms involved in nutrients release from microbial biomass under the influence of soil DRW or FT stresses cannot be better understood without knowing how microbes would respond to these physical stresses. However multiple phenomena have been attributed in terms of how microbial biomass would respond to these stresses e.g. equilibration, anhydrobiosis, and dormancy. As for example, equilibration involves minimising cellular water to avoid dehydration by increasing cellular solute concentration whilst soil is going through a drying stress (Blackwell et al., 2010) or the same process is referred as 'anhydrobiosis' in cold ecosystems used by some microbial species to avoid formation of ice crystal by minimising cellular water (Storey and Storey, 2005). However, when a dried soil is rewetted microbes must have to dispose of previously accumulated solutes to avoid cell lysis due to osmotic shock (Halverson et al., 2000). These disposed of solutes can be mineralised and re-assimilated by the surviving microbial population or dispose of into soil solution which then can potentially be leached. The pulse of CO_2 generated or the flush of nutrients released following rewetting of dried soils have often been attributed to mineralisation of osmolytes (cellular solutes) (Nguyen and Marschner, 2005; Schimel et al., 2007). Figures 2.2 and 2.3 diagrammatically present microbial responses to drying-rewetting and freezing-thawing stresses respectively.

Low soil moisture content reduces microbial activity due to reduction in water dependent microbial activities (Schimel et al., 1999; Emmett et al., 2004; Cisneros-Dozal et al., 2007) as for example has been seen in shrub land soils where soil drying reduced respiration by 29% (Emmett et al., 2004). Conversely, microbial activity and respiration rate increase concomitantly with the increase in moisture content perhaps due to restoration of water dependent microbial processes (Schimel et al., 1999; Cisneros-Dozal et al., 2007). This is supported by Cisneros-Dozal et al. (2007). They observed that leaf litter decomposition rate increased from 6 to 63 mg cm^{-2} h^{-1} upon wetting as indicated by an increase in soil respiration from 5 to 37%.

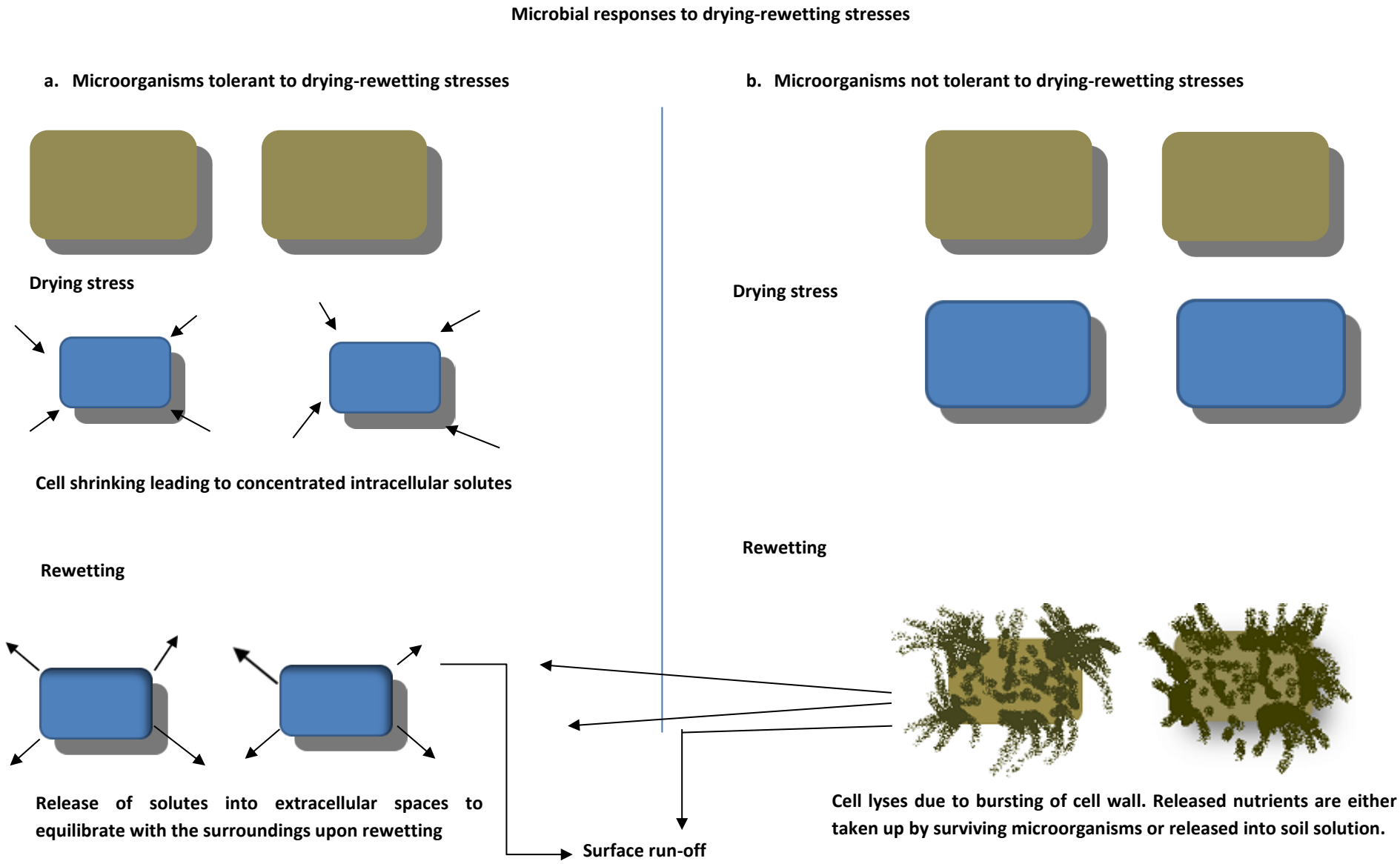

Figure 2.2: Microbial responses to drying-rewetting stresses and their contribution to nutrients release (a) Microorganism tolerant to drying-rewetting stresses, (b) Microorganism not tolerant to drying-rewetting stresses.

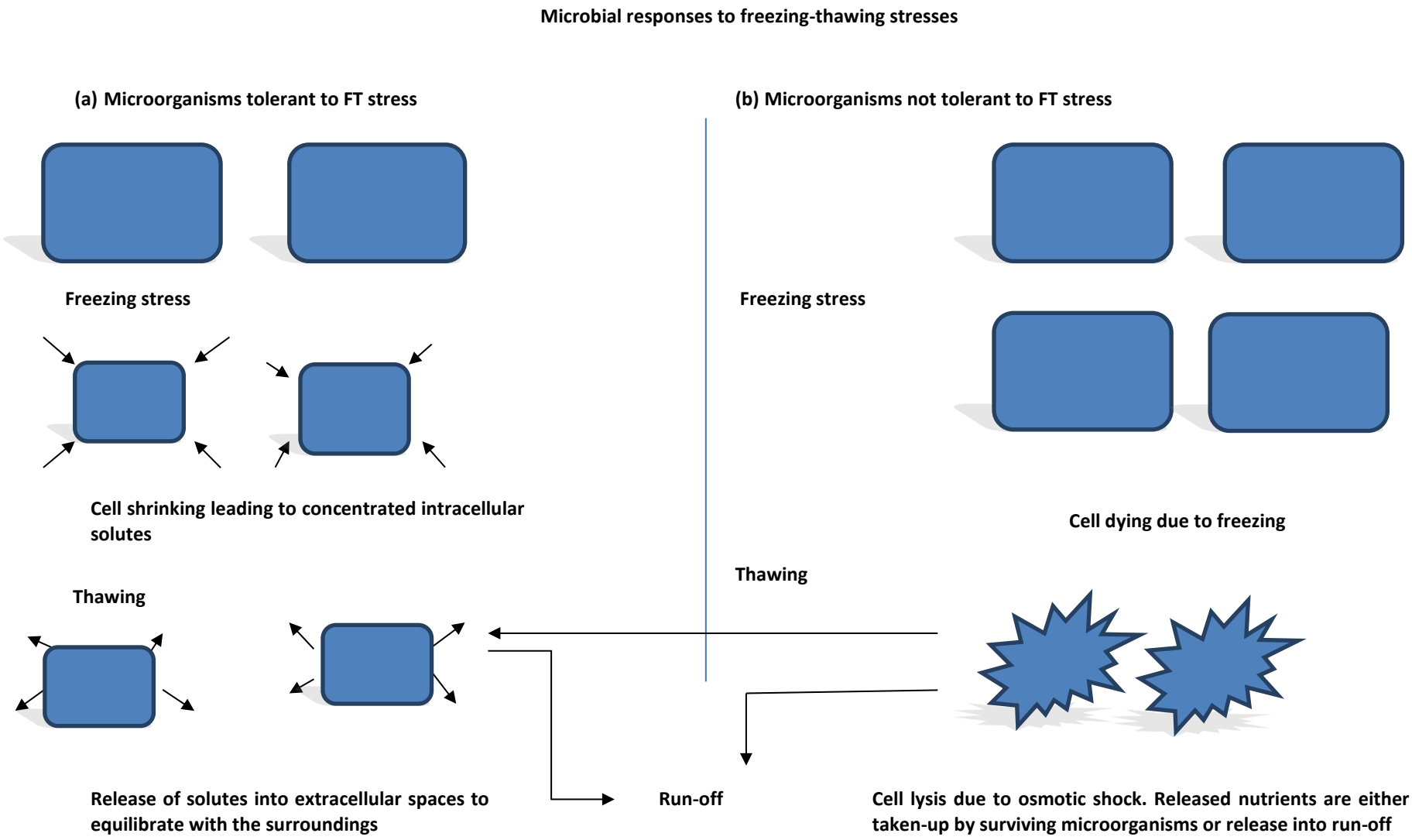

Figure 2.3: Microbial responses to freezing-thawing and their contribution to nutrients release: (a) Microorganisms tolerant to freeze-thaw stresses, (b) Microorganisms not tolerant to freeze-thaw stress.

2.3.2 Microbial contribution to nutrients release

Although soil microbial biomass comprises only a small percentage (3%) of soil organic carbon, it may contain large quantities of nutrients (Blackwell et al., 2010). Brookes et al. (1984) estimated that the size of mean annual flux of P through biomass (biomass P) may range from 23 kg P ha^{-1} $year^{-1}$ in grassland to 7 kg P ha^{-1} $year^{-1}$ in arable soils. In recent years, numerous studies have tested the effect of soil DRW and FT disturbances on microbial biomass. Birch leaves litter study with significantly low respiration rates in dried litter leaves raised many questions (Schimel et al., 1999) but now it has mostly been cleared that reduction in respiration rates following a drying stress is perhaps associated with reduction in microbial biomass or loss of enzymatic activity (Wo and Brookes, 2005; Blackwell et al., 2010; Kaiser et al., 2015). Mondini et al. (2002) for instance reported decrease in the size of microbial biomass by up to 13% for microbial biomass carbon (C) and 30% for ninhydrin reactive nitrogen (N) relative to the moist controls in a DRW study. Reduction in microbial biomass following freeze-thaw experiments has also been reported (Yanai et al., 2004). Yanai et al. (2004) for instance reported that four freeze-thaw cycles (-13°C, freeze and +4°C, thaw) significantly reduced the amount of soil microbial biomass C and N by 6 to 40% in arable and forest soils respectively. Nevertheless, rewetting of dried soils is often followed by a pulse of respiration due to restoration of microbial activity and recovery of biomass – the Birch effect (Schimel et al., 2018).

Microbial contribution to the enhanced nutrients extraction following a DRW or FT stress has long been known (DeLuca et al., 1992; Vaz et al., 1994). Since then many studies have added to the increasing body of knowledge assessing negative effects of DRW and FT stresses on microbial biomass (Gordon et al., 2008; Turner and Romero, 2010; Achat et al., 2012a, b). Koopmans et al. (2006) and Bunemann et al. (2013) reported increase in extraction of molybdate unreactive phosphorus (MUP) from dried soils. Likewise, Turner and Haygarth (2003) observed that air-drying (30°C for 7d) increased mean bicarbonate extractable inorganic P from 14.8 to 22.5 mg P kg^{-1} and the mean bicarbonate extractable organic P from 17.4 to 25.7 mg P kg^{-1} in 29 permanent lowland pasture soils from England and Wales. The increased extractability of P in these studies was attributed to drying-rewetting induced microbial cell lysis. Similar results of increased nutrients extractability following freeze-thaw manipulations have also been reported by Herrmann and witter (2002). However, studies reporting increased leaching of nutrients following drying-rewetting or freeze-thaw manipulations are limited (Blackwell et al., 2013). Both laboratory and field-based studies determining the extent of

nutrient leaching would perhaps give a clear idea, how climate change driven soil processes are likely to affect nutrients mobilisation and their potential leaching.

2.4 Non-microbial Sources

2.4.1 Aggregate breakdown

Soil aggregates stabilise soil organic matter within soil structure and protect it from decomposition (Six et al., 1998; 2000). Soil aggregation is important in controlling nutrients sequestration (Six et al., 2000; Nesper et al., 2015). Findings from both Fonte et al. (2014) and Nesper et al. (2015) suggest that organic P retention in soils is associated with greater protection of organic matter in soil aggregates. Nesper et al. (2015) and Fonte et al. (2014) found that organic P concentrations were 37% and 40% respectively higher in soils of productive pasture compared to those measured in degraded pasture. Reduced physical protection of organic matter in degraded pasture soils due to a fewer large macro-aggregate could be the reason of loss of organic P in degraded pastures. Drying-rewetting and freeze-thaw are known to be associated with physical disruption of soil aggregates (Denef et al., 2001b; Six et al., 2002; Oztas and Fayetorbay, 2003; Austin et al., 2004). Oztas and Fayetorbay (2003) reported reduction in aggregate stability by up to 51.7% due to freeze-thaw effect.

Aggregate disruption under the influence of water - known as ‘slaking’, occurs due to various mechanisms e.g. breakdown of aggregates due to compression of air entrapped during wetting, micro-cracking due to differential swelling of clays, mechanical breakdown by running water or rain drop impact (Chenu et al., 2000; Bunemann et al., 2013). Slaking have been seen to play a crucial role in accelerating mineralisation of organic matter and mobilising nutrients after wetting or thawing of soils (Turner and Haygarth, 2003; Fierer and Schimel, 2002; Xiang et al., 2008; Xu et al., 2011; Bunemann et al., 2013). Xu et al. (2011) reported increased extractability of resin-P by up to 121% in organic matter rich forest soils. Likewise, Bunemann et al. (2013) and Koopmans et al. (2006) observed that rewetting of dried soils significantly increased the concentration of molybdate reactive phosphorus (MRP) and molybdate unreactive phosphorus (MUP) in water extracts. These studies attributed this increase to the exposure of new surfaces and organic matter that were previously not available for microbial decomposition (Chenu et al., 2000; Borken and Matzner, 2009). Increased extractability of nutrients has also been reported in laboratory and field studies due to disruptive effect of freezing-thawing on aggregate stability (Hentschel et al., 2007).

Nevertheless, it is also evident that DRW or FT induced breakdown of aggregate besides accelerating mineralisation by provision of labile organic compounds, also provides new surfaces for nutrients adsorption and thus somehow reduces nutrients availability (Nguyen and Marschner, 2005; Fonte et al., 2014; Nesper et al., 2015). However, it is still unclear which two of these mechanisms predominately influence nutrients dynamics.

The effect of physical stresses in promoting mineralisation of organic matter by breakdown of organic matter can be best understood by tillage and non-tillage management practices. Non-tillage practices are known to increase water stable-aggregates and improve soil aggregation and retention of soil organic matter (Six et al., 1998; 2000). On the contrary, tillage practices promote decomposition of organic matter and influence nutrient losses by continually exposing soil surfaces to freeze-thaw or drying-wetting and, thus promoting aggregate breakdown (Six et al., 1998; 2000). Further, tillage practices are known to promote mineralisation of SOM by aggregate breakdown and thus allowing microbial access to SOM previously protected within soil aggregates. This process alone is known for maintaining higher amounts of SOM in no tillage or reduced tillage soils. Previously, Beare et al. (1994) have shown that organic carbon was 18% higher in non-tilled than in conventional tilled soils. Higher retention of organic carbon in non-tilled management system was suggested to be linked with higher amounts of macro-aggregates. The disruptive effect of thawing or wetting is more detrimental for macro-aggregates than for micro-aggregates because increase in aggregate size reduces soil surface resistance to breakdown by thawing or rewetting effect. Figure 2.4 highlights DRW and FT induced sources and sinks of nutrients.

2.4.2 Disruption of organic matter and organo-metallic complexes

Drying soils has often been seen to increase solubilisation of organic matter, which could either be contributed by microbial biomass (Suda et al., 2009), desorption from soil new exposed surfaces (Ashworth and Alloway, 2004; 2007; Provin et al., 2008) or drying induced breakdown of organo-metallic complexes (Koopmans and Groenenberg, 2011).

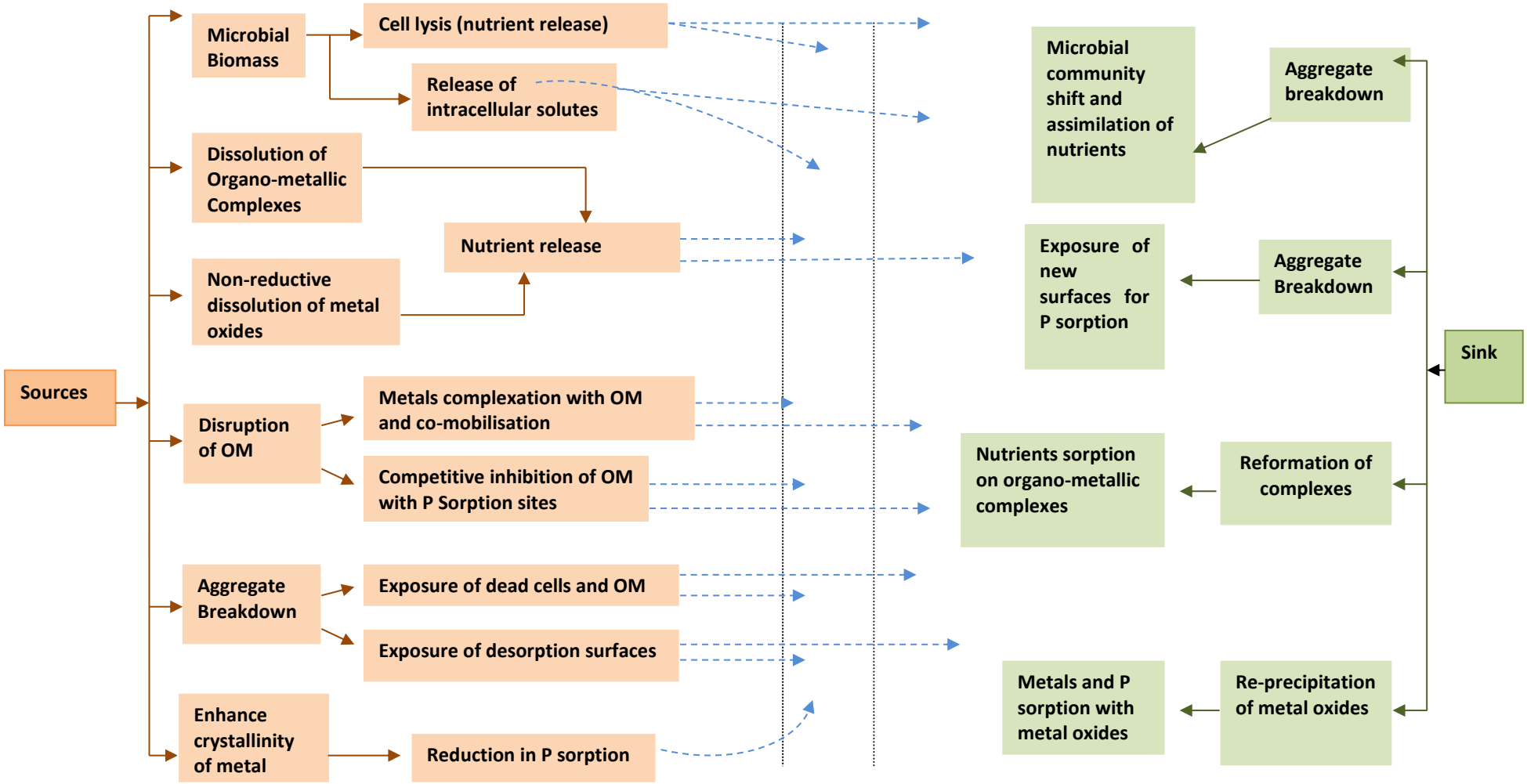

Figure 2.4: DRW and FT induced sources and sinks of nutrients

Soils with high proportion of organic matter (OM) have been seen to be more influenced by drying-rewetting stresses and tend to solubilise greater quantities of phosphorus and micronutrient metals (Styles and Coxon, 2006; Achat et al., 2010). However, the mechanisms involved are not entirely clear. Erich et al. (2002) proposed that organic matter effects phosphorus desorption either by competing with phosphorus binding sites on soil surfaces or breakdown of organo-metallic complexes. Phosphorus is unlikely to make direct bond with organic matter due to the presence of negative charges on both phosphate and organic matter at pH in the neutral and alkaline range (cf., Novak and Watts, 2006). However, negatively charged organic matter can bind positively charge metal ions (Fe, Al, Mn, Ca and Mg) which then bind phosphate anions to create an organo- metallic-P complex. Many metals like Co, Cu and Ni show high binding affinities for organic matter (Ashworth and Alloway, 2004; 2007; Zhao et al., 2007). There is considerable evidence in literature suggesting that soil drying-rewetting increases solubilisation of phosphorus and metals through breakdown of organo-metallic complexes (Peltovuori and Soinne, 2005; Styles and Coxon, 2006; Koopmans and Groenenberg, 2011). Peltovuori and Soinne (2005), for instance showed that air-drying increased extractability of P, Al, Fe and Mn in mineral soils presumably due to drying induced breakdown of organo-mineral complexes. Koopmans and Groenenberg (2011) also reported drying induced significant increase in the concentrations of total dissolved organic matter and total dissolve concentrations of Cu, Ni and P in forest soils. They attributed the release of dissolved organic matter along with adsorbed metals and P to the drying induced disruption of organo-metallic complexes. Soinne et al. (2010) linked increase in NaOH extractable MUP with drying induced breakdown of organo-mineral complexes and degradation of organic matter. The underlying mechanism involved is drying induced breakdown of hydrogen bond, shrinkage and reduced stability of organo-metallic complexes (Raveh and Avnimelech, 1978; Turner and Haygarth., 2003; Styles and Coxon, 2006; Soinne et al., 2010).

Another mechanism by which organic matter can potentially increase soil solution phosphorus concentration is competitive inhibitory effect of organic matter or its decomposition products (low molecular weight organic acids, humic acids and fulvic acids) with phosphorus sorption sites (Daly et al., 2001). This could be one of the reasons that soils with high quantity of organic matter (peat soils) tend to have poor phosphorus sorption capacity and have poor P reserves relative to soils with low organic matter contents i.e. mineral soils (Daly et al., 2001). This notion can be better understood by studies where enhanced phosphorus availability to crops has been attributed to the addition of organic residues to soils (Yan et al., 2013). This is further supported by Erich et al. (2002) who claimed that addition of organic

residues to soils increased P availability to plants likely by reducing soil P sorption capacity and inducing P desorption. Increased P availability in soils greater than plants requirements may lead to P losses through runoff which may have consequences for soil fertility and surface water quality.

Soils with high quantities of organic matter often host high microbial population and tend to release greater quantities of nutrients derived from microbial biomass (Achat et al., 2010; Achat et al., 2012a, b). This is supported by a study where drying induced increase in water soluble inorganic P from forest soils with varying organic matter and metal (Al/Fe) oxides content, was correlated with OM/metal-oxide ratio (Achat et al., 2012a,b).

However, the role of organic matter in nutrients availability seems to be dual. Studies reporting increased extractability of nutrients upon soil drying have also reported decreased extractability upon rewetting of previously dried soils. This reversible effect could be linked with the reformation of freshly formed organo-metallic complexes and adsorption of trace metals and P with these complexes. Peltovuori and Soinne (2005) reported that drying induced increase in resin-P pulse was short-term and disappeared after a week of moist incubation. Previously, Haynes and Swift (1991) showed that oven and air-drying New Zealand soils increased extractability of micronutrients (Cu, Zn, Fe and Mn) and organic matter. However, the concentrations of extractable Cu, Zn and Fe rapidly decreased to their original field moist concentrations when soils were rewetted. The reversible drying effect on micronutrients extractability was suggested to be linked with physical disruption and reformation of organ-mineral complexes.

2.5 Factors affecting leaching of nutrients

While soil undergoes through thawing or rewetting, the quantity of nutrients mobilised is determined by several plausible factors: quantity of soil organic matter, soil nutrient content, soil hydrophobicity and microbial adaptation to withstand stresses, aggregate stability, intensity, duration, frequency and rate of physical stresses.

2.5.1 Quantity of soil organic matter

Soils rich in organic matter provide better protection to microbial biomass (Austin et al., 2004; Gordon et al., 2008) and can maintain their original biomass and microbial activity following drying-rewetting or freeze-thaw stresses (Yanai et al., 2004). Better adaptability of soil to maintain its original biological functions (biomass and activity) due to high organic matter content might be due to several reasons e.g. soil high in organic matter have reduced wettability due to hydrophobic characteristics of organic compounds and have a higher resistance to disaggregation following DRW or FT stresses (Zhao et al., 2010) (soil hydrophobicity is discussed in detail in section 2.5.2). Soil hydrophobicity provides microbial communities with enough time to equilibrate with surroundings by releasing compatible solutes into cellular surroundings upon rewetting or thawing, while aggregate resistance to disaggregation protects organic matter from microbial decomposition by reducing the effect of slaking. High protection of microbial biomass in organic matter rich soils often renders less nutrients releases from microbial origin (Gordon et al., 2008).

Nevertheless, it has also been seen that soils rich in organic matter are more susceptible to release nutrients under the influence of FT or DRW stresses (Borken and Matzner, 2009). A plausible explanation to this discrepancy could be that, although soils with high organic matter content provide better protection to soil organic matter and microorganisms due to aggregation, such soils tend to release greater quantities of nutrients upon rewetting or thawing via destruction of microbial biomass and exposure of labile organic matter through aggregate disruption (Nguyen and Marschner., 2005; Styles and Coxon., 2006; Achat et al., 2012a,b). This is supported by Xu et al. (2011) who reported that air-drying effect was more pronounced in forest soils with high organic matter content. Air-drying increased resin-P extractability by 31% for soils low in organic matter (sterile soil) and by 121% for soils rich in organic matter (forest soil). These results are in good agreement with those of Nguyen and Marschner (2005), Styles and Coxon (2006) and Achat et al. (2012 a,b); they showed that soil drying increased water-soluble inorganic phosphorus relative to the moist controls in soils rich in organic matter.

2.5.2 Soil hydrophobicity

Soil hydrophobicity or soil water repellence (SWR) is the reduction in the rate of wetting of dried soils after a rainfall event (Borken and Matzner, 2009). Organic compounds causing SWR are alkanes, waxes, fatty acids originating from fungal hypae, microbial biomass or plants litter (Goebel et al., 2007). Since hydrophobicity is linked to the soil organic matter

content, as in mineral soils it is less pronounced due to smaller organic matter content but is well pronounced in soils with high proportion of organic matter (Borken and Matzner, 2009).

Soil water hydrophobicity prevents water from infiltrating into the soil matrix and may increase surface run-off (Miyata et al., 2007). Soil hydrophobicity increases aggregate stability and protect SOM by stabilising them against water slaking (Goebel et al., 2007). Moreover, hydrophobicity slows down mineralisation rates as part of the soil organic matter have little or no contact with the water during wetting phase (Hentschel et al., 2007). Water repellent aggregates slowdown the increase in water potential following a wetting event. This slow increase in the water potential gives microorganisms more time to equilibrate with their surroundings by releasing compatible solutes and restore their metabolism. In strongly water repellent soils water tends to form droplet rather than a continuous water film, which is highly essential for the free movement of nutrients, enzymes and substrates for sustaining microbial activities (Goebel et al., 2007). Nevertheless, hydrophobicity is a short-lived process, eventually the soil does get properly wet and saturated.

Alongside organic matter, soil moisture content and soil temperature also control SWR. That means the longer the soil remains dry and higher is the soil temperature, the greater is the soil water repellence.

2.5.3 Microbial adaptation to withstand stresses

Microbial species appear to have different mechanisms of tolerance to soil physical stresses. For instance, fungi are more adapted to low water potentials (dry conditions) presumably because soil bacteria are immobile and are highly dependent on diffusion of soluble constituents for nutrition (Voroney, 2007). However, fungi have been seen to be more susceptible to repeated DRW or FT stresses compared with bacterial communities, possibly because fungal biomass is predominantly located on the surfaces of soil aggregates (Blackwell et al., 2010) and is potentially vulnerable to DRW and FT induced aggregate disruption, while the bacterial population is predominantly located in the centre of soil aggregates (Blackwell et al., 2010). Greater susceptibility of fungi to repeated DRW or FT stresses appears to make them a potential source of nutrients mobilisation under changing climate especially in soils which have high fungi to bacterial ratio.

It appears that soils which are rarely exposed to physical stresses either naturally or anthropogenically (intensive agricultural activities) are more likely to be affected by climate

change induced increased frequency of physical stresses since microbial biomass is not yet adapted to tolerate such stresses and even a mild single episode of these physical stresses can be extremely detrimental to microbial biomass and may increase nutrients mobilisation driven from microbial sources (Styles and Coxon, 2006; Bunemann et al., 2013). Fierer and Schimel (2002) have already shown that the smaller CO_2 pulse from the grassland soils was possibly linked with the better adaptation of their microbial communities to endure drying stresses, while a larger pulse from Oak soils was reflective of their less ability to tolerate drying-rewetting stress. Since Oak soils were stated to be rarely exposed to drying-rewetting events due to the presence of thick litter layer and canopy shading. Both Bunemann et al (2013) and Styles and Coxon (2006) linked increase extractability of microbially driven-P from dried soils due to lack of adaptability of biomass to tolerate DRW stresses. Table 2.1 highlights microbial responses to FT and DRW stresses in soils from varying land-uses and geographical locations.

Table 2.1: Microbial responses to FT and DRW stresses in soils from varying land-uses and geographical locations

Land use and geographical location	Stress Type	Temperature (°C)	Number of cycles	Microbial response to FT or DRW stresses	References
Tropical forest soils from Republic of Panama	FT	F* = -35˚C	-	– Decrease in microbial biomass P – Microbes were not adapted to cold temperatures	Turner and Romero (2010)
NSW Australia	DRW	Dried to < 0.012 g H_2O g^{-1} Soil Rewetted to 70% water content	3	– Reduction in biomass C and P after first DRW event – Microbial community shift in subsequent cycles with reduction in fungi and increase in Gram positive bacteria	Butterly et al. (2009)
Arable and forest soils from temperate and tropical regions	FT	F= -13°C (12 hrs) T**= +4°C (12 hrs)	4	– Microbial C and N decreased – Microbes were not adapted to cold temperatures	Yanai et al. (2004)
Subarctic heath soils	FT	T= +2ºC F= -4ºC	18	– Moderate temperature fluctuations had little effects on microbial biomass – Microbial activity remained high during thaw	Larson et al. (2007)
NE Italy, Arable, Woodland and Grassland soils	DRW	Dry = 25˚C (5d) Rewet = 40% WHC	1	– Decrease in microbial biomass C and N – Microbes were not adapted to DRW stress	Mondini et al. (2002)
Colorado alpine dry meadow soils	FT	T= +3ºC F= -5˚C	-	– Microbial biomass showed tolerances against temperature fluctuations and remained unaffected at moderate freeze-thaw events	Lipson et al. (2000)

Where *F and **T indicate freezing and thawing temperatures, respectively

2.5.4 Intensity of drying-rewetting and freezing-thawing stresses

Intensity of soil drying may be defined as the minimum amount of free moisture left in the soil before the next rewetting or thawing event. Microorganisms require a critical range of temperature and moisture to sustain their growth and cellular activities. Too high and too low moisture and temperature may not be ideal for microbial growth and may affect cellular activities due to inactivation of enzymes unless microorganisms are adapted to these unfavourable conditions (Van Gestel et al., 1993a,b; Sardans and Penuelas, 2005). Soil moisture has a strong effect on microbial biomass and microbial activity and in general, organic matter mineralisation rate decreases concomitantly with the increasing intensity of drying (Borken and Matzner, 2009). The effect of soil moisture reduction on mineralisation and microbial biomass has long been known, with (Schimel et al., 1999) reporting slightly larger flush of microbial activity and mineralised carbon from Birch leaves litter dried to 10% of water holding capacity relative to the litter dried to 25% of water holding capacity (WHC) which they suggested was due to a larger die-off of microbial population in the 10% WHC treatment. Since then the initial flush of respiration and nutrients have been reported by many studies on soils and litter studies (Fierer and Schimel, 2002; Xiang et al., 2008) and is believed to be originated from mineralisation of organic substrates released by microbes via cytoplasmic release as a method of equilibration or cell disruption due to osmotic shock upon rewetting. It is also believed that upon rewetting, soils which were previously dried to a greater intensity tend to have higher mineralisation rates and release greater quantities of nutrients upon rewetting. The most plausible explanation to this could be that more intense soil drying causes a greater stress to microbes (alongside soil structure), and release greater quantities of nutrients upon rewetting or thawing as is evident by many studies (Grogan et al., 2004; Turner and Haygarth, 2001; Sardans and Penuelas, 2007; Bunemann et al., 2013). Bunemann et al. (2013) reported significantly greater release of resin extractable phosphorus (7.6 mg P kg^{-1}) upon rewetting from soils dried to low water contents relative to the soils dried to higher water contents (0.7 mg P kg^{-1}). Likewise, in a FT study, Brookes et al. (1998) reported significantly greater NO_3 leaching (1.14g NO_3-Nm^{-2}) from discontinuous snow cover with frost temperature reaching -11°C compared to the continuous snow cover (0.27g NO_3 Nm^{-2}) with frost temperature reaching -5°C. Grogan et al. (2004) concluded that moderate freeze-thaw fluctuations can have only minimal effects on microbial biomass pool in sub-arctic heath tundra and result in small quantities of inorganic N in leachates. In contrast, severe freeze-thaw regimes can result in a significant increase in N mineralisation and losses (De Luca et al., 1992).

The underlying mechanism of enhanced nutrients release at extreme low temperatures is that when soil water freezes below 0°C, there is still a thin film of water around soil particles in which microbes not only survive but continue their activity. As the freezing intensity increases progressively more water from cell solution contribute to the formation of ice-crystals leaving behind highly concentrated solutes.

2.5.5 Duration of drying-rewetting and freezing- thawing stresses

As the freezing proceeds, progressively more water from the soil solution is converted into ice crystals, leaving soil aggregates more dehydrated (Blackwell et al., 2010). Just like as the soil progressively dries, the water film surrounding the soil particles becomes thinner, disrupting and limiting diffusion of solutes (Schimel et al., 2019). Extended periods of drying or freezing may cause substantial reduction in microbial activity and microbial biomass due to reduction in diffusive transport of soluble nutrients and water dependant activities for prolong period (Blackwell et al., 2010). The detrimental effect of extended period of drying on microbial biomass has long been known as for instance Schimel et al. (1999) showed that two weeks drying of Alaskan taiga birch litter reduced microbial biomass and activity more than the shorter periods of drying (Schimel et al., 1999). Soil extended drying or freezing and subsequent reduction in microbial biomass could reduce mineralisation of organic matter, but may also increase nutrients losses upon wetting or thawing due to several reasons e.g. accumulated dead plant residues and dead microbial cells during drying phase, release of intracellular solutes as a method of equilibration during wetting, aggregate disruption and exposure of previously protected labile organic matter due the effect of slaking (Borken and Matzner, 2008). Previously, in a freeze-thaw study, Vaz et al. (1994) showed that extended period of freezing from 3 hrs to 50 days increased concentrations of soluble phosphorus in organic peaty podzol grassland soil.

Prolonged drying will not only kill microbial cells but can slow down growth rate of the surviving population by causing sub-lethal damages to DNA, proteins, membranes and cell walls (Meisner et al., 2015; Rahman et al., 2018). However, no information so far is available suggesting if there is any link between reduced microbial growth after being exposed to prolong drought and enhanced nutrients availability upon rewetting. Some microorganisms produce extracellular polymeric substances (EPS) to increase their survival by retaining moisture under drought (Schimel et al., 2018) and most of the microorganisms are known to produce higher concentrations of EPS at a temperature range of between 25ºC to 30ºC (More et al., 2014).

Table 2.2: Summary of the effects of DRW and FT frequency on nutrients mobilisation in soils with varying textures, land-use types and geographical locations

Land use and geographical location	Soil Texture	Stress type	Temperature (ºC) and Duration	Frequency (number of cycles)	Conclusive remarks	Sources of nutrients	References
Forest, Northeast China	-	FT	Freeze = -5 or - 25 Thaw= + 5	15	– Increased total inorganic nitrogen	– Aggregate disruption – Microbial cell lysis	Zhou et al. (2011)
Annual grassland, California, USA	Loam	DRW	Dry = 5% MC Moist = 35% MC	4, 6 and 12	– Increased CO_2 pulse – Increased N, C and P mineralisation	– Aggregate disruption	Xiang et al. (2008)
Western Alps, Pennines	Sandy	FT	Freeze = -9 (12 hrs) Thaw= +4 (12 h)	4	– Increased extractability of nitrogen and phosphorus	– Microbial cell lysis	Freppaz et al. (2007)
Perennial oak and annual grassland, California, USA	Loam (oak) and Clay loam (Grass)	DRW	Dry = -30 MPa (3 days) Rewet= -0.01MPa (6 days)	1	– Rapid pulse of CO_2 – Increased extractable organic carbon	– Mineralisation of intracellular solutes	Fierer and Schimel (2002)
Grassland, New Zealand	Silt loam	DRW	Dry = 22°C (48h) Rewet = 32% W/W	2	– Increased extractability of phosphorus	– Aggregate disruption – Increased mineralisation of OM due to exposure of new surfaces	Chepkwony et al. (2001)
Grassland, UK	Organic peaty podzol	FT	Freeze = -12 (3h) Thaw = +4 (17h)	8	– Increase in phosphorus solubilisation	– Aggregate disruption – Disruption of biomass	Vaz et al. (1994)

It is also likely that rewetting of soils after being exposed to prolonged drought triggers release of nutrients (e.g. phosphorus and metals) from these structures since a large amount of phosphorus is accumulated in EPS by phosphorus accumulating microorganism (Zhang et al., 2013).

2.5.6 Frequency of drying-rewetting and freezing-thawing cycles

Increased nutrient extractability concomitantly with the increased frequency of DRW and FT stresses has been reported by many workers (Chepkwony et al., 2001; Bechmann et al., 2005; Freppaz et al., 2007) (Table 2.2). For example, Freppaz et al. (2007) reported increased extractability of total dissolve nitrogen (TDN) and total dissolve phosphorus (TDP) in Alpine soils after 4 freezing cycles. Bechmann et al. (2005) reported that repeated freezing and thawing stresses significantly increased release of water extractable phosphorus (WEP). In a DRW study Chepkwony et al. (2001) claimed that subjecting a New Zealand silt loam grassland soil to two DRW cycles increased extractability of phosphorus. A combination of mechanisms seems to be involved in causing increased extraction of nutrients when dried soils are rewetted e.g. aggregate disruption (Denef et al., 2001b; Six et al., 2002; Oztas and Fayetorbay, 2003; Austin et al., 2004), microbial cell lysis (Turner et al., 2003; Turner and Haygarth, 2003; Styles and Coxon, 2006; Achat et al., 2010; Turner and Romero, 2010; Achat et al., 2012a,b; Bunemann et al., 2013), dissolution of organo-metallic complexes (Raveh and Avnimelech, 1978; Turner and Haygarth., 2003; Styles and Coxon, 2006; Soinne et al., 2010), and reduced nutrients sorption on metal oxides due to drying induced increased crystallinity (Qiu and McComb, 2002; Schonbrunner et al., 2012; Dieter et al., 2015).

Nevertheless, studies reporting increased nutrients extractability also noted decrease in nutrients extractability with successive DRW or FT cycles. They attribute this decrease to the limitation in labile organic matter pool (Fierer and Schimel, 2002; Butterly et al., 2009, 2011), resistance to aggregate slaking (Denef et al., 2001a) and microbial community shift (Butterly et al., 2009, 2011). Pezzolla et al. (2019) reported that microbial biomass phosphorus decreased after the first DRW event but recovered after the second event likely due to resilience of certain species of microbes to these perturbations. Zhang et al. (2007) observed a decrease in soil respiration rate after first wetting drying cycle but noticed recovery in respiration rates in the subsequent wetting drying cycles. Microbial species surviving the first rewetting event are considered to be more adapted and able to increase their biomass by assimilating available substrates. This causes a shift in community which is more capable to withstand stresses

(Butterly et al., 2009, 2011). However studies (Magid et al., 1999, Butterly et al., 2009, 2011; Zhou et al., 2011) have also shown that microbial population partly regain biomass and activity upon rewetting but not to the extent maintained by microorganism in continuously moist or non-dry conditions likely due to drying induced sub-lethal damages to cellular structure (Meisner et al., 2015; Rahman et al., 2018).

Another factor contributing to the reduced mineralisation rates and nutrients release in successive FT or DRW cycles is aggregates resistance to slaking (Hentschel et al., 2007; Zhou et al., 2011). Slake resistance of aggregates prevents exposure of labile organic matter from microbial decomposition. For instance, Zhou et al. (2011) state that N mineralisation rate decreased with repeated freeze-thaw cycles, FTCs (15 cycles) and Hentschel et al. (2007) reported reduced concentration of DOC in the percolates after the first FT cycle. In both studies, reduced N mineralisation rates and release of DOC concurrent with the number of freeze-thaw cycles were thought to be associated with aggregates resistance to slaking.

Depletion in the quantity of labile organic matter held within micro-aggregates could also be another reason causing reduced nutrients release with repeated DRW or FT cycles. Nevertheless, limitation of labile organic matter seems to be an unlikely phenomenon under natural field conditions with a continuous input of organic matter in form of dead leaves, litter and soil macro-and micro-organisms. So, if limitation of organic matter is unlikely factor, slake resistance of aggregates and microbial community shift would be the likely controlling factors determining the concentration of nutrients to be leached in natural field environments. Also, it appears that history of soil drought (intensity and duration) determine how soils are likely to respond in terms of nutrients mobilisation in subsequent rewetting cycles. However, extended period of soil drying is most likely to cause shift in microbial community structure due to destructive effect of extended drying on microbes and only species which are capable of surviving such intense conditions e.g. spore formers (Meisner et al., 2015) are capable to increase their biomass rapidly by assimilating freshly available substrate – thus lesser mineralisation rate, assimilation of available substrates by surviving microorganisms and a shift in community structure due to growth of certain more adapted species of microbial population would undoubtedly explain reduced leaching of nutrients in subsequent rewetting events. Nevertheless, based on the current knowledge it is difficult to explain if the interaction of drought history (duration and intensity) and frequency of DRW/FT stresses has any combined effect on mobilisation of nutrients. There is no conclusive agreement among recent studies with some reporting increased nutrients extraction with increasing frequency of DRW

cycles, whilst other reporting decreased nutrients extraction. Clearly, further research would help understand many unanswered questions.

2.5.7 Rate of drying-rewetting and freeze-thaw stresses

The rate at which soils are rewetted or thawed affects quantities of nutrients in the leachate (Blackwell et al., 2009; 2013). One of the logical explanations to such differences in nutrients mobilisation could be associated with varying microbial responses to fast and slow rewetting or thawing. In slow rewetting and thawing, slow change in water potential allows microorganism enough time to equilibrate with their surroundings and may cause less stress to microbial cells. In contrast, rapid rewetting or thawing – with a sudden increase in water potential cause cell lysis due to osmotic shock (Blackwell et al., 2010). Slow rewetting allows less microbial biomass destruction thus a greater surviving microbial population will be available to mineralise freshly exposed labile organic matter resulting in greater concentration of nutrients in leachate.

Rapid rewetting whilst promoting aggregate destruction and particle detachment also promotes preferential flow. Thus, allowing water to move through the soil profile by making channels instead of percolating evenly through the soil profile, subsequently interacting with less surface area for nutrients release. This could be one of the reasons that in rapid rewetting, particulate nutrient forms predominate because of quick fixation of dissolve forms with soil minerals, whereby particulate forms escape from fixation and are easily transported (Toor et al., 2004; Blackwell et al., 2009). This explanation is supported by a study conducted by Toor et al. (2004). They observed higher P losses in particulate unreactive forms in leachate from intact soil monoliths during high intensity flood irrigation season. However, in non-irrigation season, the amount of dissolve unreactive P was higher than particulate unreactive P, perhaps due to much lower amount of natural rainfall and subsequently much less mobilisation of particles. This is supported by the findings of highest dissolve P concentrations in the leachate from slow rewetting treatments, while particulate P concentrations were highest in the leachate from fast rewetting treatment (Blackwell et al., 2009). The same logic seems to be realistic as under natural field conditions whereby heavy torrential rains have been reported to mobilise greater quantities of nutrients in particulate forms (Drewry et al., 2009; Chen et al., 2013), whilst nutrients loading from land to catchment water has been projected to increase (Hagg et al., 2014; Huttunen et al., 2015), with more intense episodic rainfall events (Kostaschuk et al., 2003; Zhang and Nearing, 2005).

2.6 Impacts of extended period of drying followed by flooding on nutrients mobilisation

Soils act as a natural sink for nutrients and many other anthropogenic contaminants due to their organic and inorganic constituents (organic matter, clay, Fe/Mn-oxy hydroxides etc.). Changing pattern of climate can influence soil processes, potentially making soil systems a source of nutrients/contaminants loss to water. More intense episodic rains, as predicted (IPCC, 2014) can cause soil saturation or flash floods, especially in low-lying landscapes. Extended periods of flooding can impair quality of surface waters by mobilising and transporting nutrients and trace metals from land to surface waters (Kashem and Singh, 2001; Van Vliet and Zwolsman, 2008; Shaheen et al., 2014a; Amarawansha et al., 2015). Mobilisation and transportation of phosphorus from seasonally flooded soils is a concern as phosphorus is eutrophication-limiting nutrient (Carpenter, 2008; Lewis et al., 2011; Kolahchi and Jalali., 2013).

In flooded soils phosphorus dynamics (retention-release) across soil-water interface are governed by a number of biological e.g. release of P from mineralisation of organic matter, microbial cell lysis, activity of facultative bacteria (Wright et al., 2001) and non-biological mechanisms e.g. reductive dissolution of Fe/Mn oxy/hydroxides or non-reductive dissolution of Fe- and Al-phosphate (Chacon et al., 2005), solubilisation of metal-OM-P complexes, solubilisation of Ca/Mg-phosphate) (Bostrom et al., 1988; Reddy et al., 1999). These mechanisms are controlled by a number of factors such as: soil type, soil moisture content prior to flooding, temperature, pH, redox potential, P concentrations in native soil and overlying water, and microbial biomass (Bostrom et al., 1988; Baldwin et al., 2000; Wright et al., 2001; Novak and Watts, 2006; Unger et al., 2009; Dieter et al., 2015). The following sections consider the underlying factors involved in mobilisation of P and trace elements in flooded soils.

2.6.1 Mineralisation of organic matter under anaerobic conditions

As flooding proceeds, within a few hours of saturation, microorganisms utilise dissolve O_2 present in water and trapped in soil, thus rendering flooded soil devoid of molecular O_2. In the absence of O_2, facultative and obligate anaerobes sequentially use alternative electron acceptors such as NO_3^-, Mn^{+4}, Fe^{+3}, SO_4^{-2} (Ponnamperuma, 1972) and reduce them as follows: NH_4^+, Mn^{+2}, Fe^{+2}, H_2S respectively. Absence of oxygen, presence of organic matter and anaerobic microbial activity are the basic requirements for the development of reducing conditions in flooded soils. The extent and rate of reduction depends upon the amount of organic matter, soil temperature, pH and redox potential (Kogel-Knabner et al., 2010). Under oxygen deficiency intermediate products like simple fatty acids, hydroxyl-carbonate and polycarboxylic acid, alcohols and ketons are produced due to incomplete decomposition of organic compounds, which are further decomposed into CO_2, methane and hydrocarbons. Thus, flooded soils may contain both organic and inorganic compounds in reduced forms, which have tendency to donate electrons and combine with oxygen (Kogel-Knabner et al., 2010).

Soil organic matter mineralisation decreases with increasing moisture content or in other words mineralisation rates are lower in anaerobic conditions compared to aerobic as demonstrated by Lewis et al. (2014) where soil carbon mineralisation decreased with increasing soil saturation and inundation. This is perhaps because of less energy demands of anaerobic microorganisms and production of fatty acids. These products inhibit the growth of bacteria and subsequently slow down organic matter consumption process (Kogel-Knabner et al., 2010). Under flooding induced reducing conditions, mineralisation of organic matter stops at the ammonium stage because of the absence of oxygen required in ammonia nitrification. Consequently, ammonium accumulates under anaerobic conditions (Ponnamperuma, 1972). Thus, end products of anaerobic decomposition are CO_2, hydrogen, methane, ammonia, amines, hydrogen sulphides and partially humified residues. While in aerobic decomposition end products are CO_2, nitrate, sulphate and resistant residues (humus) (Ponnamperuma, 1972).

2.6.2 Drivers of nutrients mobilisation under flooding induced anaerobic conditions

Phosphorus exists in soils and water in dissolved and particulate forms as iron and aluminium phosphate, phosphate adsorbed or co-precipitated with Fe (III) and Mn (IV) hydrous oxides, calcium and magnesium phosphate, and organic phosphates (Reddy et al., 1999; Sondergaard et al., 2001). In flooded soils nutrient dynamics (retention and release) are governed by a number of abiotic (e.g. pH, redox potential, metal content (Fe, Al, Mn, Ca and Mg), anions competing with phosphate for ligands exchange (sulphate), and soil organic matter content), and biotic factors (microbial contribution) (Loeb et al., 2008; Bruland and DeMent, 2009; Schonbrunner et al., 2012; Alloway, 2013). Figure 2.5 shows a schematic diagram summarising biotic and abiotic factors controlling redox induced nutrients (P and trace-metals) mobilisation in flooded soils. Table 2.3 highlights factors contributing to enhanced P solubilisation in flooded soils. In the following sections, these factors are critically discussed with supporting references from recent laboratory and field studies.

Table 2.3: Studies illustrating factors contributing to enhanced P release from flooded soils previously dried

State of soil/sediments	Flooding duration	Drying temperature (ºC) and duration	Water column depth and nature of floodwater used	Factors contributing to increase in P upon flooding	Source
Sieved sediments artificially packed in cores	36 Weeks	20ºC for 4 weeks	Depth = 25cm Tap water (50µg $SRPL^{-1}$)	– Mineralisation of organic P – Drying induced reduced P sorption – Reductive dissolution of P bearing minerals – SO_4^{-2} mediated P release	Dieter et al. (2015)
Sieved (10 mm)	8 Weeks	-	Depth = 5cm Reverse osmosis water	– Dissolution of Ca and Mg phosphate – Reductive dissolution of Fe and Mn phosphate	Amarawansha et al. (2015)
Soil Cores	133 days	-	Lake water Depth = 75 cm	– Fe and Al bound P – Organic P mineralisation	Aldous et al. (2007)
Soil Cores	28 days	25ºC	Depth = 10cm Creek water (0.08 mg P L^{-1})	Dissolution of Fe and Al bound P	Pant and Reddy (2003)
Soil cores	-	40 days 8%-14% water content of air-dried sediments	Lake water	– Microbial cell lysis – Mineralisation of organic matter – Drying induced reduction in P sorption	Qiu and McComb (1994)

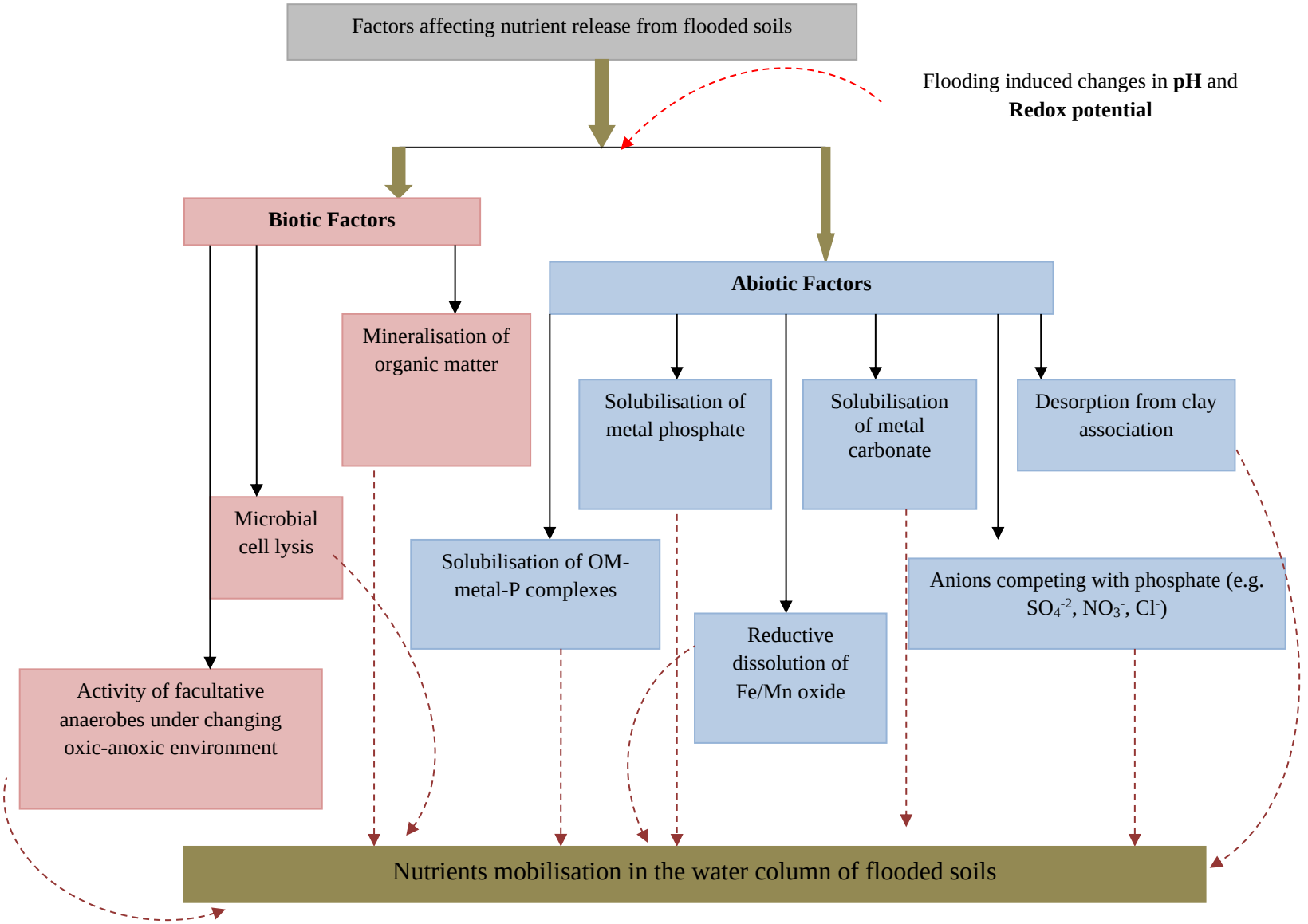

Figure 2.5: Flow chart highlighting biotic and abiotic factors involved in mobilising nutrients in flooded soil.

2.6.3 pH

Soil flooding renders drastic electrochemical changes - a decrease in redox potential, increase in pH of acidic soils and a decrease in pH of alkaline soil, changes in specific conductance, sorption and desorption of ions (Alloway, 2013). The pH of an acidic soil starts to increase rapidly after flooding because of removal of hydrogen ions from the soil solution and stabilises at pH 6.5 or just above if sufficient Fe is present, but the pH rise is delayed if temperature, percentage of organic matter and the amount of ferric iron are too low to produce sufficient iron for $Fe_3(OH)_8$-$FeCO_2$ buffer to become operative (Ponnamperuma, 1972; Alloway, 2013).

In acidic pH soils phosphate associated with Fe and Al are predominant P sorbents, while in alkaline to neutral soils calcium phosphate predominates. Release of P by hydrolysis of Fe and Al phosphate and desorption from metal carbonates, OM-metal–P complexes, and clay association is mainly controlled by flooding induced variations in pH, while reduction of Fe(III) to Fe(II) and subsequent solubilisation of P is controlled predominantly by flooding induced reduction in redox potential. The pH value effecting equilibrium of hydroxide, carbonates, sulphides, phosphates and silicates subsequently regulates precipitation-dissolution and sorption-desorption of ions (Pannamperma, 1972).

In acidic soils, flooding induced increase in pH decreases P binding capacity of Fe and Al minerals primarily due to ion exchange reactions in which OH^- replaces orthophosphate (PO_4^{-3}) (Bruland and DeMent, 2009). Moreover, aluminium is known to make Al-OM-PO_4 complexes. These complexes are sensitive to flooding induced variation in pH and release P into the water column (Darke and Walbridge et al., 2000).

However, in alkaline soils decrease in pH favours solubilisation of P (Newman and Pietro, 2001). Berryman et al. (2009) claim that in wetland soils rich in $CaCO_3$, decrease in pH due to soil saturation contribute to release of carbonate-bound P to the pore water. Previously, Diaz et al. (1994) have also shown that 75-90% of inorganic P which was originally precipitated as Ca-phosphate under higher pH solubilised into water column as pH dropped to about 7.

2.6.4 Redox potential

Redox potential (Eh) is the tendency of a solution to accept electrons from an oxidisable substance or donate electrons to a reducible substance (Alloway, 2013). Under reducing condition soils redox potentials can range from 0.2V to -0.4V, while the redox potentials of oxidised soils generally range from 0.8 to 0.3V (Alloway, 2013). Soil redox potential gives an

indication of soil oxygen status and decreases with increasing flood duration (Unger et al., 2009).

The rate of redox reduction depends upon the amount of soil organic matter, temperature, reducible metal content and duration of flooding. Redox conditions influence adsorptive capacities of anions and cations in soil by affecting precipitation and dissolution of metal oxides (Alloway, 2013). Unlike Al, Fe (III) is redox sensitive and reduces to Fe (II) with the onset of flooding induced reducing conditions. Reductive dissolution of Fe releases previously sorbed phosphorus and trace metals into soil solution thus increasing their concentration in the water column. Redox induced dissolution of metal oxides and mobilisation of P and metals is discussed in detail in the section 2.6.5.

2.6.5 Metal oxides

Metal oxy/hydroxides are important in retaining soil nutrients and trace elements (Dong et al., 2005; Tack et al., 2006; Alloway, 2013; Schulz-Zunkel et al, 2015). In flooded soils, with the development of low redox potential, one of the strongest phosphates sorbent Fe^{+3} oxyhydroxide becomes unstable and results in reductive dissolution of Fe^{+3} oxides. Fe^{+3} hydroxides are not only the strongest P sorbent but also acts as a terminal electron acceptor for anaerobic soil organic matter decomposition. Microorganisms enzymatically oxidise organic matter in the presence of Fe^{+3} and reduce it to soluble Fe^{+2}. The critical redox potential for iron reduction and dissolution at pH 5 is +300 mV; pH 6 is between +300 mV and +100 mV; and pH 7 is 100 mV (Gotoh and Patrick, 1974; Kogel-Knabner et al, 2010).

The reductive dissolution of Fe causes release of P and other previously sorbed trace metals in soil solution. Phosphorus mobilisation from flooded soils into the overlying water column as a result of reductive dissolution of P bearing minerals has been studied in term of its importance in nutrient availability and release, and reported in flooding experiments on soils from flooded forests (Chacon et al., 2005), wetlands (Surridge et al., 2007; Lai and Lam, 2008; Berryman et al., 2009) and floodplains (Loeb et al., 2008; Shaheen et al., 2014a,b). Berryman et al. (2009) reported elevated pore-water concentrations of dissolved reactive phosphorus (DRP) and associated this increase with reduction of Fe oxyhydroxide. Surridge et al. (2007) and Schonbrunner et al. (2012) showed positive correlations between phosphorus and Fe concentrations suggesting coupled mobilisation of Fe and P due to reductive dissolution of Fe (hydro) oxide.

Trace metals like Co, Fe, Ni, Cu and Mn show high adsorption affinities for metal oxides (Ashworth and Alloway, 2004; 2007; Alloway, 2013). Redox induced dissolution of metal oxides causes mobilisation of Fe and Mn ions along with adsorbed trace metals (Grybos et al., 2007; Shaheen et al., 2014b). Grybos et al. (2007) and Shaheen et al. (2014b) linked increased mobilisation of Co and Ni in soil solution of wetland and contaminated flood plain soils at low redox with reductive dissolution of Fe/Mn-oxides

However, the presence of high proportion of $Al(OH)_3$ in flooded soils has been seen to reduce P mobilisation in the water column perhaps by adsorbing P liberated from dissolution of $Fe(OH)_3$ since $Al(OH)_3$ has a high P sorption capacity and is not sensitive to altering redox (Kopacek et al., 2000; Huser and Rydin, 2005; Kopacek et al., 2005).

Presence of high levels of nitrate in flooded soils has also been reported to prevent release of phosphate into water column likely because nitrate buffers redox potential to a level high enough to prevent dissolution of P bearing minerals (Bostrom et al., 1988). Higher concentrations of nitrate cause a delayed release of Fe^{+2} and dissolved P likely due to preferential use of nitrate instead of Fe^{+3} as an electron acceptor (Surridge et al., 2007).

Salinity appears to be another controlling factor (alongside Al $(OH)_3$ and nitrate) affecting P mobilisation in flooded soils. Anions Cl^- and SO_4^{-2} compete with phosphate for binding sites, thus may increase P concentrations in the soil solution. Bruland and Dement (2009) showed that increasing surface water salinity of the wetlands in Hawaii decreased phosphorus sorption index (PSI). PSI of sediments decreases due to increase in anions (Cl^- and SO_4^{-2}) concentrations, competing with phosphate for binding sites**.**

Studies reporting initial increase in P concentration in flooded soils have also reported decrease in P pulses during later stages (Chacon et al., 2005; Amarawansha et al., 2015). Phosphorus transportation to the surface layer might be restricted due to oxidation of Fe^{+2} when it diffuses upward in the water column towards the oxygenated zone and bind P (Wilson and Baldwin, 2008), since freshly precipitated or amorphous iron oxide provides more reactive surface area and porosity for phosphate and trace elements sorption relative to the more crystalline forms (Tack et al., 2006; Zhang et al., 2010; Alloway, 2013). However, it is worth noting that in laboratory flooding experiments, solubilised Fe^{+2} would likely re-oxidise across reduction-oxidation zone along with sorbed P and trace metals. In contrast, Fe^{+2} solubilised in natural systems would likely leave the system, subsequently reducing available sorption sites for P and trace metals.

2.6.6 Organic matter

Soil organic matter improves soil's ability to retain P by increasing P sorption capacity (Bruland and Rishardson, 2006). In acidic soils organic matter predominantly make complexes with Al and Fe, while in alkaline soils organic matter complexation with Ca and Mg is more important in retaining P (Bruland and Rishardson, 2006). Soils with high quantity of organic matter seems to be more susceptible in releasing phosphorus upon flooding through a number of mechanisms - blocking or occluding P binding sites on mineral surfaces (Dieter et al., 2015), competing with phosphate anion for adsorption sites on mineral surfaces and dissolution of organo-metallic-P complexes (Hutchison and Hesterberg, 2004; Hunt et al., 2007; Abeit, 2013). Concentrations of dissolve reactive phosphorus increased up to seven-folds during reduction of coastal plain soils, which was suggested to be linked with dissolution of Fe (III) and Al (III) oxide minerals and competitive ligand exchange of organic matter for mineral adsorbed phosphate (Hutchison and Hesterberg, 2004).

Unlike Fe-OM-P complexes, Al-OM-P and Ca-OM-P are not sensitive to flooding induced redox changes and are not likely to release P with altering redox (Bruland and Rischardson, 2006). However, flooding induced changes in pH may result in solubilisation of humic-Al complexes and release of P (Darke and Wallbridge, 2000**).** Darke and Wallbridge (2000) suggested that Al complexation and P co-precipitation are feasible in pH range of 2-5 in non-flooded Ogeechee floodplain soils and flooding induced increase in pH over 5.4 can lead to solubilisation of OM-Al-P complexes and release of P.

Flooding induce reducing conditions increase solubilisation of organic matter through production of organic metabolites by microbes, reductive dissolution of OM-metal complexes, and desorption of organic matter from soil minerals due to rise in pH (Grybos et al., 2009). As the pH increases towards neutrality deprotonation of hydroxyl groups at humus surfaces decreases positive surface charges and organic molecules become more electronegative, as a result repulsive forces between anions and organic matter solubilise organic matter along with bound metals (Grybos et al., 2009). Solubility of organic matter controls mobility of trace metals. Sorption of trace metals by DOM increases with rise in pH since trace metals preferentially bind with ionised functional group formed with increasing pH (Kashem and Singh, 2001). Copper (Cu), for instance, make strong complexes with OM becomes more available at high pH (Ashworth and Alloway, 2004; 2007).

2.6.7 Sulphate (SO_4^{-2}) mediated nutrients mobilisation

Flooding of dried soils may increase mobilisation of P by affecting Fe-S-P cycling (Baldwin et al., 2000; Dieter et al., 2015). Drying induced oxidation accelerates formation of sulphate. Thus when dried soil is flooded, sulphate (SO_4^{-2}) is reduced to sulphide (S^{-2}) at significantly lower redox potential, which may react with heavy metals to give insoluble sulphides of metals e.g. FeS, CuS, ZnS etc (Bostrom et al., 1988; Kashem and Singh, 2001; Shaheen et al., 2014b). Formation of insoluble iron sulphides compounds reduces ability of P to bind with iron and stimulate P release into soil solution (Baldwin et al., 2000). In a drying-flooding study, Dieter et al. (2015) linked increase pore-water P concentration with drying-flooding mediated oxidation-reduction of sulphate and formation of insoluble FeS_x.

However, unlike P, the mobility and availability of metals in flooded soils seems to be reduced by the reduction of sulphate to sulphides and formation of insoluble metal sulphides at very low redox. In a flooding experiment on contaminated flood plain soils, Shaheen et al. (2014b) observed reduction in Cu and Zn concentrations at low redox (-85 mV) which was suggested to be due to reduction of SO_4^{-2} to sulphide and precipitation of Cu and Zn as insoluble sulphides. Kashem and Singh (2001) also associated decreased solubility of Ni and Zn, in flooded soils at extremely low redox (< -100 mV) with the reduction of sulphate and formation of insoluble metal sulphides.

2.6.8 Phosphorus mobilisation from Ca/Mg-P system in alkaline flooded soils

In calcareous flooded soils, phosphorus association with Ca and Mg ions as Ca/Mg phosphate reduces P release to soil solution. Shenker et al. (2005) observed that upon submergence of calcareous soils, Ca-P precipitation effectively retained much of the released P in soil solution. High concentrations of Ca and Mg combined with alkaline pH (> 9) make suitable conditions for P co-precipitations with $CaCO_3$ and formation of Ca-phosphate. Ca-phosphate formation enhances P removal from the water column as long as soil remains flooded combined with higher concentration of Ca and high pH (Newman and Pietro, 2001). However, a decline in pH liberates phosphate due to reduced precipitation of these minerals at lower pH. Diaz et al. (1994) observed that Ca-phosphate formed in Otter Creek and Dry Lake waters under high pH and rapidly dissolved as the pH dropped. About 90% of the original P in the Otter Creek and 75% in Dry Lake solubilised and brought back into soil solution as the pH dropped to about 7, suggesting increased solubility of freshly precipitated mineral of Ca-phosphates at lower pH. These studies imply that Ca-P system could be an important P

retention mechanism in alkaline aquatic environment restricting P releases. However, flooding induced decrease in pH leads to solubilisation and release of phosphate along with other adsorbed metals into water column, which could subsequently be released into surface run-off.

2.6.9 Microbial contribution to P release

In flooded soils a combination of factors may contribute to the release of microbial driven P in water column e.g. decreased biological P demand under anaerobic conditions and release of labile P from microbial cells either through cell lysis or the activity of facultative anaerobes which are adapted to tolerate oxic-anoxic fluctuations (Wright et al., 2001).Unger et al. (2009) reported negative effect of stagnant flooding on Gram-negative bacteria, Gram positive bacteria, mycorrhizal fungus and reduction in microbial biomass due to development of anaerobic conditions. Wright et al. (2001) suggested that release of labile P from microbial biomass could be the reason of increase P availability in flooded soils.

Studies have shown that some microbial species may release or uptake P while shifting between aerobic and anaerobic conditions (Bostrom et al., 1988; Reddy et al., 1999; Wright et al., 2001). Under oxic environment and adequate nutrients supply, microorganisms assimilate dissolve P for their growth and store any surplus P in the form of polyphosphate. However, as conditions become anaerobic, this stored P is released into soil solution as a protective mechanism to survive oxic-anoxic fluctuations (Khoshmanesh et al., 1999; Wright et al., 2001; Khoshmanesh et al., 2002). In a study carried out on wetland sediments, Khoshmanesh et al. (1999) observed that under aerobic condition 100% of the added P was taken up biologically, of which 34-45% was microbial uptake. However, when these aerobic experiments were made anaerobic, sediments released 30% of the initially added P, suggesting that these sediments took up P for cell growth and stored some of the P as polyphosphate (Poly-P). This stored P was then released under anaerobic conditions. These studies suggest that microbes play an important role in up taking P under aerobic conditions and releasing P back to water column under flooding induced anaerobic conditions.

Microbial species appear to have different tolerances to variations in water content. Fungi, for instance, are more adapted to low water potentials but highly sensitive to prolonged flooding relative to the bacterial biomass (Drenovsky et al., 2004; Mentzer et al., 2006; Voroney, 2007). Drenovsky et al. (2004) observed significantly lower ratios of fungal to bacterial biomarkers in flooded soils. The decrease in fungal biomass parallel to the increasing soil water content suppressed fungi but had a little effect on bacterial biomass. Similar results were reported by Mentzer et al. (2006). They observed that arbuscular mycorrhizal fungal was

negatively affected by prolonged flooding, while Gram-positive and anaerobic microbial indicators were more abundant in continuous flooding treatments compared to the unflooded controls. Relative abundance of Gram-positive and anaerobic microbial indicators is perhaps reflective of their greater stress tolerance ability to water content fluctuations.

These studies elucidate that increase in P release concomitantly with increasing drying duration is the result of larger number of dead microbial cells combined with enhanced mineralisation rate and accumulation of nutrients in a state ready to be released with the onset of flooding (Schonbrunner et al., 2012). Phosphorus mobilisation from microbial biomass due to drying-flooding may contribute to water pollution.

Significance of dried wetland sediments (exposed sediments due to lowering of water level) in releasing considerable quantities of P to the overlying water column relative to control continuously inundated sediments (unexposed) has been reported by quite a few studies (Baldwin et al., 2000; Gilbert et al., 2014; Dieter et al., 2015) with Gilbert et al. (2014) and Qiu and McComb (1994) reporting significantly higher quantities of P released to the overlying water column from dried exposed sediments relative to the continuously inundated sediments. Nevertheless, no one has yet explored how climate-change predicted (Bates et al., 2008) soil drying followed by heavy rains with high risks of flash flooding, can potentially alter P dynamics in terrestrial soils which are rarely dried-flooded. Factors controlling enhanced nutrients mobilisation from dried sediments upon inundation have been reported are: enhanced mineralisation of organic matter with the advent of flooding due to availability of moisture when conditions are still oxic (Qiu and McComb, 1994), microbial cell lysis due to osmotic shock (Wright et al., 2001; Unger et al., 2009) and increased minerals crystallinity due to sediment drying and reduced nutrients sorption (e.g. P) when dried sediments were reflooded (Qiu and McComb, 1994; Baldwin et al., 2000; Schonbrunner et al., 2012; Dieter et al., 2015). Crystalline metal oxides become more dehydrated and less strongly charged with time during drying induced oxidation in a process known as '*mineral aging'* (Alloway, 2013; Schulz-Zunkel et al., 2015). So, when dried soils are flooding, loss of sorption capacity due to increased mineral crystallinity prevents the adsorption of P originated from microbial cell lyses or organic matter mineralisation. This process could also contribute to the general increase in overall water column concentration of P often seen in flooded soils.

The duration of soil drying also seems to control the water column P concentration, with soils dried for an extended period get more dehydrated and crystalline leading to reduced surface area and number of sorption sites. In a drying-flooding experiment 200 hours drying resulted in greater total P water concentrations relative to sediment dried for a shorter period

(100 hours) (Schonbrunner et al., 2012). Higher degree of crystallinity provides a fewer binding sites for P sorption thus reduces P retaining capacity of soils and results in high water column concentrations. These findings imply that flooding of soils, in particular those, which were previously dried, can potentially increase water column concentrations of soil P by reducing P sorption capacity of soils, though this is one of the several processes discussed previously which together are responsible of increased P dissolution in flooded soils. Nonetheless, this is one of the possible contributory factors, and together these factors/processes may have implications for soil fertility and surface water quality under changing climate – droughts followed by flash floods.

2.7 Conclusion

Amongst common soil stresses, drying-rewetting, freeze-thaw and flooding have been recently given much attention perhaps because of their potential contribution to enhance mineralisation of organic matter and mobilisation of nutrients and trace metals. These mobilised nutrients and trace metals can potentially be transported to surface waters through surface runoff.

It is clear from recent laboratory and field studies that DRW and FT induced mobilisation of nutrients and trace metals have mostly been attributed to a number of intriguing factors: microbial biomass destruction, disaggregation of soil structure, exposure of previously protected organic matter and dead cells, dissolution of organic matter, competitive inhibitory effect of organic molecules for phosphate sorption sites, disruption of organo-metallic complexes, dissolution of metal oxides and activity of facultative bacteria to release nutrients under changing redox (in case of flooded soils). Nevertheless, studies witnessed nutrients mobilisation upon wetting or flooding of dried soils have also observed decrease in nutrients release. The underpinning mechanisms contributing to nutrients sequestration have been reported, and include: microbial community shift and microbial assimilation of released nutrients; exposure of new surfaces for nutrients sorption upon disaggregation; re-formation of organo-metallic complexes and co-precipitation of nutrients; nutrients sorption to freshly precipitated metal oxides and reduction of sulphate and formation of insoluble metal sulphides at very low redox (in case of extended period of flooding). It seems likely that in case of soil drying-rewetting nutrients mobilisation is mainly governed by microbial biomass and aggregate breakdown; whilst reductive and non-reductive dissolution of metal-oxides and organo-metallic complexes are the most predominant factors controlling mobilisation of nutrients and metals in flooded soils.

The responses of soils in terms of nutrients mobilisation may vary depending on soil type, quantity of organic matter, metal oxide content and microbial adaptations to withstand soil stresses. It can be concluded from the literature that climate change driven soil drying-rewetting, freezing-thawing or drying-flooding process have potential to increase mobilisation of nutrients and trace elements from soil solid phase into solution and thus have implications for soil fertility and surface water quality.

Chapter 3 - Materials and methods

3.1 Site and soil description

The study site is in North Wyke, Devon, UK (Figure 3.1) and is described in Harrod and Hogan (2008). The first soil was typical non-calcareous typic hapla quept (USDA) of Hallsworth series (FAO Stagni-vertic cambisol) (hereafter referred to as Hallsworth-I soil). The second soil (hereafter referred to as Hallsworth-II soil) was the same soil type/series as Hallsworth-I but collected at different time and location (see section 3.2). The third soil was a typical brown earth dystric eutrochrept (USDA) of the Crediton series (FAO dystric cambisol) (hereafter referred to as Crediton soil). Soils are described in detail in Harrod and Hogan (2008). Crediton soils are free draining permeable soils on soft sandstone substrates with relatively high permeability and have low runoff potential (Harrod and Hogan, 2008). Hallsworth soils are slowly permeable, seasonally waterlogged soils and have high runoff potential (Harrod and Hogan, 2008).

3.2 Soil sample collection and preparation

A bulk sample of Hallsworth-I soil was collected from the top 10 cm depth in August 2014 from sheep and beef cattle grazed permanent grassland field comprising an area of approximately 3.47 hectares. Two bulk soil samples, one each of Hallsworth series (hereafter referred to as Hallsworth-II) and Crediton series were collected again in March 2016 to carry out the flooding experiment (Chapters 7 and 8). Hallsworth-II soil was collected from the peripheral area situated at the up-slope side of the field whilst Hallsworth-I was collected from the middle area of the field situated at the down-slope side. The reason of collecting Hallsworth-I and II soils from different locations was that during the second time of sample collection in March 2016, livestock slurry had just been applied at the previous location of sampling, so that area was avoided and instead sampling was carried out at the peripheral up-slope side which might have received fewer inputs of slurry and fertilizers over the time. So, although Hallsworth-I and II are the same soil type, they had received different quantities of fertilisers/manure historically and differ spatially. The spatial, seasonal and landscaping variability along with the differences in fertilizer/manure history and livestock pattern can affect soil properties not only between fields but also within individual fields (Brubaker et al., 1993; Hook and Burke, 2000; Page et al., 2005; Shi et al., 2013), which may explain the differences seen in the soil properties (Tables 4.2 and 7.2). However, Hallsworth-I soil was

used to carry out the DRW experiments (work carried out in Chapters 4, 5 and 6), whilst Hallsworth-II soil was used to carry out the drying-flooding experiment (see Chapters 7 and 8). Both experiments are stand-alone pieces of work and the results are not being compared across these two types of experiments.

Soils were prepared using the method of Carter and Gregorich (2008). Briefly, soil was crushed and passed through 2 mm sieve, removing all non-soil materials (e.g. grass, roots, earthworms and stones). For the purpose of analysis, soil sub-samples were oven dried at 40°C till no further loss in the moisture was observed. For carrying out the DRW experiment, Hallsworth-I soil was maintained at 54% of maximum water holding capacity (WHC) moisture content prior to use. While in case of the flooding experiment both Crediton and Hallsworth-II soils were maintained at 25 % of maximum WHC moisture content prior to their use.

Figure 3.1: a) Sampling location – Sheep and beef cattle grazed permanent grassland in North Wyke, Devon, UK. b) Soil sampling. c) Crediton series soil. d) Hallsworth series soil.

3.3 Laboratory analysis

3.3.1 Determination of soil pH

Soil pH was measured in 1:2.5 (w/v) soil water suspensions, using a pre-calibrated pH meter. Each soil sample had 3 replications.

3.3.2 Determination of soil moisture content (MC)

Soil moisture content was measured by removing soil moisture by oven-drying a soil sample at 105°C for 16 hours until the weight remains constant. The moisture content (%) was then calculated from the sample weight before and after drying. Each soil sample had 3 replications.

3.3.3 Determination of soil texture

Soil texture was determined by hydrometer method. Briefly the method involved soil pre-treatment with 30% H_2O_2. Triplicate soil samples were then shaken with 5% (w/v) hexametaphosphate (Calgon) solution for 1 hour. The mixture was then transferred to a 1000 ml graduated cylinder, marked to 1000 ml with DI water, and thoroughly mixed with plunger for 3 minutes. Subsequently the hydrometer was introduced 20 seconds before each measurement. Readings were taken at 40 seconds (finer than 5 µm fraction), 4 minutes 48 seconds (finer than 20 µm fraction) and after 5 hours (finer than 2 µm fraction). After each reading the temperature of the suspension was recorded using a thermometer. Since the hydrometer graduations refer to a temperature at 20°C, corrections for temperature and for solution viscosity were made. The reading at 4 min 48 sec seconds gave the % Clay + Silt and that at 5 hours the % Clay only; % Silt was found by the difference. The sand fraction was then determined directly by taking the % clay + silt fraction from 100%.

3.3.4 Determination of bulk density

Bulk density for packing the soil in the funnels was calculated as BD = s / v as described in Glendell et al. (2014). Where BD is the bulk density of the dry soil (g cm^{-3}), s is the mass of the dry soil (g) and v is the volume of the funnels (cm^3)

3.3.5 Determination of orthophosphate (P) in water samples

In all cases orthophosphate (commonly known as dissolved reactive phosphorus) in water samples was determined by Murphy and Riley (1962) method. Briefly, reagent A was prepared by the following method: 12 g of ammonium paramolybdate was dissolved in 25 ml of distilled water (18 MΩ) and 0.2908 g potassium antimony tartrate was dissolved in 100 ml of distilled water (18 MΩ). Both solutions were then added to 1000 ml of 2.5 M H_2SO_4 (Aristar grade) in a 2000 ml volumetric flask, diluted to 2000 ml with distilled water (18 MΩ). Subsequently, after mixing, molybdate solution was stored in a pyrex bottle in a cool dark area. Reagent B was prepared as required by dissolving 1.056 g ascorbic acid in 200 ml Reagent A.

Phosphorus stock solution was prepared using anhydrous KH_2PO_4 in a 1000 ml volumetric flask. Calibration standards were prepared by pipetting appropriate amounts of the working phosphorus solution and diluting with DI (18 MΩ) in appropriate volumetric flasks.

For analysis, 20 ml of filtered (0.45 µm cellulose nitrate membranes) sample was transferred to a 25 ml volumetric flask. Following this, 4 ml of the freshly prepared reagent B was added to the flask; made up the volume with DI (18 MΩ), mixed and left for 10 minutes before reading the absorbance by spectrophotometer

3.3.6 Determination of Cation Exchange Capacity (CEC)

For the determination of soil cation exchange capacity 4 g of sieved (< 2 mm) dried soil was mixed with 33 ml of 1 M sodium acetate solution in 50 ml polyethylene centrifuge tube. Sealed tubes were then shaken for 10 min, centrifuged and the supernatant was decanted. In this way triplicate soil samples were treated with 2 additional 33 ml aliquots of sodium acetate solution discarding the supernatant after each centrifugation. Subsequently, soil samples were suspended in 30 ml ethanol, shook for 5 minutes, centrifuged and the supernatant was discarded. This washing treatment was repeated for another two times. This was followed by addition of 33 ml of 1M ammonium acetate solution to the soil samples. Samples were then shaken for 10 minutes, centrifuged and the supernatant was decanted into a 100 ml volumetric flask. The extraction procedure was repeated twice more and carefully made the contents of the flask to the 100 ml mark with de-ionised water (18 MΩ). The sodium (Na) content of the solution was then determined in the flask by flame emission spectrometry.

3.3.7 Determination of soil organic carbon (OC)

Soil organic carbon was determined by Walkley-Black method (Walkley and Black, 1934). Briefly, 0.4 g sieved (< 2 mm) dried soil was added to a 500 ml conical flask. In a similar

manner two more replicates and triplicate blanks (without soil) were prepared. Subsequently, 1 M potassium dichromate solution was added to all flasks. 20 ml of concentrated sulphuric acid was then added to all flasks. After swirling, flasks were allowed to stand for 30 min. in the fume cupboard. Consequently, approximately 200 ml deionised water (18 MΩ) and 10 ml of concentrated ortho-phosphoric acid was added to all flasks, swirled, and left to cool for another 30 min. This was followed by addition of 12 drops of the indicator solution (barium diphenylamine sulphonate) to the mixture. After the flasks swirled several times, excess of dichromate was titrated with 0.5 M ammonium ferrous sulphate solution. At the end point, the solution changed from blue to green. Total volume (in ml) of ferrous ammonium sulphate used in the sample and the reagent blank was recorded. The titre was then inversely related to the amount of organic carbon (C) present in the soil sample

3.3.8 Determination of soil total phosphorus (TP)

Soil total phosphorus was determined by digesting triplicate samples of finely ground soil in perchloric acid ($HClO_4$) on hot plate for about 40 min at 203°C (boiling point of $HClO_4$) until the dark colour of organic matter disappeared. In addition to three sample replicates, three blanks (without soil) were also included. Subsequently after cooling, digests were filtered (Whatman 541) and diluted to 250 ml with de-ionised water (18 MΩ) and determined for total phosphorus by inductively coupled plasma atomic emission spectrometry (ICP-AES).

3.3.9 Determination of soil total organic phosphorus (TOP)

For the determination of soil total organic phosphorus triplicate crucibles with 1 g of soil (< 2 mm) were placed in a cool muffle furnace. The furnace temperature was then slowly raised to 550°C over a period of 1-2 hours. Temperature was maintained at 550°C for 1 hour, allowed the crucibles to cool, and transferred the ignited soil to 100 ml polypropylene centrifuge tubes. Subsequently, 3 replications of 1 g unignited soil were prepared in separate 100 ml polypropylene bottles. 50 ml of 1N H_2SO_4 (0.5 M) was then added to both samples. Tubes were then shaken for 16 hours and centrifuged for 15 min. Subsequently, the extracts were filtered using acid resistant filter paper (Whatman 541) and transferred into acid-clean polyethylene bottles. The samples were then determined for phosphorus by ICP-AES. Total organic phosphorus was then calculated as the difference between phosphorus content of ignited and unignited samples (Carter and Gregorich, 2008).

3.4.0 Determination of microbial biomass phosphorus

Microbial biomass phosphorus was determined by the method described by Brookes et al. (1982). Briefly, three sets of repeats were required, prior to extraction, the first was fumigated using chloroform in a desiccator and incubated for 24 hours at 25˚C, the second and third were incubated for the same period and temperature. The third set was then dosed with a phosphate solution of concentration 250 µg P ml^{-1}. All samples were then extracted by the method described by Olsen et al. (1954). Briefly, 0.5 M sodium hydrogen carbonate solution was prepared by dissolving 42 g sodium bicarbonate and 0.72 g sodium hydroxide pellets in 900 ml deionised water. The solution pH was adjusted to 8.5 using saturated solution of sodium hydroxide or concentrated sulphuric acid and making the final volume up to 1 litre with DI water. Determination of microbial biomass phosphorus involved following four major steps:

i. Fumigation

For sample fumigation a sheet of moist blue paper was placed at the bottom of desiccator underneath the desiccator shelf. Subsequently, jars of soil for fumigation and a 50 ml beaker containing at least 30 ml $CHCl_3$ and 2-3 anti-bumping granules were placed in desiccator. The whole procedure was carried out in a well-ventilated fume cupboard. The lid of the desiccator was replaced. Silicone sealant was used to improve the seal. The desiccator was then evacuated; the $CHCl_3$ quickly started to boil. $CHCl_3$ was boiled vigorously for 2 minutes. Consequently, the valve on the top of the desiccator was closed; releasing the pipe and vacuum pump was turned off. The desiccator was covered with a black plastic bag and left in the switched-on fume cupboard for 24 hours.

Non-fumigated samples were left at laboratory temperature in a desiccator covered with a black plastic bag 24 hours at the same time as the fumigated samples.

ii. Defumigation

For de-fumigating soil samples, the black bag was removed followed by vacuum release from the desiccator. Subsequently, after removing $CHCl_3$ jar and the moistened blue paper, the desiccator was evacuated for 5 minutes. The vacuum was then released, and this process was repeated for 3 to 5 times until $CHCl_3$ was no longer detectable by smell. Once de-fumigated, soil was transferred to acid washed sample bottles.

iii. Extraction

For control samples, 2 ml of de-ionised water, 100 ml of the 0.5 M sodium bicarbonate extractant and 1 g acid washed activated charcoal was added to a plastic bottle containing 5g (DWE) non-fumigated soil. In this way two more replicates and three blanks (without soil) were prepared. Sample bottles were then placed on the shaker for 60 minutes.

For P-spiked samples, 2 ml of P spiked solution of concentration 250 μg P ml^{-1}, 100 ml of the 0.5 M sodium bicarbonate extractant and 1 g acid washed activated charcoal was added to a plastic bottle containing 5g (DWE) non-fumigated soil. In this way two more replicates and three blanks (without soil) were prepared. Sample bottles were then placed on the shaker for 60 minutes.

Finally, de-fumigated soil (5 g DWE) was transferred into acid washed plastic extraction bottles and dosed with 2 ml of de-ionised water, 1 g activated charcoal and 100 ml of bicarbonate extractant. In this way two more replicates and three blanks (without soil) were prepared. Sample bottles were then placed on the shaker for 60 minutes.

iv. Analysis

After shaking, soil extracts were filtered (Whatman 42), acidified (0.25 M H_2SO_4) and analysed for orthophosphate within 24 hours by the method described by Murphy and Riley (1962). The results were then used within a calculation to determine the amount of phosphorus within each soil sample that was attributable to the microbial population. The mean of the three replicates of each soil sample was then calculated.

3.4.1 Determination of total phosphorus (TP) in water samples

Total phosphorus in water samples was determined by persulfate digestion method. Briefly, the method involved addition of 1 ml of sulphuric acid solution (11 N) and 0.4 g of ammonium persulfate to a 50 ml sample in a 125 ml Erlenmeyer flask. Mixture was then boiled gently on a pre-heated hot plate until a final volume of about 10 ml was reached. Subsequently, samples were cooled and diluted to 30 ml with deionised water (18 MΩ). One drop of phenolphthalein indicator solution was then added to the sample and neutralised with NaOH. Orthophosphate was determined by the Murphy and Riley, (1962) method.

3.4.2 Determination of soil total metal concentration

Soil total metal content was determined by concentrated nitric acid method. Briefly, 1.5 g finally ground soil was weighed into 100 ml conical flask, 20 ml concentrated nitric acid was added to each flask. The mixture was then allowed to digest overnight at room temperature. Flasks were then placed on hot plate and heated at 50°C for 30 min under reflux condition. The temperature was then increased to 120°C for 60 minutes and finally held at 170-180 °C for 2 hours. The flasks were then allowed to cool. The contents of the flasks were filtered (through Whatman no. 42 filter paper) into 100 ml volumetric flasks, diluted (with DI water) and analysed by ICP-AES (Jobin Yvon Horiba – ULTIMA 2C / 2CE). Certified reference material (CRM005) was included in the digestion as a check for quality control.

3.4.3 Ammonium oxalate-oxalic acid extraction of soil

Metal oxides (amorphous Fe, Mn and Al) in the soil were extracted by the acid ammonium oxalate method (Carter and Gregorich, 2008). Briefly, reagent A (0.2 M Ammonium oxalate) was prepared by dissolving 14.15 g of ammonium oxalate to 500 ml of distilled water in a volumetric flask. Reagent B (0.2 M oxalic acid) was prepared by dissolving 12.6 g of oxalic acid to 500 ml of distilled water in a volumetric flask. Extracting solution was then prepared by mixing 350 ml of reagent A and 267.5 ml of reagent B; its pH was adjusted to 3.0 by adding either A or B. Subsequently, 0.25 g air-dried soil was weighed into 100 ml foil covered plastic bottles. 25 ml of extractant reagent was then dispensed into each bottle, including repeats and blanks. Samples were then shaken for 4 hours, placing them upright in a reciprocating shaker, filtered (Whatman 42), diluted (x10) and analysed for Fe, Mn, and Al by ICP-AES (Jobin Yvon Horiba – ULTIMA 2C / 2CE).

3.4.4 Water extractable phosphorus

For the determination of water extractable P, 20 ml of DI water was added to 2 g DWE (dry weight equivalent) soil and the mixture was then shaken for 1 hour at 10 rpm, followed by centrifugation at 3000 g, filtration (Whatman no. 42) and determination of P colorimetrically (Murphy and Riley, 1962).

3.4.5 Quality control

Metals (Fe, Mn, Cu, Co, Ni, Zn) in the leachates and water samples from the flooding experiment were determined by ICP-MS with appropriate quality control assurance protocols. For quality control four approaches were followed – (a) all glassware was always washed with 10% HNO_3 (analytical grade) to remove contaminants adsorbed to the glass surfaces followed by rinsing with DI water (18 MΩ) thrice prior to use (b) blanks were run to estimate levels of procedural contamination, (c) 6 to 7 randomly selected samples, in a batch of 20 samples, were spiked with a known concentration of metals. In the case of DRW experiment, concentrations were raised to either 50 µg L^{-1} for Fe and Mn or 5 µg L^{-1} for Cu, Co, Zn and Ni. In case of flooding experiment, samples were spiked at two levels with an increasing concentration (10 mg L^{-1} and 20 mg L^{-1} for Fe and Mn, and 30 µg L^{-1} and 60 µg L^{-1} for Cu, Zn, Co and Ni. Both spiked and unspiked samples were analysed and percent recoveries were calculated (d) Each sample was split into two replicates and analysed separately to estimate the level of precision or reproducibility of the analytical equipment.

Chapter 4 - Microbial biomass responses to soil drying-rewetting and phosphorus leaching

4.1 Introduction

Predicted changes in climate will potentially increase the intensity and frequency of soil drying-rewetting (DRW) cycles (IPCC, 2014), with implications for nutrient dynamics (plant availability, sequestration and leaching), crop productivity and catchment water quality. Recent studies have tested the effects of soil DRW cycles on nutrient release. There is some evidence that soil DRW cycles can increase the extractability of soil-nutrients (Bunemann et al., 2013; Forber et al., 2017; Sun et al., 2017; Dinh et al., 2018; Homberg and Matzner, 2018). For instance, Bunemann et al. (2013), Styles and Coxon (2006) and Koopmans et al. (2006) reported increased extractability of phosphorus (P) as a result of soil drying. Gordon et al. (2008) reported that DRW induced significant increases in dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved inorganic nitrogen (DIN) in the leachates from two UK grassland soils. These findings support the notion that the changing pattern of climate has the potential to alter soil nutrient dynamics.

Enhanced nutrient extractability following soil drying-rewetting has been attributed to the disruptive effects of these soil stresses on the soil microbial biomass (Achat et al., 2010; Turner and Romero, 2010; Bunemann et al., 2013; Dinh et al., 2018; Brodlin et al., 2019), aggregate stability (Koopmans et al., 2006; Xiang et al., 2008; Bunemann et al., 2013), and stability of organic matter and organo-metallic complexes (Peltovuori and Soinne, 2005; Styles and Coxon, 2006; Butterly et al., 2009, 2011; Soinne et al., 2010). However, the response to wetting in terms of quantities of nutrients released varies with soil type (Zhao et al., 2010; Achat et al., 2012a,b), microbial tolerance to withstand soil stresses (Styles and Coxon, 2006; Gordon et al., 2008; Bunemann et al., 2013; Dinh et al., 2017), rate of soil rewetting (Turner and Haygarth, 2001; Blackwell et al., 2009, 2013), intensity of drying (Sardans and Penuelas, 2007; Bunemann et al., 2013; Sun et al., 2017), and drying duration (Forber et., 2017).

The soil microbial biomass plays an important role in nutrient cycling by acting as a source (e.g. mineralisation of soil organic matter, releasing nutrients from cytoplasm as a mechanism to equilibrate with surroundings and cell bursting) or sink by immobilising

nutrients (Achat et al., 2010; Dinh et al., 2017; Wang et al., 2017; Zhang and Marschner, 2018). Reductions in soil microbial biomass following soil DRW have been reported in recent years (Mondini et al., 2002; Blackwell et al., 2013; Chen et al., 2016). Mondini et al. (2002) reported a decrease of 13% for microbial biomass C and 30% for ninhydrin reactive N relative to a moist control following drying. Wu and Brookes (2005) reported a reduction in biomass C of 44% as a result of soil DRW, while Blackwell et al. (2010) reported that soil air-drying and rewetting may kill up to 70% of the total soil microbial biomass. Reduction in microbial biomass following a DRW stress is attributed to the rapid hydration of microbial cells. If microorganisms do not quickly equilibrate to sudden changes in water potential, their cell walls can burst, and cell lysis takes place. Biomass surviving soil drying are typically a better adapted part of the microbial population which can survive drying stresses by making protective structures e.g., spores or cysts (Borken and Matzner, 2009; Blackwell et al., 2010).

Although the soil microbial biomass typically comprises only a small percentage (1-3%) of soil organic matter (Blackwell et al., 2010), it may contain large quantities of phosphorus (Brookes et al., 1984). Currently studies reporting microbially driven soil phosphorus leaching are either limited (Blackwell et al., 2009, 2013) or have considered only a single drying temperature or duration. The novelty of the research reported here lies in how phosphorus leaching is affected by increasing the intensity and duration of drying. The selection of drying temperatures of 30°C and 40°C may seem unrealistic within the UK context, but surface soils have been reported to experience such high temperatures during summer (c.f. Blackwell et al., 2009). Moreover, to our knowledge, the effects of chloroform fumigation on soil microbial biomass phosphorus leaching have not yet been studied in pre- and post-dried soil samples. This work investigates how the soil microbial biomass responds to fumigation in the presence or absence of soil moisture and impacts on dissolved reactive phosphorus (DRP) leaching. In the above context, the basic objectives of this controlled laboratory experiment were to: (1) test the hypothesis that increases in the intensity and duration of soil drying will increase concentrations of DRP in leachates, (2) determine the soil microbial biomass phosphorus contribution to enhanced leaching of DRP following DRW, and (3) examine how the soil microbial biomass responds to sterilisation in pre- and post-drying soil samples – this will help understand how soil drying-rewetting influences phosphorus leaching when biomass was killed before or after drying. Phosphorus leaching from soils occurs in the forms of DRP, dissolved organic P and particulate-bound P (Hooda et al., 1999). This work, however, considers only DRP leaching because this is the most important factor in

terms of water quality and eutrophication as noted in the Water Environment Regulations, 2015.

4.2 Material and Methods

4.2.1 Sample collection and preparation

A bulk sample of Hallsworth-I soil was collected from sheep and beef cattle grazed permanent grassland field located in North Wyke, Devon, UK (see Section 3.1 for site and soil description). Soil was prepared as described in Section 3.2.

4.2.2 Drying-rewetting effects on fumigated and non-fumigated soil samples

Soil sub-samples were fumigated with chloroform either before or after oven drying at 30°C or 40°C for either 2-days or 14-days. Soil was weighed before and after drying to measure the moisture loss at different temperatures. The average moisture contents after 2-days drying were 3.7% and 1.1% at 30°C and 40°C, respectively and after 2-days no further loss in moisture was observed. A control soil was stored moist in vented plastic bags at 3°C until required and maintained at room temperature (21°C) for a day before subjecting to leaching. Each sample was then split into two sub-samples, with one sub-sample used for extracting microbial biomass phosphorus based on the method described by Brookes et al. (1982), while the other sub-sample was leached with deionised (DI) water (18MΩ) as follows: three replicates, each comprising 30 g dry-weight equivalent (DWE) of soil, gently compacted (to 1.2 g/cm^3 density) in plastic Buchner funnels with internal diameter of 76 mm and capacity 186 ml. Grade 1 qualitative filter paper (GE Healthcare Whatman) was placed at the bottom of funnels. In total 60 replicates of the moist unfumigated (MUF), moist fumigated (MF), dried unfumigated (DUF), dried fumigated (DF) and fumigated dried (FD) treatments were prepared in the leaching experiment for the measurement of DRP. Soil was watered with 105 ml of deionised water applied gently in several portions (to mitigate by-pass flow and high hydrophobicity effects) over a period of two hours, using a syringe, simulating an intense rainfall event. Collected leachates (~ 65 ml/funnel) were filtered through Whatman 0.45μm cellulose nitrate membrane filters and analysed within 24 hours of collection for DRP by the molybdate blue method. All soil treatments, including the control soil, were rewetted to the same moisture content. Table 4.1 shows the treatments imposed.

Table 4.1 Types of treatments imposed (storage conditions, fumigation and drying)

Treatments	
MUF - Moist unfumigated	Reference moist control (stored at 3°C and maintained at 54% of the maximum WHC), neither dried nor fumigated, maintained at 21°C (room temp.) for one day before subjecting to leaching.
MF - Moist fumigated	Maintained at 54% of the maximum WHC before subjecting to chloroform fumigation, followed by either leaching or extraction for microbial biomass phosphorus.
DUF – Dried unfumigated	Oven-dried at either 30°C or 40°C for 2-days or 14-days, followed by leaching.
DF - Dried fumigated	Dried at 30°C or 40°C for either 2-days or 14-days, and then fumigated, followed by leaching or extraction for microbial biomass phosphorus.
FD - Fumigated dried	Fumigated and then dried at 30°C or 40°C for either 2-days or 14-days, followed by leaching or extraction for microbial biomass phosphorus.

4.2.3 Laboratory analyses

Soil was characterised in detail (Table 4.2) using standard procedures and quality controls. In all cases triplicate soil samples (n = 3) were analysed. Soil pH was measured in 1:2.5 (w/v) soil-water suspensions, using a pre-calibrated pH meter (Oakton™ pH 700 Benchtop Meter). Soil texture was determined by the hydrometer method following pre-treatment with hydrogen peroxide (H_2O_2), as outlined in Rowell (1994). Soil organic carbon was determined by the Walkley-Black potassium dichromate oxidation method (Walkley and Black, 1934). Cation exchange capacity of the soil was determined by sodium saturation (sodium acetate) and displaced sodium (using ammonium acetate) was then analysed by flame emission spectrometry (Chapman, 1965). Soil total phosphorus was determined by digesting finely ground soil in perchloric acid ($HClO_4$) on a hot plate for about 40 min at 203°C until the dark colour of organic matter disappeared. Digests were filtered (Whatman 541), diluted to 250 ml with de-ionised water and phosphorus in the digestates determined by ICP-AES (Inductively coupled plasma atomic emission spectroscopy; JobinYvon Horiba - ULTIMA 2C /2CE) (Carter and Gregorich, 2008). For the measurement of soil organic phosphorus, soil was ignited at 550°C for 2 hours. Both ignited and unignited samples were extracted by 0.5 M H_2SO_4 before analysing them by ICP-AES. Total organic phosphorus was then calculated as the difference between phosphorus content of ignited and unignited samples (Carter and Gregorich, 2008).

Bicarbonate extractable phosphorus was measured based on the method described by Olsen et al. (1954). Briefly, 5 g DWE of soil was extracted in 100 ml of 0.5 M $NaHCO_3$ solution

adjusted to pH 8.5 along with 1g acid washed charcoal on a shaker for 1h. Extracts were then filtered (Whatman No. 42), acidified (0.25 M H_2SO_4), and determined colorimetrically by the Murphy and Riley (1962) method. Microbial biomass phosphorus was measured in both moist and dry soil replicates using the chloroform fumigation-extraction method as described by Brookes et al. (1982). Briefly three sets each comprising three replicate samples of the soil (5g DWE) were prepared (all moist and dry soil treatments). One set was sterilised using chloroform fumes under vacuum in a desiccator jar and incubated for 24 hours in the dark at 25°C. The second and third sets were incubated in the dark without chloroform in desiccator jars for the same period and at the same temperature. The second unfumigated set was used as a control and the third unfumigated set was spiked with 250 µg P ml^{-1} solution (1.0975g KH_2PO_4 into 1L DI water). All samples (control, fumigated and spiked) were then extracted with 0.5 M $NaHCO_3$ (100 ml) adjusted to pH 8.5 along with 1g acid washed charcoal. Each sample was then acidified (5-6 drops of 0.25 M H_2SO_4/50 ml of sample). The amount of orthophosphate in the $NaHCO_3$ extracts was measured colorimetrically by the Murphy and Riley (1962) method.

Table 4.2 Initial soil properties (mean ± standard deviation, n = 3) of Hallsworth-I soil

Soil Properties	
pH	5.82 ± 0.07
Cation exchange capacity (c.mol kg^{-1})	29 ± 2.67
Organic carbon (%)	3.95 ± 0.12
Bicarbonate extractable P (mg P kg^{-1})	32 ± 0.4
Moist soil microbial biomass P (mg P kg^{-1})	125 ± 10
Total soil P (mg P kg^{-1})	1,929 ± 7
Total organic P (mg P kg^{-1})	928 ± 63
Sand (%)	66
Silt (%)	14
Clay (%)	20

4.2.4 Statistical analysis

The significance of differences between treatments was determined by one-way ANOVA (significance reported as $p < 0.05$) by SPSS (IBM SPSS statistics 23). Tukey's post-hoc test was employed for multiple comparisons. The data are reported as mean ± standard deviation. Dependent variables were normalised using $\log_{10}$ transformation, when they did not follow a normal distribution to meet the ANOVA assumptions. Effect of interaction between

independent variables (e.g. drying intensity, duration and fumigation) on dependant variables (e.g. DRP concentrations or microbial biomass phosphorus) was determined using three-way ANOVA (Table 4.3). Pearson's correlation analysis was performed to explore relationship between DRP and microbial biomass P in both DF and FD treatments using SPSS (IBM SPSS statistics 23).

4.3 Results

4.3.1 Effect of drying-rewetting on DRP leaching

Overall, soil drying intensity had a significant ($p < 0.05$) effect on DRP leaching (Table 4.3). The largest percentage increase in DRP occurred in the soil dried at 40°C for 14-days (300%), relative to the moist unfumigated control. The smallest increase in DRP was observed in the leachate derived from the soil dried at 30ºC for 2-days (72% increase) relative to the unfumigated moist control (Figure 4.1).

Table 4.3 Summary statistics of three-way ANOVA. Assessing the effects of independent factors (Drying intensity, drying duration, and fumigation) on microbial biomass phosphorus and DRP concentrations.

Tests of between-subjects' effects	**Microbial biomass P***		**DRP***	
Source	**F**	**P**	**F**	**P**
Drying intensity	16.034	< 0.05	8	< 0.05
Drying duration	18.307	< 0.05	396	< 0.001
Fumigation	105.13	< 0.001	58	< 0.001
Drying intensity*duration	2.693	NS	1	NS
Drying intensity*Fumigation	3.344	NS	0	NS
Duration*Fumigation	0.018	NS	156	< 0.001
Intensity*Duration*Fumigation	1	NS	1	NS

*Values of microbial biomass phosphorus and DRP concentrations were normalised by $\log_{10}$ transformation prior to ANOVA analysis. The data from both fumigation treatments (DF and FD) were combined to see an overall influence of post- and pre-fumigation treatments. Note: 'NS' indicates not significant ($p > 0.05$).

For DUF treatments, DRP concentrations in the leachates derived from soils dried for 14-days at 30°C and 40°C (0.9 ± 0.2 mg P kg^{-1} and 1.0 ± 0.2 mg P kg^{-1} respectively) were almost double the corresponding DRP concentrations in the leachates from soils dried for 2-days at 30°C and 40°C (0.43 ± 0.01 and 0.59 ± 0.10 mg P kg^{-1} respectively; Figure 4.1). DRP concentration from the moist fumigated (MF) soil was as much as 4 times ($p < 0.001$) more than from the corresponding moist unfumigated (MUF) samples (Figure 4.1).

Two-days drying followed by fumigation treatment (DF) leached 88% and 46% more DRP in the leachates from the soils dried at 30ºC and 40ºC respectively (Figure 4.2), relative to 2-days dried unfumigated controls (0.4 ± 0.01 mg P kg^{-1} and 0.59 ± 0.10 mg P kg^{-1} respectively). Similarly, 14-days drying followed by fumigation (DF) treatment released 11% and 10% more DRP (with concentrations of 1.0 ± 0.2 mg P kg^{-1} and 1.1 ± 0.2 mg P kg^{-1}) at 30ºC and 40ºC respectively, relative to their 14-days dried unfumigated controls (0.9 ± 0.2 mg P kg^{-1} and 1.0 ± 0.2 mg P kg^{1}, respectively). However, these differences in DRP (DF at 30°C and 40°C for 14-days) were not statistically significant ($p > 0.05$) compared to their dried unfumigated (DUF) counterparts (Figure 4.3).

In the 2-days FD treatment, DRP concentrations in the leachates from soil dried at either 30ºC were below the level of detection (< LOD) (Figure 4.2). In contrast, significantly greater quantities of DRP ($p < 0.001$) were leached in the same treatment (FD) when drying duration was increased to 14-days, with concentrations of 1.7 ± 0.3 mg P kg^{-1} and 1.9 ± 0.2 mg P kg^{-1} at 30ºC and 40ºC, respectively (89 and 90% increase relative to that from dried unfumigated counterparts; Figure 4.3).

4.3.2 Microbial biomass contribution to enhanced DRP leaching

Results from the leaching experiment showed that fumigating pre- or post-drying generally increased DRP leaching, with the exception of the 2 days FD (fumigated dried) leaching treatment where DRP was not detected (< LOD) in the leachates from soils dried at 30ºC (Figures 4.2 and 4.3).

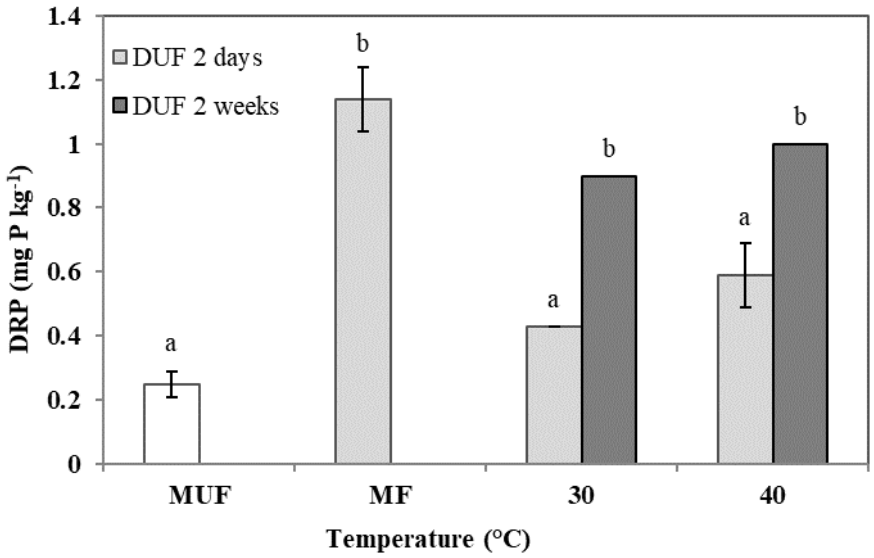

Figure 4.1 DRP (mg P kg^{-1}) leached in 2-days and 14-days dried unfumigated (DUF) treatments at 30°C and 40°C. Error bars represent standard deviation (for some columns error bars are too small to be seen), n = 3. MUF, moist unfumigated soil and MF, moist fumigated soil. Means with the same letters are not significantly different from each other ($p > 0.05$) as determined using Tukey's post-hoc test.

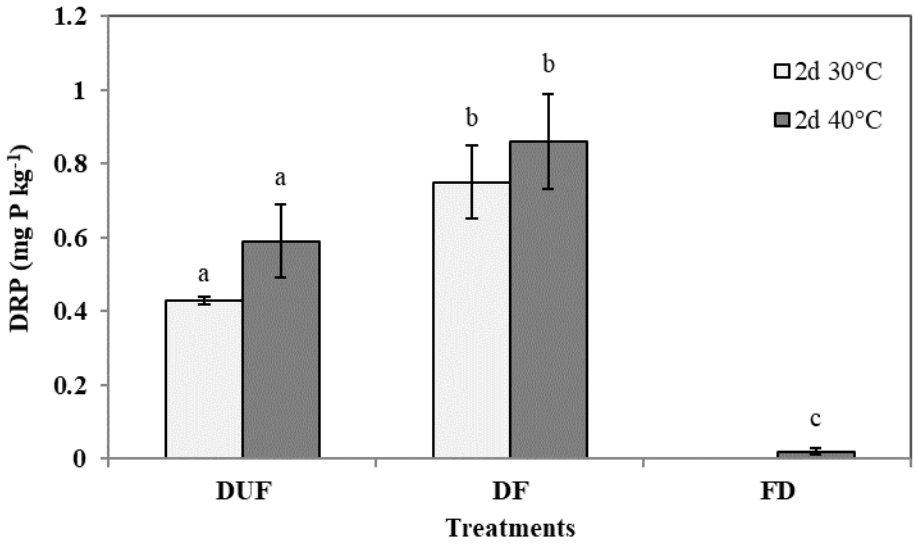

Figure 4.2 Comparing DRP (mg P kg^{-1}) leached in 2-days drying, unfumigated (DUF), with drying followed by fumigation (DF), and fumigation followed by drying (FD) treatments. Error bars represent standard deviation (for some columns error bars are too small to be seen), n = 3. Means with the same letters are not significantly different from each other ($p > 0.05$) as determined using Tukey's post-hoc test.

Comparing DRP in leachate from unfumigated and fumigated treatments suggest that increased DRP leaching could in part be caused by microbial cell lysis. To investigate the role of the soil microbial biomass in this phenomenon, biomass associated phosphorus was extracted. Results from the sodium bicarbonate extraction experiment showed that microbial biomass phosphorus in the control soil sample was significantly ($p < 0.05$) higher than in all treatments with dried soils (Figure 4.4).

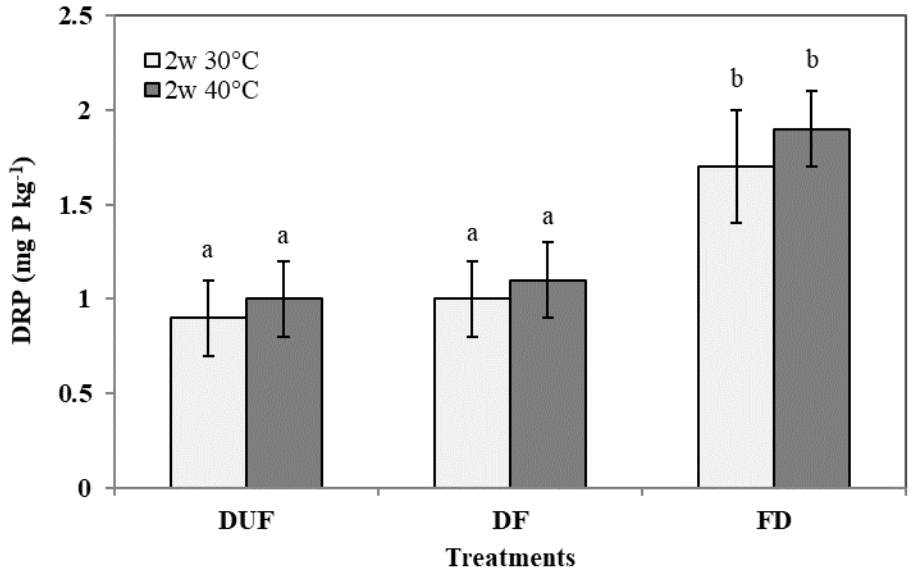

Figure 4.3 Comparing DRP (mg P kg^{-1}) leached in 14-days drying, unfumigated (DUF), with drying followed by fumigation (DF), and fumigation followed by drying treatments (FD). Error bars represent standard deviation, n = 3. Means with the same letters are not significantly different from each other ($p > 0.05$) as determined using a Tukey's post-hoc test.

In the case of the 2-days DF treatment, soil drying at 30ºC and 40ºC reduced microbial biomass phosphorus by 52% and 61% respectively, with concentrations of 60 ± 27 mg P kg^{-1} and 49 ± 10 mg P kg^{-1} respectively, relative to that of the control soil (125 ± mg P kg^{-1}) (Figure 4.4). In general, soil drying for a longer duration and at a greater intensity caused a significant reduction in microbial biomass phosphorus ($p < 0.05$), relative to the control. The greatest decrease occurring in the microbial biomass phosphorus in soil dried at 40ºC for 14-days (70% decrease relative to the control soil microbial biomass phosphorus), in the DF treatment (Figure 4.4). In the case of the 2-days DF treatment, microbial biomass phosphorus was reduced by 18% and 28%, with the increase in drying intensity (from 30ºC to 40ºC) and duration (from 2-days to 14-days), respectively, the concentrations ranged from 60 ± 27 mg P kg^{-1} to 49 ± 10 mg P kg^{-1} and 60 ± 27 mg P kg^{-1} to 43 ± 11 mg P kg^{-1}, respectively. However, these differences in microbial biomass P due to soil drying intensity and duration were not statistically significant (Figure 4.4)

Fumigation followed by drying (FD) treatments generally showed similar trends, with a reduction in microbial biomass phosphorus following fumigation and drying relative to the control soil. However, no reduction in microbial biomass P was observed when the soil was dried for 2-days at 30ºC (Figure 4.5).

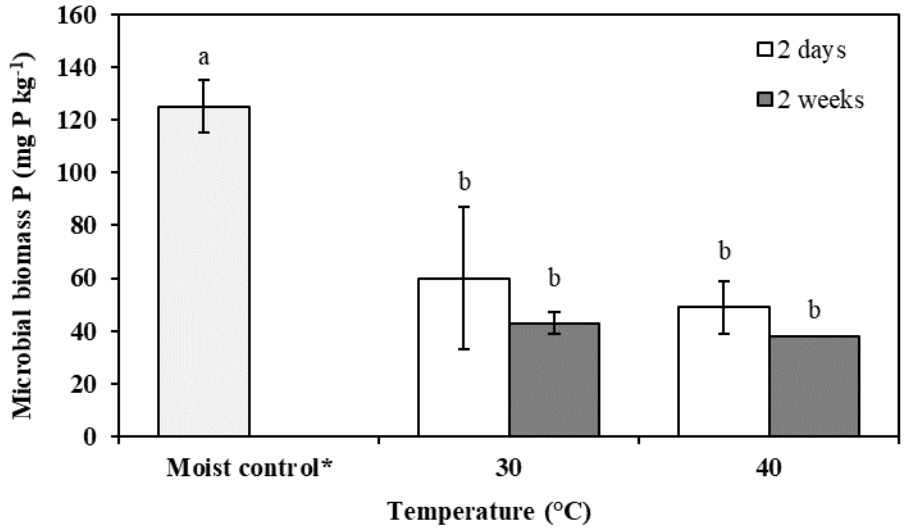

Figure 4.4 Microbial biomass phosphorus (mg P kg^{-1}) extracted in samples dried for 2-days and 14-days (2 weeks) followed by fumigation (DF) treatments at 30°C and 40°C. Error bars represent standard deviation (for some columns error bars are too small to be seen), n = 3. Means with the same letters are not significantly different from each other ($p > 0.05$) as determined using Tukey's post-hoc test. *Moist control is reference moist soil.

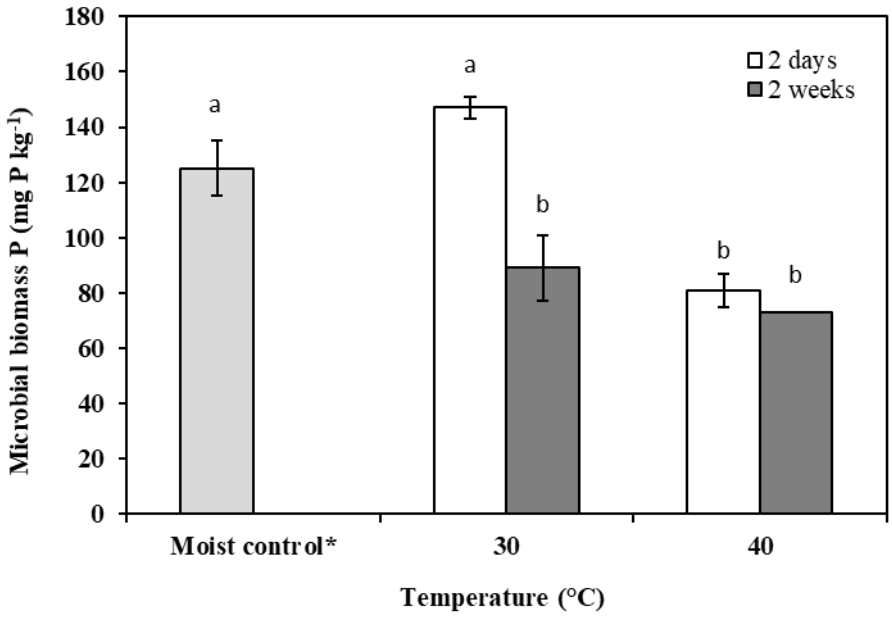

Figure 4.5 Microbial biomass phosphorus (mg P kg^{-1}) extracted in samples fumigated followed by drying for 2-days and 14-days (FD) treatments at 30°C and 40°C. Error bars represent standard deviation, n = 3. Means with the same letters are not statistically significant from each other i.e., $p > 0.05$ as determined using Tukey's post-hoc test. *Moist control is reference moist soil.

4.4 Discussion

Our results demonstrate that DRP quantities significantly ($p < 0.05$) increased in the leachates from soils dried for a longer duration (14-days) and at a greater intensity (40ºC) compared to the corresponding soil samples which were subjected to drying at a lower temperature (30ºC) and for a shorter duration (2-days) (Figure 4.1). We can therefore accept the hypothesis that increases in the duration and intensity of drying of soil significantly increases the concentrations of DRP in leachate from the soil used in this experiment. It is likely that more intense soil drying exerts a greater stress on the microbial biomass and thus renders greater release of nutrients upon rewetting. In this study the observed reduction in microbial biomass phosphorus upon drying (Figures 4.4 and 4.5) explains, at least in part, the increase in DRP in leachates (Figures 4.1-4.3). This is further supported by negative relationship between DRP and microbial biomass phosphorus, however, the Pearson's correlation was significant ($r = -0.604$, $p < 0.05$) only for the DF treatment. Drying-rewetting induced reduction in microbial biomass has been reported previously (Kaiser et al., 2015; Chen et al., 2016; Brodlin et al., 2019). Results from the $NaHCO_3$ extraction experiment showed that drying at a greater intensity caused a greater reduction in soil microbial biomass phosphorus compared to drying at the lower temperature (30ºC) (Figure 4.5).

Drying duration had a greater impact on DRP leaching relative to drying intensity. Extended periods of drying may cause substantial reductions in microbial activity and microbial biomass due to reduced diffusive transport of soluble nutrients and water-dependant activities for prolonged periods (Blackwell et al., 2010). Extended drying and subsequent reduction in microbial biomass could increase nutrient losses upon rewetting due to several factors e.g., accumulated dead microbial cells, release of intracellular solutes, aggregate disruption, and exposure of previously protected labile organic matter and mineralization of organic matter in the soil (Borken and Matzner, 2009). Results from the leaching experiments showed that soil drying for 14-days generally resulted in the higher concentrations of DRP in leachates relative to the corresponding samples where soil was dried for 2-days (Figure 4.1). One of the factors contributing to the increase in DRP concentrations in the leachates derived from soil dried for the longer duration was likely to be due to the greater destruction of microbial biomass, as indicated by the microbial biomass phosphorus measurements, which showed significant reductions ($p < 0.05$) in soils dried for 14-days compared to the control soil (Figures 4.4 and 4.5). Moreover, prolonged drought slows down microbial growth rate and enzymatic activity by causing sub-lethal damages to cell structure (e.g., DNA, proteins, cell wall; Meisner et al.,

2015; Rahman et al., 2018), subsequently increasing potentially leachable phosphorus. This could be another reason of higher leaching of phosphorus in the 14-days treatments.

Fumigating soils also influenced DRP in leachates relative to the non-fumigated counterparts (Figures 4.2 and 4.3). However, the influence was not straightforward e.g., 2-days FD or 2-weeks DF drying treatments, where either no DRP was detected in the leachate (Figure 4.2) or it was similar to that from the control soil counterparts (Figure 4.3). Nonetheless, these results are generally consistent with Bunemann et al. (2013). They observed that drying-rewetting of sterilised soil samples (gamma irradiation and autoclaved) significantly increased resin extractable phosphorus. The observed increased DRP concentrations in leachates following soil fumigation with chloroform in this study are most likely due to lysis of microbial cells and consequent release of phosphorus. Moreover, microbes that survive fumigation (Kieft et al., 1987) can increase their population size by utilisation of nutrients made available by subsequent hydrolysation of organic compounds released from microorganisms killed during sterilisation (Bunemann et al., 2013). Such a shift in microbial community following soil drying has been observed in drying-rewetting studies (e.g. Butterly et al., 2009, 2011; Pezzolla et al., 2019). This process can also result in an increase in mineralised phosphorus (DRP) availability for leaching. Hydrolysis of organic phosphorus compounds could also be one of the possible reasons of increased DRP in the leachate. This explanation, however, remains speculative since in this study leachate dissolved organic phosphorus (DOP) was not measured. Measuring DOP would have allowed a better understanding of the underlying processes, particularly the role of microbial biomass-P and transformation between inorganic and organic P.

Soil drying followed by fumigation (DF) resulted in greater concentrations of DRP relative to the DUF treatments (though the concentration increase was significant only in the case of the 2-days DF and two-weeks FD treatments; Figures 4.2 and 4.3). This may be because soil drying kills microbial species less adapted to drying. Thus, the biomass surviving drying stress represents a relatively more adapted fraction of the microbial population which can survive drying stress by making protective structures e.g., spores or cysts (Bottner et al.,1985; Schimel et al., 1999). Subjecting dried soil to chloroform fumigation could have resulted in death of microbial species which survived the original drying stress, consequently enhancing DRP concentrations in the leachate.

However, all DF treatments leached lower concentrations of phosphorus relative to the moist fumigated counterpart (MF) treatment (Figure 4.1-4.3). The greater DRP concentrations in leachates from moist fumigated soils probably occurred because of the presence of a larger

microbial biomass, since fresh moist soil microbial biomass is always greater than the dried soil microbial biomass, as drying kills some of the native biomass (Blackwell et al., 2013). Moreover, drying before fumigation potentially opens-up the soil aggregates more, exposing more surfaces for phosphorus binding so that when the soil is fumigated after drying, more of the phosphorus released gets bound to the surfaces of newly exposed/accessible soil particles (e.g., clay surfaces), potentially reducing the amount of phosphorus availability for leaching.

In the 2-days FD leaching treatment DRP was not detected in the leachate (< LOD) from the soil dried at 30°C. It is possible that phosphorus assimilation by the recolonising microbial population as well as retention on soil colloids due to drying induced crystallisation (Schonbrunner et al., 2012; Dieter et al., 2015) could be cause of this result (Figure 4.2).This is supported at least partially by the $NaHCO_3$ extraction results, which showed no reduction in microbial biomass phosphorus in the soil samples which were fumigated before subjecting to oven drying at 30°C for 2-days (FD treatment; Figure 4.5), unlike the DF treatment (Figure 4.4). Immobilisation of nutrients by the recolonising microbial population has also been previously reported (Brookes et al., 1982; Yevdokimov et al., 2016). It is possible that upon warming of soil as the soil drying proceeded, microbial activity at least initially increased causing assimilation of phosphorus released from dead cells as well as soil available mineral phosphorus. The highest concentrations of DRP in leachates came from the 14-days (2 weeks) FD treatments dried at both 30°C and 40°C (Figure 4.3). Significant increases in DRP concentrations in leachates could be associated with the greater impact of prolonged soil drying and subsequent rewetting on a fraction of the microbial biomass that are poorly adapted to drying stresses. The reduction in microbial biomass phosphorus in the 14-days FD treatment at 30°C and 40°C was less (Figure 4.5) relative to the reduction observed in the 14-days DF treatments at 30°C and 40°C (Figure 4.4). This may be due to the effectiveness of the fumigation process, whereby in a dried soil the chloroform has better access to all the micropores, which in a moist soil will be at least partially filled with water preventing the access of chloroform, meaning more microbes survive the fumigation process. Moreover, it is also likely that fumigation followed by drying (FD) for an extended period of time (14-days) triggered microbial growth due to the availability of nutrients from the dead biomass and moisture during moist incubation in the initial stages of oven-drying before the moist-dry threshold was reached. This explanation can be supported by Voroney (2007) and Dinh et al. (2017) who state that fungal communities are better adapted to survive drying stress due to their thick cell wall structure limiting water loss and can increase their biomass due to their

greater ability to utilise nutrients released from other microbes. However, fungal communities are less adapted to drying-rewetting stresses likely due to their location at the surfaces of soil aggregates (Blackwell et al., 2010). This work did not consider repeated drying-wetting; nonetheless, some of the observed enhanced leaching of DRP in the 14-days FD treatment might have been contributed by less adapted fungal communities to drying-rewetting stresses.

4.5 Conclusion

The results clearly support the hypothesis that increase in the duration and intensity of drying of soils increases the concentration of DRP in leachate relative to the control moist soil used in this experiment. A reduction in microbial biomass phosphorus as a result of soil drying is the most likely reason that the quantities of DRP in leachates increased relative to the control, concurrent with the drying intensity and duration. The results show that soil drying at higher intensity and for prolonged duration affect the microbial biomass to a greater extent than low intensity-short duration drying, and subsequently cause the leaching of higher concentrations of DRP following rewetting. However, drying duration seems to have a greater influence on DRP concentrations in leachates relative to drying intensity. This means high intensity-short duration drying conditions may not mobilise as much phosphorus as more persistent drying at moderate temperatures. The results also showed that fumigating soil samples before or after drying exhibit similar trends with reduction in microbial biomass phosphorus concurrent with drying intensity and duration. However, the effect of chloroform fumigation was more pronounced in terms of microbial biomass reduction in the DF treatments. These results indicate that the patterns of soil drying and rewetting under future climate change could have impacts on DRP leaching from soils and add to the growing body of evidence on this topic. However, there remains a need to understand soil drying-rewetting impacts on DRP leaching on a wider scale and in a range of soil types, as well as under natural field conditions, to obtain a more realistic assessment of nutrient leaching.

Chapter 5 - Influence of soil drying and rewetting cycles on leaching of phosphorus

5.1 Introduction

Soil drying-rewetting (DRW) cycles are naturally existing soil processes which can mobilise soil nutrients (e.g. P, N, C) and thus potentially can cause their increased leaching. The frequency and intensity of these naturally occurring soil processes will increase due to changing pattern of climate e.g. variations in the rain-fall pattern and increase in ambient temperature (Stocker et al., 2013; IPCC, 2014). It may have consequences for soil fertility and surface water quality due to increased leaching of nutrients.

So far most of the work on soil drying-rewetting processes have been reported by evaluating the results obtained from soil extraction experiments. This approach can overestimate the extent of nutrients mobilisation due to physical/mechanical breakdown of aggregates during extraction experiments. Enhanced nutrients extraction following drying-rewetting is known to be linked with a number of factors e.g. increased mineralisation of soil organic matter (Wu and Brookes, 2005), microbial biomass destruction (Gordon et al., 2008; Turner and Romero, 2010; Bunemann et al., 2013; Brodlin et al., 2019), disaggregation and exposure of previously protected organic matter (Fonte et al., 2014), and breakdown of organo-metallic complexes (Peltovuori and Soinne, 2005; Styles and Coxon, 2006; Soinne et al., 2010).

Phosphorus is an important nutrient required for the growth and development of all living organisms, including crop plants; its adequate availability/supply in farmland soils is important for sustaining a healthy ecosystem. Phosphorus makes an important part of soil's both microbial (Zhang et al., 2013) and non-microbial environments. This inorganic nutrient is unlikely to make direct bond with organic matter due to the presence of negative charges on both phosphate and organic matter at pH levels in the neutral to alkaline range (cf., Novak and Watts, 2006). However, negatively charged organic matter can bind positively charge metal ions (e.g. Fe and Mn) which then bind phosphate anions to create organo-metallic-P complexes. Soil drying induced disruption of aggregates/organo-metallic complexes and the death of microbial biomass thus could result in the mobilisation of phosphorus from both microbial and non-microbial sources (Soinne et al., 2010; Koopmans and Groenenberg, 2011), which upon rewetting can potentially leach out of the soil system.

Soil drying and rewetting micro/mesocosm study under controlled laboratory conditions is a useful alternative to actual field situations. Natural phenomenon is much more complicated, as nutrients mobilised may be retained in the sub-soil or redistribute over the

landscape. The outcomes from controlled laboratory studies, however, are likely to provide a mechanistic understanding of the extent the nutrients can be mobilised/leached under the imposed conditions.

The work in this chapter is the extension of work presented in Chapter 4. This experiment was conducted using the same Hallsworth-I soil as used in Chapter 4 but the work was carried out separately and independently. The work presented in Chapter 4 showed that the intensity and duration of soil drying increased dissolved reactive phosphorus (DRP) leaching which was partly contributed by lysis of microbial biomass as indicated by the reduction in microbial biomass phosphorus, and also that the decrease in microbial biomass phosphorus and subsequent increase in the DRP leaching was concurrent to the increase in drying duration and drying intensity (Khan et al., 2019). The research in Chapter 4 was primarily designed to understand soil microbial biomass response to drying and rewetting. However, in that experiment (Chapter 4), just two somewhat harsh drying temperatures (30°C and 40°C) were selected, thus raising a question, how intermediate drying temperatures may influence phosphorus leaching. To address these uncertainties, in the current study four consecutive soil drying intensities (25°C, 30°C, 35°C and 40°C) were chosen. Moreover, soils were rewetted using a rapid rewetting rate in Chapter 4. In contrast, here two contrasting rewetting rates were chosen – slow or rapid (see Section 5.2.2) to see if the rate by which dried soils are rewetted affects DRP concentrations in the leachates. In Chapter 4 the soil was rewetted once but here soil was rewetted up to three rewetting cycles (see Section 5.2.2) to see how DRP leaching could be influenced in subsequent rewetting cycles.

The aim of this experiment was to understand how naturally occurring soil drying rewetting cycles in terms of variations in rain-fall patterns and rising ambient temperature may influence soil phosphorus leaching, with the following research questions: (1) How drier periods are likely to alter leaching of phosphorus. The novelty of this research lies in how phosphorus leaching is affected with increasing intensity of soil drying. (2) How variability in rain-fall patterns would affect phosphorus leaching. To examine this, two rewetting rates were chosen – a slow rewetting mimicking low intensity rainfall and a rapid rewetting mimicking heavy rainfall. (3) Will increase in the number of drying days have any effects on phosphorus leaching? For this purpose, two drying durations (2-days or 14-days) were imposed. (4) Will succeeding rain-fall events likely to influence phosphorus leaching – for this purpose soils were subjected to three rewetting cycles (n = 3).

5.2 Material and methods

5.2.1 Site description, sample collection and preparation

This experiment was carried out using the same soil (Hallsworth-I) as in Chapter 4. See Section 3.1 for the site and soil description. Soil was prepared as described in Section 3.2.

5.2.2 Soil drying rewetting and leaching experimental design

Soil drying was carried out at four temperatures (25°C, 30°C, 35°C, and 40°C) in an oven with controlled temperature for either 2-days or 14-days. Reference control moist soil was not dried and kept stored at 3°C until required and maintained at 54% of maximum water holding capacity at room temperature (21°C) for a day before subjecting to rewetting. Each drying- rewetting cycle consisted of either 2-days or 14-days drying followed by either rapid rewetting or slow rewetting and soil was rewetted up to three rewetting cycles (n=3).

The soil was weighed before and after oven drying to measure the moisture loss at different temperatures. Subsequently, two replicates of each treatment, each comprising 30 g dry-weight equivalent (DWE) of the soil, were placed in plastic Buchner funnels with internal diameter of 76 mm and 186 ml capacity, as shown in Figure 5.1. Grade 1 qualitative filter paper (GE Healthcare Whatman) was placed at the bottom of the funnels to prevent soil particles from passing through the funnel. Soil was gently compacted (to 1.2 g/cm^3 density - although the field bulk density for this series soil is reported to be 0.99 g/cm^3 for the 0-27cm depth (Harrod and Hogan, 2008). For laboratory leaching trials, prepared/disturbed soils are packed to bulk densities ranging from 1.1 to 1.6 g/cm^3, depending upon soil texture, as recommend by OCED (1993), at least partly to prevent bypass flow in such controlled studies. Deionised water (18MΩ) was applied evenly on the soil surface using a syringe so that 30 g dry-weight equivalent soil received 105 ml of water (This is the same experimental set-up described in Section 4.2.2 for microbial biomass responses to soil drying-rewetting and phosphorus leaching experiment). However, this experiment was carried out separately and independently, with entirely new objectives as outlined in Section 5.1.

In the rapid rewetting treatment, soil was watered with 105 ml of deionised water gently (to mitigate by-pass flow and high hydrophobicity effects) but in one application. While in slow rewetting 105 ml of water was applied in small portions (5 ml) after every 20 min over a period of seven hours. All treatments were subjected to three rewetting cycles. Before subjecting the soil to the second and third rewetting events, they were allowed to air-dry at room temperature (21°C to 22°C). The second rewetting cycle was carried out on the seventh

day after the first rewetting cycle. Similarly, the third rewetting was carried out on the seventh day after the second rewetting cycle. Here no attempt was made to estimate the remaining moisture content before the second and the third rewetting, assuming all treatments would have similar soil moisture content. Collected leachates (~ 65 ml) were filtered through Whatman 0.45 μm cellulose nitrate membrane filters and analysed within 24 hours for dissolved reactive phosphorus (DRP) by the molybdate blue method.

Table 5.1
Types of treatments imposed (drying intensity, drying duration, rewetting rates, and rewetting cycles).

Moist control (MC)	Soil was not dried and kept stored at 3°C until required and maintained at 54 % of maximum WHC at room temperature (21°C) for a day before subjecting to rewetting
2D	Soil was oven-dried (25°C, 30°C, 35°C, or 40°C) for 2-days
14D	Soil was oven-dried (25°C, 30°C, 35°C, or 40°C) for 14-days (2 weeks)
R	Rapid rewetting –105 ml of water was applied gently but in one application
S	Slow rewetting –105 ml water was applied in small portions (5 ml) after every 20 min over a period of 7-hours
C1	First rewetting cycle
C2	Second rewetting cycle – commenced on the seventh day after the first rewetting cycle
C3	Third rewetting cycle – commenced on the seventh day after the second rewetting cycle

Note: Based on the above information, treatment 2DRC1 refers to 2-days drying rapid rewetting first cycle, and 14DSC2 refers to 14-days drying slow rewetting second cycle.

Figure 5.1: Soil drying-rewetting leaching experimental set-up: Two soil replicates were placed in plastic Buchner funnels. Grade 1 qualitative filter paper (GE Healthcare Whatman) was placed at the bottom of funnels to prevent soil particles passing through the funnel. Deionised water was applied evenly on the soil surface using a syringe. Leachate was collected in acid washed 250 ml plastic bottles as shown.

5.2.3 Laboratory analyses

Soil was characterised (see Table 4.2 for physical and chemical properties of the Hallsworth-I soil) using standard procedures and quality controls. The soil sampling strategy, sample preparation and storage protocols are described in Chapter 3.

5.2.4 Statistical analysis

The significance of differences between treatments were determined by one-way ANOVA (significance reported as $p < 0.05$) by SPSS (IBM SPSS statistics 24). Fisher's LSD (Least Significant Difference) post-hoc test was employed for multiple comparisons. The data were reported as mean ± standard deviation. Dependent variables were normalised using $\log_{10}$ transformation, when they did not follow a normal distribution to meet the ANOVA assumptions. The effect of interactions among the independent variables (drying intensity, drying duration, rate of rewetting, and rewetting frequency) on the DRP concentration was determined using univariate ANOVA (Table 5.2).

Table 5.2
Summary statistic factors of univariate ANOVA assessing the effects of independent factors (drying intensity, drying duration, rewetting rate and rewetting frequency) on the DRP leachate concentrations. The means difference is significant at $p < 0.05$ level.

Source	DF	F	P
Drying intensity	4	149.340	< 0.001
Drying duration	1	52.024	< 0.001
Rewetting rate	1	41.770	< 0.001
Rewetting frequency	2	45.809	< 0.001
Drying intensity*Drying duration	4	9.778	< 0.001
Drying intensity*Rewetting rate	4	0.646	NS
Drying intensity*Rewetting frequency	8	12.433	< 0.001
Drying duration*Rewetting rate	1	6.256	< 0.05
Drying duration*Rewetting frequency	2	25.703	< 0.001
Rewetting rate*Rewetting frequency	2	13.686	< 0.001
Drying intensity*Drying duration*Rewetting rate	4	2.909	< 0.05
Drying intensity*Drying duration*Rewetting frequency	8	4.438	< 0.001
Drying intensity*Rewetting rate*Rewetting frequency	8	1.634	NS
Drying duration*Rewetting rate*Rewetting frequency	2	0.392	NS
Drying intensity*Drying duration*Rewetting rate*Rewetting frequency	8	1.085	NS

Values of DRP concentrations were normalised by $\log_{10}$ transformation prior to ANOVA analysis. Note: 'NS' indicates not significance ($p > 0.05$).

5.3 Results and discussion

5.3.1 Soil responses to varying intensity of drying

Drying soil at 35°C and 40°C may be considered relatively high, but soils experience such high temperatures in summers in the UK (Blackwell et al., 2009) e.g. the highest temperature recorded in the UK (38.7°C on 29 Jul 2019; www. metoffice.gov.uk). The average moisture content of the dried soils before rewetting them were 4.7%, 3.7%, 2.2%, and 1.1% at 25ºC, 30ºC, 35ºC, and 40ºC, respectively.

Overall, soil drying intensity had a significant effect ($p < 0.001$) on the DRP leaching (Table 5.2), and following rapid or slow rewetting, first cycle, the DRP concentrations in the leachates collected from soils previously dried for either 2-days or 14-days at all temperatures (25ºC, 30ºC, 35ºC, and 40ºC) significantly ($p < 0.05$) increased relative to the control moist counterparts (Figures 5.2 a-b). For instance, in case of 2-days drying at 40°C, followed by rapid and slow rewetting, first cycle (2 DRC1 and 14 DSC1), the DRP concentrations were about 2.8 and 3.1 times, respectively higher than their respective control moist counterparts (Figure 5.2a-b). As with the 2-days drying, 14-days drying followed by rapid or slow rewetting, first cycle (14 DRC1 and 14 DSC1) significantly ($p < 0.05$) increased DRP concentrations at all temperatures (25ºC, 30ºC, 35ºC, and 40ºC) relative to the control moist counterparts (Figure 5.2a-b). Here, for example, the DRP concentrations leached from the soils dried for 14-days at

40ºC followed by rapid and slow rewetting, first cycle were about 6.6 and 9 times, respectively higher than the control moist soil counterparts (Figure 5.2a-b).

The DRP that leached from the soil dried for either 2-days or 14-days in the first (rapid or slow) rewetting cycle showed some increasing trends with increasing drying intensity (Figure 5.2a-b). However, the DRP concentrations leached from two consecutive drying temperatures (e.g. 25°C and 30°C) were not significantly ($p > 0.05$) different from each other in almost all treatments (Figure 5.2a-b), with the exception where the soil was dried at 40°C for 14-days followed by rapid and slow rewetting (Figure 5.2 a-b). In this latter treatment, the DRP concentrations leached at 40°C were significantly ($p < 0.05$) greater than the corresponding 35°C counterparts. Furthermore, in the treatment where soil was dried for 2-days followed by rapid rewetting first cycle, the DRP concentration leached at 35°C was significantly greater than its 30°C counterpart (Figure 5.2a).

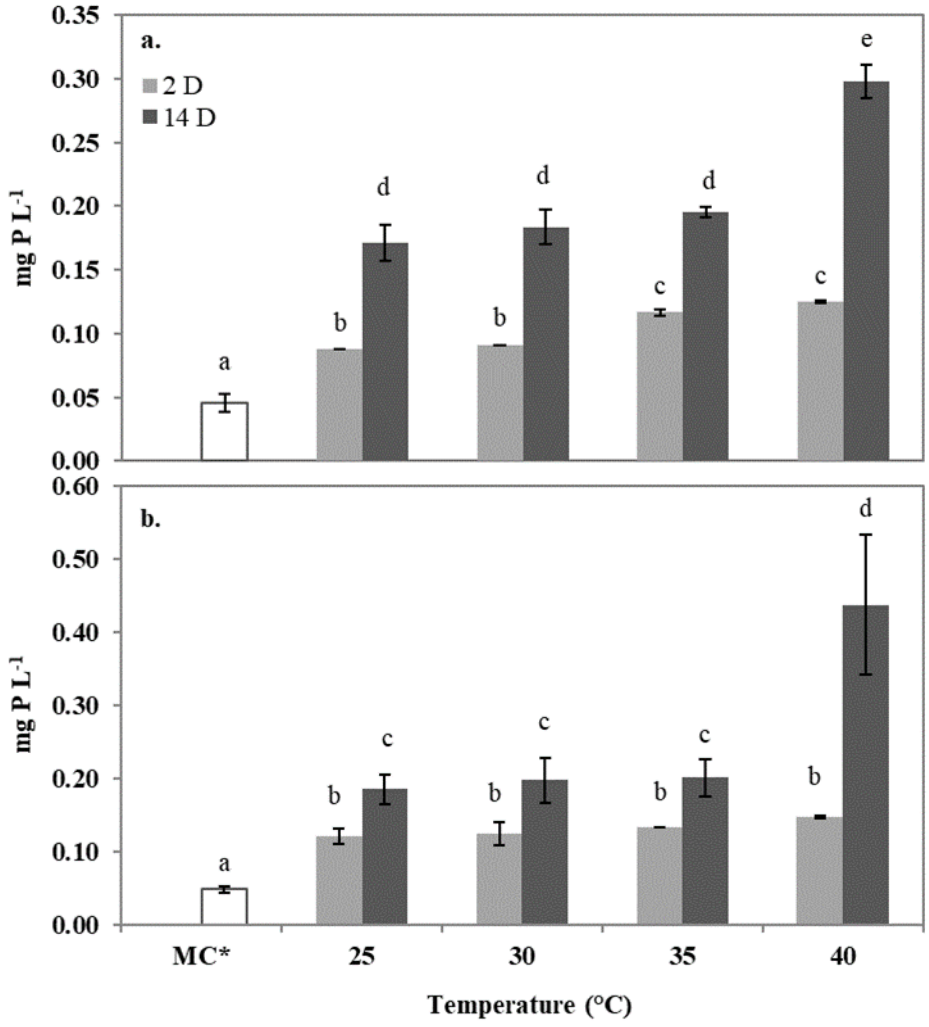

Figure 5.2a-b: DRP (mg P L^{-1}) leached in 2-days (2 D) and 14-days (14 D) drying treatments (**a.** Rapid rewetting, first cycle, **b.** Slow rewetting, first cycle). Error bars represent standard deviation, n = 2. Bars labelled with the same letters are not significantly different ($p > 0.05$) from each other. * MC is the moist control soil.

In the second slow or rapid rewetting cycle, the 2-days of soil drying resulted in significantly ($p < 0.05$) greater DRP leaching relative to the control moist soil at all temperatures (25°C, 30°C, 35°C, and 40°C) (Figures 5.3a-b). As with the 2-days drying, the 14-days drying at 25°C, 30°C, 35°C, and 40°C followed by rapid or slow rewetting second cycle, leached significantly ($p < 0.05$) greater DRP concentrations relative to the control moist soil (Figure 5.3 a-b) with the exception of the 14-days drying at 25°C followed by slow rewetting (Figure 5.3b).

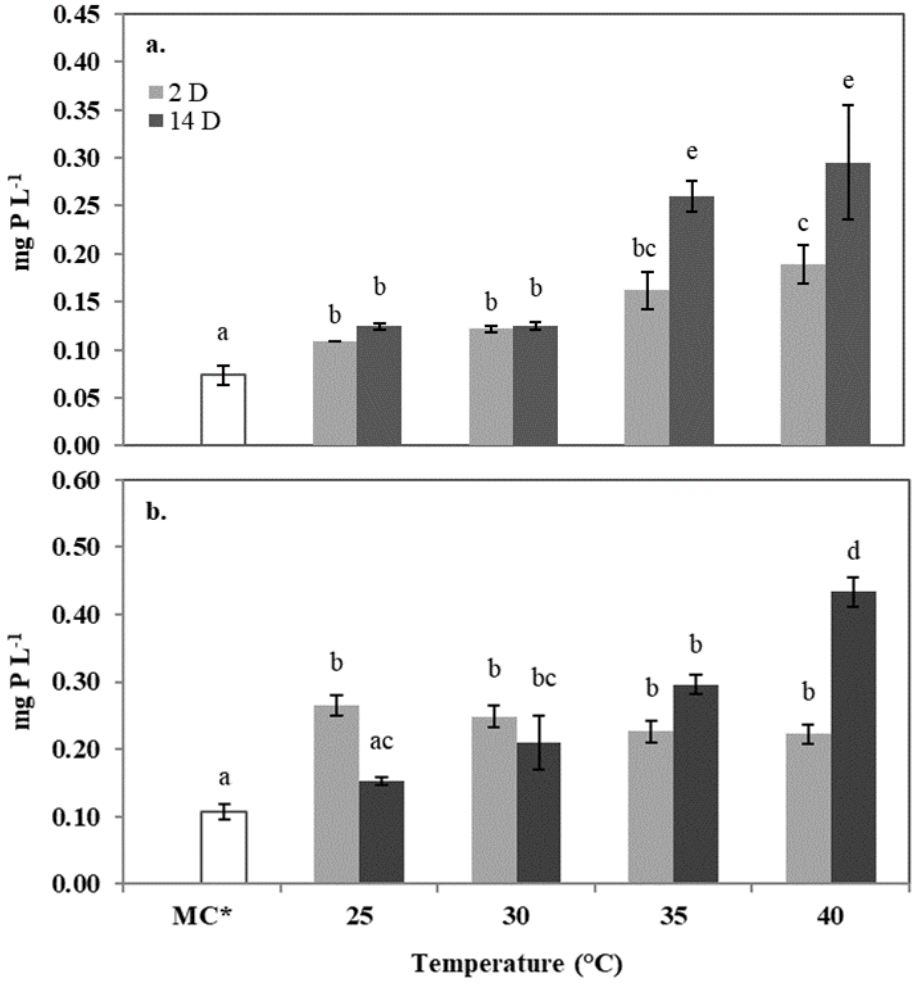

Figure 5.3a-b: DRP (mg P L^{-1}) leached in 2-days (2 D) and 14-days (14 D) drying treatment (**a.** Rapid rewetting, second cycle, **b**. Slow rewetting, second cycle). Error bars represent standard deviation, n = 2. Bars labelled with the same or coinciding letters are not significantly ($p > 0.05$) different from each other e.g. **bc** is not statistically different from either **b** or **c**. Just like, **bc** is not statistically different from **ac** or **b**. Note: * MC is the moist control soil.

The effect of pre-drying remained up to the third rewetting cycle, which means although the soils were dried once before subjecting to the first rewetting cycle, but this influenced DRP leaching relative to the moist control up-to the third rewetting cycle, although to a variable extent across the treatments (Figure 5.4a-b). In the third rewetting cycle, relative to the control moist counterparts, significantly ($p < 0.05$) greater DRP concentrations were leached from the

soils previously dried at 25°C, 30°C, 35°C, or 40°C for 2-days followed by rapid or slow rewetting (Figure 5.4a-b); however, in treatment where soil was dried for 2-days at 25°C followed by rapid rewetting, the concentration increase relative to the control moist soil was not statistically significant (Figure 5.4a). As with the 2-days drying, soils previously dried for 14-days at 25°C, 30°C, 35°C, or 40°C followed by rapid or slow rewetting third cycle, leached significantly ($p < 0.05$) greater DRP concentrations relative to the control moist counterparts (Figure 5.4a-b); however, the concentration increase relative to the control moist soil was not statistically significant at 30°C (14 DSC3; Figure 5.4b).

The greater DRP concentrations in the leachates derived from the dried soils could partly be contributed by DRW induced breakdown of organo-metallic-P complexes (Soinne et al., 2010) and lysis of microbial cells (Denef et al., 2001a; Peng et al., 2007; Achat et al., 2012a,b; Bunemann et al., 2013; Rahman et al., 2018; Pezzolla et al., 2019). Previous studies have provided some evidence of phosphorus solubilisation through drying-induced breakdown of organo-metallic-P complexes with which P was previously sorbed, since soil organic macromolecules are aggregated due to hydrogen bonds and drying decreases the stability of soil organic matter by reducing the interaction caused by hydrogen bond and causing shrinkage (Soinne et al., 2010; Koopmans and Groenenberg, 2011).

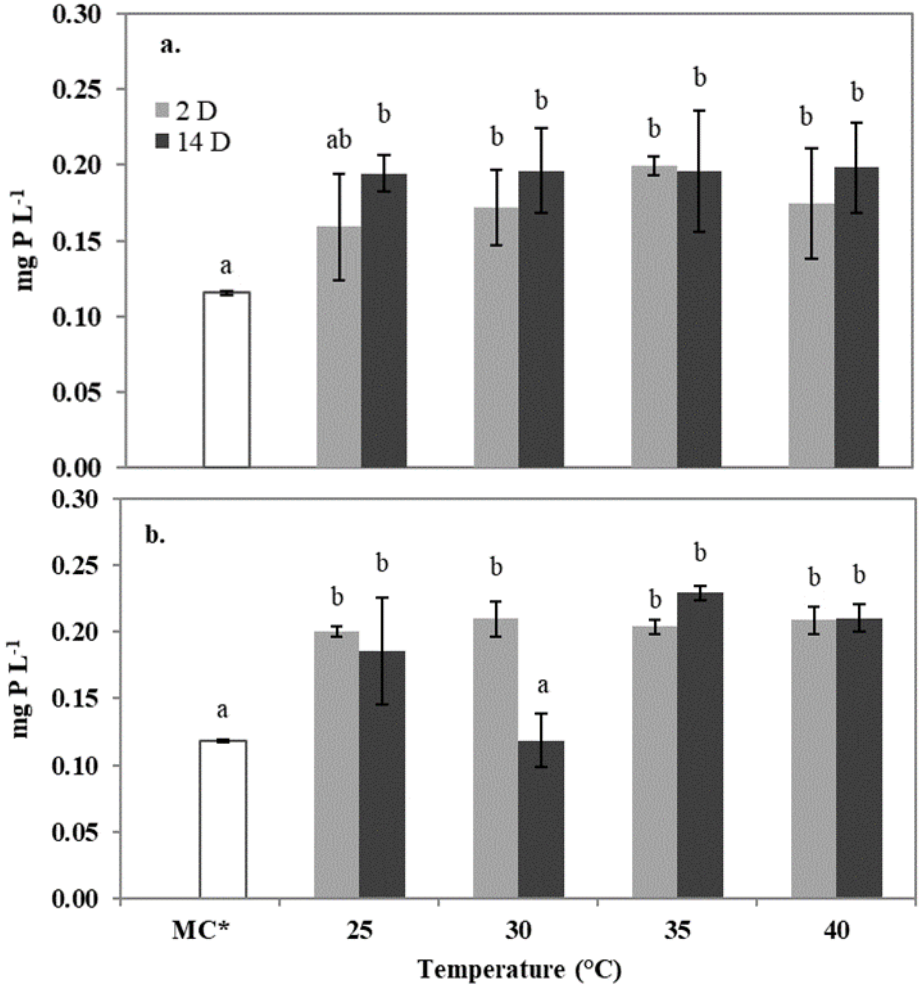

Figure 5.4a-b: DRP (mg P L^{-1}) leached in 2-days (2 D) and 14-days (14 D) drying treatment (**a.** Rapid rewetting third cycle, **b.** Slow rewetting third cycle). Error bars represent standard deviation, n = 2. Bars labelled with the same or coinciding letters are not significantly ($p > 0.05$) different from each other e.g. **ab** is not statistically different from either **a** or **b**. Note: * MC is the moist control soil.

Microbial contribution to the phosphorus pulses after DRW event has also been widely reported (Achat et al., 2012a,b; Bunemann et al., 2013; Chen et al., 2016; Dinh et al., 2017; Brodlin et al., 2019; Khan et al., 2019). Dinh et al. (2017) suggested that the Gram-negative bacteria, being the most sensitive part of microbial biomass, represent an important source of phosphorus solubilisation following a DRW stress. Koopmans et al. (2006) and Bunemann et al. (2013) reported increase in molybdate unreactive phosphorus (MUP), which was attributed to the drying-induced microbial cell lysis. More recently, Brodlin et al. (2019) suggested that the extent of drought-induced phosphorus mobilisation is determined by the intensity and duration of DRW stresses with harsh drying (40ºC) conditions exerting a greater osmotic stress to microbial biomass relative to the mild drying stress (20ºC). Bunemann et al. (2013) reported significantly greater release of resin extractable phosphorus upon rewetting from soils dried to

low moisture contents (2-5%) relative to the soils dried to higher moisture contents (15-40%). They associated this increase to microbial cell lysis since this increase was concurrent with the reduction in microbial biomass phosphorus. Turner and Haygarth (2001) reported that DRW solubilised more water-soluble phosphorus at 30ºC relative to 15ºC in UK grassland soils. The underlying mechanism of enhanced phosphorus release at the high temperature could be that when the soil undergoes through the drying process, there is still a thin film of water around soil particles in which microbes not only survive but continue their activity. As the drying intensity increases, more and more water from the soil solution evaporates leaving behind highly concentrated solutes. Microorganisms require a critical range of temperature and moisture to sustain their growth and cellular activities. Too low moisture will not be ideal for microbial growth and may affect cellular activities due to inactivation of enzymes unless microorganisms are adapted to such unfavourable conditions (Van Gestel et al., 1993a,b; Sardans and Penuelas, 2005), thus lowering organic matter mineralisation rate concomitantly with the increasing intensity of drying (Borken and Matzner, 2009). However, upon rewetting, soils which were previously dried to a greater intensity tend to have high mineralisation rate and release greater quantities of phosphorus partly because of greater impact of intense drying on microbial biomass and soil structure (Khan et al., 2019).

An additional mechanism other than destruction of organo-metallic-P complexes and microbial biomass destruction which could have contributed to the increase in DRP leaching might be drying-induced aggregate shrinkage and exposure of additional soil surfaces with subsequent increased overall porosity compared with the moist soil, with tightly held interstices (Brodlin et al., 2019). This allows water an opportunity to contact with more and new soil surfaces, thus dissolving any dislodged or mobilised nutrients before leaving the soil compartment. This could be another possible reason for higher phosphorus concentration in the leachates at higher temperatures compared to the moist control counterparts.

Although it is difficult to provide evidence in terms of which of the above-mentioned processes may have played a key role, the evidence presented indicates that more intense soil drying exerts a greater impact on microbial biomass and soil structure and thus releases greater concentrations of DRP upon rewetting.

5.3.2 Duration of soil drying

Overall, soil drying duration had a significant ($p < 0.001$) effect on DRP leaching (Table 5.2). In the first rapid rewetting cycle, the leachate DRP concentrations from the soils dried for 14-days at 25ºC, 30ºC, 35ºC and 40ºC were significantly ($p < 0.05$) greater than their 2-days

drying counterparts (Figure 5.2a). Here, the percent increases in DRP concentrations for soils dried for 14-days at 25ºC, 30ºC, 35ºC, and 40ºC (14 DRC1) were 96%, 101%, 67%, 138%, respectively relative to their 2-days drying counterparts (2 DRC1) (Figures 5.2a).

As with rapid rewetting, in the slow rewetting, first cycle, the DRP concentrations leached from the soils dried for 14-days at 25ºC, 30ºC, 35ºC and 40ºC were significantly ($p < 0.05$) greater than their 2-days drying counterparts (Figure 5.2b). For instance, the DRP concentration leached from the soil dried for 14-days at 40ºC followed by slow rewetting first cycle (14 DSC1) was 198% higher than its 2-days drying counterpart (2 DSC1) (Figures 5.2b).

A longer period of drying may have stronger effects on microbial mortality and may cause substantial reduction in microbial biomass due to unavailability of moisture for a longer period (Borken and Matzner, 2009). In this study, microbial biomass was not measured but the results presented in Chapter 4 using the same soil (Hallsworth-1) suggest that the drying-induced reduction in microbial biomass phosphorus was parallel to the increase in drying intensity and drying duration, with decrease in microbial biomass phosphorus at 30ºC soil drying for 2-days and 14-days being 52% and 66% respectively relative to the moist control soil. The reduction in microbial biomass phosphorus at 40ºC soil drying for 2-days and 14-days duration were 61% and 70%, respectively, relative to the moist control counterparts (Khan et al., 2019). These results suggest that relatively higher microbial mortality and subsequent release of microbial phosphorus from lysed microbial biomass at higher drying temperature could have at least partly contributed to higher concentrations of DRP in the leachates. These results are also supported by a recent DRW study on UK soils (Forber et al., 2017). The authors reported 28% more soluble reactive phosphorus in the soil dried up to 15 days. Extended drying and a subsequent reduction in microbial activity and microbial biomass could reduce mineralisation of soil organic matter due to unavailability of moisture for a prolonged period of time, but may increase nutrients losses upon wetting due to accumulated dead microbial cells and release of intracellular solutes from the better adapted microbial species as a surviving mechanism (Borken and Matzner, 2009). As the soil progressively dries, the water film surrounding the soil particles becomes thinner, disrupting and limiting diffusion of solutes (Schimel et al., 2018). Some microorganisms produce extracellular polymeric substances (EPS) to increase their survival by retaining moisture under drought (Schimel et al., 2018) and most of the microorganisms are known to produce higher concentrations of EPS at temperatures between 25ºC to 30ºC (More et al., 2014). It is also likely that rewetting of soils after prolonged drought tends to release phosphorus from these structures since a large amount of phosphorus is accumulated in EPS by phosphorus accumulating microorganism (Zhang et

al., 2013). Beside these mechanism and factors, drying induced increased crystallinity of metal oxides and reduced sorption of phosphorus could also be a contributory factor in increasing DRP leaching (Schonbrunner et al., 2012; Dieter et al., 2015).

Drying duration seems to have long-term impacts on phosphorus dynamics that even after the normal soil moisture conditions and soil temperature were returned, the drying duration influenced DRP leaching up to the third rewetting cycle. As mentioned earlier, in this study the soil was dried just once before subjecting to the first rewetting event and the successive rewetting cycles were performed on the soil samples which were allowed to air-dry at room temperature (21-22ºC) for 7-days. However, greater DRP was leached in several of the treatments in the second and third rewetting cycles where soil was previously dried for a longer period, although the influence in the third rewetting cycle was less clear (Figures 5.3 and 5.4). For example, in the second slow rewetting cycle, the DRP leached from the soil dried at 40°C for 14-days was as much as twice that DRP concentration leached from its 2-days drying counterpart (Figure 5.3b). Greater DRP leachate concentrations in the successive rewetting cycles from the soil previously dried for a longer duration could likely be due to greater detrimental impact of prolonged drying on the microbial community structure and activities, particularly at higher soil drying temperatures (Nguyen et al., 2018). Prolonged drying will not only kill microbial cells, but can also cause sub-lethal damages to DNA, proteins, membranes and cell walls in the surviving microbial population (Meisner et al., 2015). Meisner et al. (2015), for example, reported that prolonged drying slowed down microbial growth rate as indicated by a longer lag period. Rahman et al. (2018) reported that long-term drought reduced bacterial growth and respiration upon rewetting. Clearly, it would seem that the influence of soil drying duration on DRP leaching can extend beyond the first rewetting, particularly when soils drying occurs at greater temperatures.

5.3.3 Rapid and slow rewetting

Overall, soil rewetting rate had a significant ($p < 0.001$) effect on the DRP leaching (Table 5.2). Generally, the leachate DRP concentrations from the soils rewetted at the slower rate tended to be higher than their corresponding DRP concentrations measured in the leachates collected from soils rewetted at the rapid rate. However, the difference between the DRP concentrations from the soils rewetted at rapid and slow rewetting rates was statistically significant ($p < 0.05$) mainly for those treatments where the soil was dried at 25°C or 30°C. For instance, the DRP leachate concentration from the soil dried for 2-days at 25°C or 30°C

followed by the first slow rewetting cycle was significantly ($p < 0.05$) greater relative to its 2-days drying, first rapid rewetting counterpart (Figure 5.2a-b). This confirms that the rate at which dried soils are rewetted affects phosphorus concentrations in the leachate, as found in an earlier work (Blackwell et al., 2013). Slow rewetting allows less microbial biomass destruction thus a greater surviving microbial population will be available to mineralise freshly exposed labile organic matter resulting in greater concentration of nutrients in leachate. In contrast, rapid rewetting alongside promoting aggregate destruction and particle detachment also promotes preferential flow. Thus, allowing water to move through the soil profile faster by making channels instead of percolating evenly through the soil profile, subsequently interacting with less surface area for nutrients detachment. This could be one of the reasons that in rapid rewetting, particulate forms of nutrients predominate because of quick fixation of dissolve forms with soil minerals, whereby particulate forms escape from fixation and are easily transported (Toor et al., 2004; Blackwell et al., 2009). In the current study although phosphorus in particulate form was not measured, the same pattern seems to be taking place as under natural field conditions whereby heavy torrential rains have been reported to mobilise greater quantities of phosphorus in particulate forms (Drewry et al., 2009; Chen et al., 2013). This is further supported by studies which projected greater nutrients loading from land to catchment waters (Hagg et al., 2014; Huttunen et al., 2015), with more intense episodic rainfall events (Kostaschuk et al., 2003; Zhang and Nearing, 2005).

Nevertheless, more studies are required to further investigate the processes and mechanisms involved in affecting nutrients leaching at different rewetting rates since so far only Blackwell et al. (2013) have investigated the effects of rewetting rate on phosphorus leaching. Clearly it is difficult to set boundaries between faster and slower rewetting rates and there is no conclusive agreement among studies (rapid rate reported by one study may not be rapid or fast for other). Nevertheless, it is worth mentioning that faster the soils are rewetted it is more likely to affect nutrients in particulate form, whilst dissolved concentrations are more likely to be affected in slower rewetting rates (Blackwell et al., 2013).

5.3.4 Frequency of rewetting cycles

In the second slow rewetting cycle, DRP concentrations leached from the soils previously dried for 2-days at all temperatures were significantly ($p < 0.05$) greater than those DRP concentrations leached from the first slow rewetting counterparts (Figure 5.5 b). In the second slow rewetting cycle, the percent increases in the DRP concentrations, leached from the

soils previously dried for 2-days at 25°C, 30°C, 35°C and 40°, were 120%, 100%, 70% and 51% respectively relative to their first slow rewetting counterparts (Figure 5.5b).

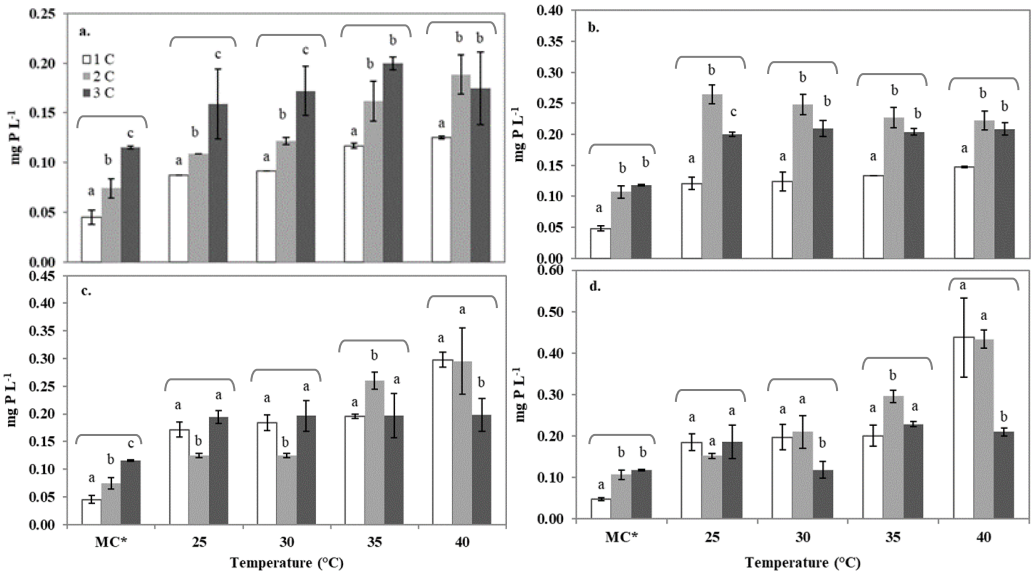

Figure 5.5a-b: Dissolved reactive phosphorus (mg P L^{-1}) leached in 2-days and 14-days drying, first/second/third, rapid and slow rewetting cycles at different imposed temperatures: a (2-days drying rapid rewetting treatment), **b** (2-days drying slow rewetting treatment), **c** (14-days drying rapid rewetting treatment) and **d** (14-days drying slow rewetting treatment). Error bars represent standard deviation, n=2. In some cases, error bars are too small to be seen. The letter 'C' represents the number of cycles e.g. the first (1C), second (2C), and the third (3C) rewetting cycle. Bars labelled with the same letters are not significantly ($p > 0.05$) different from each other. The bracket signs indicate the significant differences (as indicated by letters) among the means of 1C, 2C, and the 3C for individual treatments e.g. Significance differences among the means of 1C, 2C, and the 3C at 40°C. Note: * MC is the moist control soil.

As with the slow rewetting, in the second rapid rewetting cycle, the leachate DRP concentrations from the soils dried at all temperatures for 2-days were significantly ($p < 0.05$) greater relative to the first rapid rewetting counterparts (Figure 5.5a).

These results are consistent with other studies reporting increased extractability of phosphorus with repeated DRW cycles (Chepkwony et al., 2001; Pezzolla et al., 2019). It seems likely that a combination of physical and biological factors may have contributed to the increased leaching of phosphorus in the second rewetting cycle. For example, repeated DRW cycles have been seen to stimulate microbial activity and mineralisation of organic matter through aggregate disruption and destabilisation of soil structure, allowing access to new surfaces (Xiang et al., 2008; Bunemann et al., 2013; Pezzolla et al., 2019). It is possible that the 7-days "incubation" period at (21-22°C) after the first rewetting cycle allowed microbial

biomass to increase their biomass due to availability of moisture and freshly exposed labile organic matter either from aggregate breakdown or lysis of dead cells as microbial biomass has been reported to recover within 7-days of DRW events (Chen et al., 2016). Subsequently, when the second rewetting was employed, it triggered greater phosphorus leaching from a newly grown microbial biomass due to osmotic shock.

In treatments where the soil was previously dried for 14-days, the second rapid or slow rewetting cycle showed a different pattern with the DRP concentrations either decreased, remained similar, or showed an increasing trend relative to the first rapid or slow rewetting counterparts (Figure 5.5c-d), though remained greater than the respective moist control counterparts (Figures 5.3 a-b).

In the third rapid rewetting cycle, the soil previously dried at 25°C and 30°C for 2-days continued to leach significantly greater DRP concentrations relative to the first and second rewetting counterparts (Figure 5.5a). However, for treatments where the soil was previously dried for 2-days at 35°C or 40°C (followed by rapid rewetting, third cycle), the DRP concentrations either remained similar (not statistically different at 35°C) or showed a decreasing trend relative to the second rewetting cycle (Figure 5.5a).

In the third slow rewetting cycle, the DRP concentrations leached from the soils previously dried for 2-days at all temperatures showed a decreasing trend relative to the second slow rewetting counterparts (Figure 5.5b), though remained significantly higher than the first slow rewetting counterparts (Figure 5.5b).

In case of 14-days drying, in the third rapid rewetting cycle, the DRP concentrations either significantly increased (at 25ºC and 30 ºC) or significantly decreased (at 35ºC and 40 ºC) relative to the second rapid rewetting counterparts (Figure 5.5c). In the third slow rewetting cycle, the DRP concentrations leached from the soils previously dried for 14-days either remained similar (not statistically different at 25°C) or significantly decreased (at 30°C, 35ºC and 40 ºC) relative to the second slow rewetting counterparts (Figure 5.5d).

Studies reporting increased nutrients extractability also noted decrease in nutrients availability with successive DRW cycles. They attribute this decrease to the limitation in labile organic matter pool (Fierer and Schimel, 2002; Fierer et al., 2003; Butterly et al., 2009, 2011), resistance of aggregate to slaking (Denef et al., 2001a), rapid growth of certain spore former (Meisner et al., 2015) and microbial community shift (Butterly et al., 2009, 2011). The effect

of microbial community shift in decreasing nutrient extractability has also been reported by Butterly et al. (2009) in a DRW study. They observed reduction in microbial biomass by up to 75% after the first rewetting event. However consecutive rewetting events had less impact on the microbial biomass. It was suggested that microbes surviving the first rewetting event may be more resistant to subsequent DRW. Pezzolla et al. (2019) reported that microbial biomass phosphorus decreased after the first DRW event but recovered after the second event likely due to the resilience of certain species of microbes to these perturbations. Zhang et al. (2007) observed a decrease in soil respiration rate after the first drying-wetting cycle but noticed recovery in respiration rates in the subsequent drying-wetting cycles. They attributed this increase in respiration to a shift in the microbial community structure, since this increase coincided with the increase in microbial biomass. Moreover, it is also possible that repeated DRW events progressively depleted the quantity of available labile soil organic matter held within micro-aggregates (Fierer and Schimel, 2002; Fierer et al., 2003; Butterly et al., 2009). Nevertheless, the impact of DRW cycles varies from one cycle to the other; perhaps more research in this area is needed especially under natural field conditions to explore further the responses of both biological and non-biological factors in determining nutrients leaching.

5.4 Conclusion

Overall, soil drying intensity had a significant effect ($p < 0.001$) on the DRP leaching. The leachate DRP concentrations from the soil previously dried for 2-days or 14-days at 25ºC, 30ºC, 35ºC, and 40ºC followed by rapid or slow rewetting, first cycle significantly ($p < 0.05$) increased relative to the control moist counterparts. The leachate DRP concentrations in the first rapid or slow rewetting cycle from the soil dried for 2-days or 14-days showed an increasing trend parallel to the drying intensity. The observed increase in phosphorus leaching is likely to be caused by a combination of factors such as drying-rewetting induced aggregate destruction, breakdown of organo-metallic-P complexes, enhanced mineralisation of organic matter upon availability of moisture, and microbial cell lysis due to osmotic shock.

Overall, the duration of soil drying had a significant ($p < 0.001$) effect on the leachate DRP concentrations. The DRP concentrations leached from the soil dried at 25ºC, 30ºC, 35ºC or 40ºC for 14-days followed by either rapid or slow rewetting, first cycle were significantly greater than their 2-days drying counterparts. Overall, soil rewetting rate had a significant ($p < 0.001$) effect on the DRP leaching and generally the leachate DRP concentrations from the soil rewetted at the slower rate tended to be higher than their corresponding DRP concentrations measured in the leachates collected from soils rewetted at the rapid rate. However, the difference between the DRP concentrations from slow and rapid rewetting rate was not statistically significant ($p > 0.05$) in most of the treatments.

The second rapid or slow rewetting cycles leached significantly greater DRP concentrations relative to the first rewetting counterparts at all temperature treatments where soil was previously dried for 2-days. However, in treatments where the soil was previously dried for 14-days, the second rapid or slow rewetting cycle showed a different pattern with the DRP concentrations either increased, decreased, or remained similar.

In the third rewetting cycle, the DRP leaching showed some trends (increasing, decreasing or no change). The tendency of decreasing DRP leaching in the succeeding rewetting cycles is perhaps due to depletion in the easily leachable phosphorus. Besides this, other factors like microbial community shift, slake resistance of aggregates and limitation in labile organic matter pool could also have contributed to the decrease in DRP concentrations in the successive rewetting cycles.

The results suggest that longer-drier periods proceeded by heavy rainy events will render enhanced leaching/mobilisation of phosphorus, and the magnitude of phosphorus leaching/mobilisation will increase with the increase in the frequency of DRW cycles, under

changing pattern of climate. Nevertheless, additional work on soils from wider ecosystems may further explain how soils with different texture, microbial community structure may interact with predicted changes in climate. So far majority of the work has been done to address the effects of soil drying and rewetting on nutrients extraction, whilst only a few studies reporting the effects of repeated DRW cycles on nutrient dynamics (mobilisation and sequestration), with some contradictory results – some are reporting increase while other are reporting decrease in nutrient concentrations with increase in the frequency of DRW cycles.

Moreover, laboratory DRW manipulations can explain the significance of these processes but cannot replicate natural-field conditions as any phosphorus that is solubilised is not necessarily mobilised and transported to surface water. Laboratory leaching experiments using homogenised sieved soils also could overestimate the biomass-P quantity that is leached as soil is not structured/intact so the drying and rewetting will be uniform (Blackwell et al., 2010). The quantity of phosphorus potentially leachable under natural-field conditions will be less than the laboratory manipulations because of the following factors: (A) soil is well structured under natural-field conditions, which will not be uniformly dried and rewetted and only the top layer will be mainly exposed to drying and rewetting stresses. (B) The presence of aggregates will also mediate the effect of drying and rewetting as the microbes residing well within the aggregates will be less affected by these stresses (Blackwell et., 2010). (C) The presence of thick layer of grass/vegetation will reduce the intense drying effect by protecting moisture in the topsoil layer. (D) The presence of roots will reduce the quantity of phosphorus potentially leachable by up taking any solubilised phosphorus. However, as the intensity and frequency of DRW stresses will increase due to global warming mediated changing climate, the quantity of phosphorus potentially mobilised/leached will also increase.

Chapter 6 - Influence of soil drying and rewetting cycles on leaching of micronutrients

6.1 Introduction

In nature, soils are continuously under the influence of physical stresses, such as drying and rewetting (DRW) or freezing and thawing. These physical stresses have potential to influence soil organic matter (SOM) mineralisation rates and can mobilise soil-borne nutrients, which can potentially be transported to catchment waters through leaching and runoff. The frequency and intensity of these physical stresses is likely to increase due to the predicted changing pattern of climate e.g. longer periods of drought followed by intense rainfall events (IPCC, 2014).

Micronutrients (e.g. Fe, Mn, Zn, Cu, Co and Ni) are essential elements for all living organisms including crop plants, their availability in soils is important for sustaining a healthy ecosystem and maintaining crop production (Alloway, 2013). Climate change driven soil drying-rewetting processes may promote increased mobilization and loss of micronutrients via leaching and runoff, as seen for phosphorus in many studies (e.g. Blackwell et al., 2013). Thus, if such climate change mediated processes cause actual loss of micronutrients, this could lead to scarcity of these elements to plants, with potential implications for soil fertility and catchment water quality.

Some recent work has evidenced that rewetting of dried soils causes increased mobilisation of macronutrients (P, N and C), though much of the evidence is predominantly for phosphorus (Blackwell et al., 2013; Brodlin et al., 2019). However, most of the work to date on soil drying-rewetting processes is based on the outcomes of extraction experiments, and increased nutrients extraction following a drying-rewetting stress is believed to be linked with both biotic e.g. microbial biomass destruction (e.g. Bunemann et al., 2013; Brodlin et al., 2019) and abiotic factors e.g. increased mineralisation of soil organic matter (Wu and Brookes, 2005), disaggregation and exposure of previously protected organic matter (Fonte et al., 2014), breakdown of organo-metallic complexes (Peltovuori and Soinne, 2005; Styles and Coxon, 2006; Soinne et al., 2010). Nevertheless, extraction experiments tend to overestimate the quantities of nutrients that can be potentially released following rewetting of dried soils, due mainly to commonly used rigorous extraction protocols (shaking and centrifugation). Therefore, the nutrient quantities measured in soil extracts do not represent the quantity that potentially can be transported to surface water (Blackwell et al., 2010). It is still not clear how climate change-driven longer periods of soil drying followed by intense rainfall may influence

leaching of micronutrients. Furthermore, assuming soil rewetting following drying mobilises micronutrients as seen particularly for phosphorus (e.g. Blackwell et al., 2010; Khan et al., 2019), we do not know the role of drying-rewetting on soil micronutrient mobilisation in subsequent rewetting events. Although some previous work (e.g. Pan et al., 2016; Antic-Mladenovic et al., 2017; Shaheen et al., 2017) has shown increased metal mobilisation under anaerobic conditions with the focus on reductive dissolution of metals oxides, it is still unclear how metals mobilisation can be affected after rewetting of dried soils when conditions are predominately aerobic.

Micronutrient metals (e.g. Fe, Mn, Cu, Zn, Ni and Co) make an important part of soil's both microbial (Ledin, 2000; Gadd, 2007) and non-microbial environment. Many micronutrient metals such as Co, Cu and Ni show high binding affinities for organic matter (Ashworth and Alloway, 2007; Zhao et al., 2007). Soil drying induced disruption of aggregates/organo-metallic complexes and the death of microbial biomass thus could result in the mobilisation of these nutrients from both microbial and non-microbial sources (Soinne et al., 2010; Koopmans and Groenenberg, 2011), which upon rewetting can potentially leach out of the soil.

The primary aim of this experiment was to understand how climate change-driven soil drying-rewetting may influence soil micronutrients leaching, with the following research questions: (1) How are hotter-drier periods followed by rewetting likely to alter leaching of micronutrients (Fe, Mn, Cu, Co, Ni and Zn)? (2) Will increase in the number of drying days influence micronutrients leaching? (3) Will succeeding rainfall events likely to have any influence on the micronutrients leaching?

6.2 Material and methods

6.2.1 Site description, sample collection and preparation

This experiment was carried out using the same soil (Hallsworth-I) as in Chapters 4 and 5. See Section 3.1 for the site and soil description. Soil was prepared as described in Section 3.2.

6.2.2 Drying-rewetting leaching experimental design

This is the same experimental set-up/design as described in Section 5.2.2 for the influence of drying-rewetting cycles on leaching of DRP. Initially the DRW leaching experiment was designed to analyse leachate samples for both DRP and micronutrient metals. However, metals in the leachate samples could not be analysed due to the ICP-MS being out

of service for several months. Subsequently, the leachate samples were found to contain colloidal material, perhaps inadvertently, the leachate samples were not sufficiently acidified. Therefore, for micronutrient metals the experiment had to be carried out again. However, due to time constraints it was limited to two drying temperatures (30°C or 40°C), two drying durations (2-days or 14-days), and all the soil treatments (n=2) were rewetted by rapid rewetting rate (see Section 5.22 for detail) up to three rewetting cycles.

Table 6.1
Description of treatments imposed (drying intensity, drying duration, and rewetting cycles).

Treatments	
Moist control (MC)	Moist control soil was not dried and kept stored at 3°C until required and maintained at 54% of maximum WHC at room temperature (21°C) for a day before subjecting to rewetting
2D	Soil was oven-dried (30°C or 40°C) for 2-days
14D	Soil was oven-dried (30°C or 40°C) for 14-days (2 weeks)
C1	First rewetting cycle
C2	Second rewetting cycle – commenced on the seventh day after the first rewetting cycle
C3	Third rewetting cycle – commenced on the seventh day after the second rewetting cycle

Note: Based on the above information, treatment 2DC1 refers to 2-days drying first rewetting cycle, and 14 DC2 refers to 14-days drying second rewetting cycle.

6.2.3 Laboratory analyses

The soil was characterised in detail (see Table 4.2 for physical and chemical properties of Hallsworth-I soil) using standard procedures and quality assurance protocols.

For the determination of total dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn filtered (Whatman 0.45µm cellulose nitrate membrane filters) leachate samples were acidified (using 2-3 drops of Aristar HNO_3 per 50 ml) before being analysed by mass spectrometry using ICP-MS (Agilent 7700 series). For quality control the following approaches were followed: (a) reagent blanks were included to estimate levels of procedural contamination, (b) samples were spiked by adding metal solutions equivalent to either 50 µg L^{-1} for Fe and Mn or 5 µg L^{-1} for Co, Ni, Cu and Zn, and subsequently percent recovery was calculated, with matrix spike recovery ranged between 90-115% across the metals, (c) replicates were analysed to estimate

the level of precision (or reproducibility of the analysis) which was generally < 10% but in a few cases of very low metal concentrations it was > 10% but < 15%.

6.2.4 Statistical analysis

The significance of differences between treatments were determined by one-way ANOVA (significance reported as $p < 0.05$) by SPSS (IBM SPSS statistics 24). Fisher's LSD (Least Significant Difference) post-hoc test was employed for multiple comparisons. The data were reported as mean ± standard deviation. Dependent variables were normalised using $\log_{10}$ transformation, when they did not follow a normal distribution to meet the ANOVA assumptions. The effect of interaction among the three independent variables (drying intensity, drying duration, and the rewetting frequency) on the total dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn was determined using a three-way ANOVA (Table 6.2). Pearson's correlation analysis was performed using SPSS (IBM SPSS statistics 24) to explore relationship among metals in the leachate samples (Table 6.3).

6.3 Results and discussion

6.3.1 Soil responses to varying degrees of drying

Overall, soil drying intensity had a significant effect ($p < 0.001$) on the leaching of total dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn (Table 6.2), and following the first rewetting cycle, the leachate dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn from the soils previously dried for either 2-days or 14-days at 30ºC or 40ºC significantly ($p < 0.05$) increased relative to the control moist counterparts (Figure 6.1a-f); however, in case of Fe the concentration increase at 30°C relative to the control moist soil was not statistically significant (Figure 6.1a). Here for example, soil drying for 14-days at 40°C caused the maximum increase in leachate dissolved concentrations relative to the control moist soil, with Fe and Mn concentrations increased by 13 and 49 times, respectively. The concentration increases for other micronutrients ranged from 3.7 for Cu to 10 times for Zn (Figures 6.1a-f).

The effect of drying on micronutrients leaching at 40°C was considerably higher than at 30°C. Consequently, the dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn leached from the soil dried at 40°C followed by the first rewetting cycle were significantly ($p < 0.05$) higher than their corresponding 30°C drying counterparts (Figure 6.1); however, in case of Ni and Zn, in treatments where the soil was dried for 2-days, the concentration increase from 30°C to 40°C was not significantly different from each other (Figure 6.1d and f). For example, when

the soil was rewetted following drying for 14-days, the percent increases in dissolved metal concentrations from 30°C to 40°C were 53% for Fe, 54% for Mn, 36% for Cu, 43% for Co, 31% for Ni, 174% for Zn in the first rewetting cycle (Figure 6.1a-f).

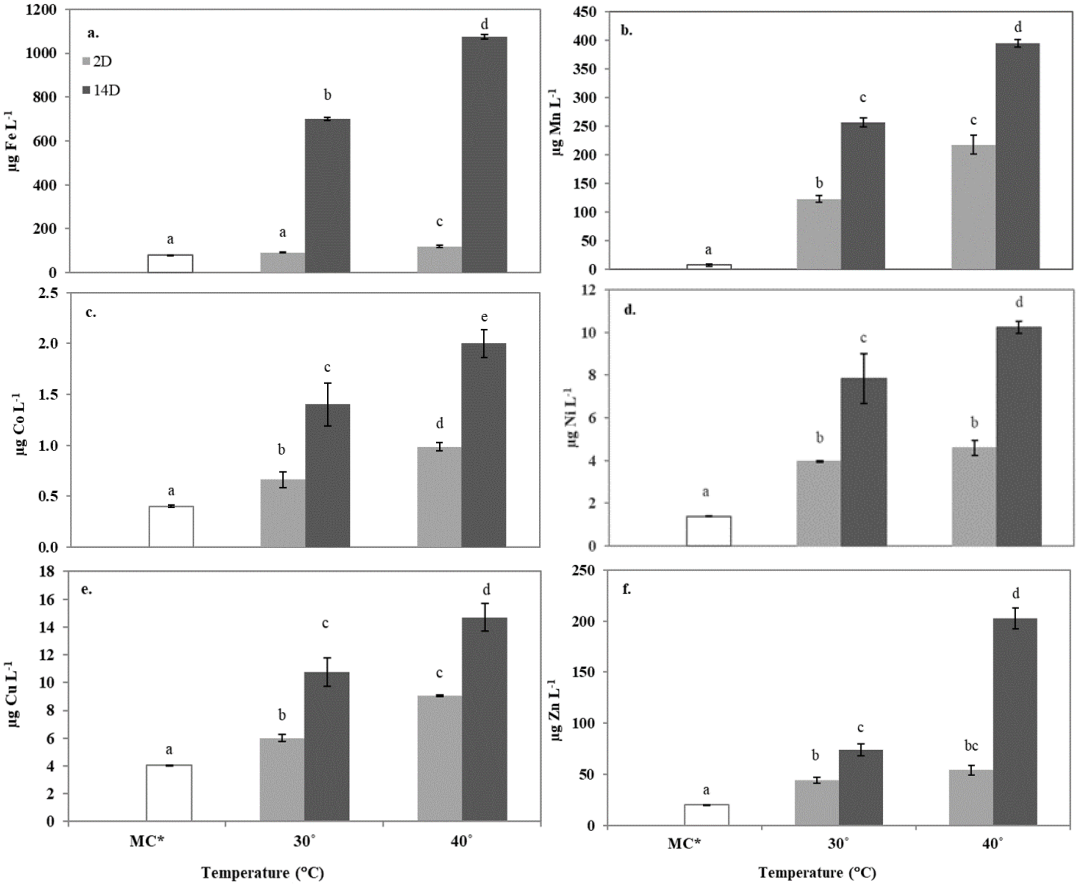

Figure 6.1a-f: Metals (µg L^{-1}) leached in 2-days (2 D) and 14-days (14 D) drying, first rewetting cycle (**a.** Iron (Fe), **b.** Manganese (Mn), **c.** Cobalt (Co), **d.** Nickel (Ni), **e.** Copper (Cu), **f.** Zinc (Zn)). Error bars represent standard deviation, n = 2. Bars labelled with the same or coinciding letters are not significantly ($p > 0.05$) different from each other e.g. **bc** is not statistically different from either **b or c**. * MC is the moist control soil.

Metal micronutrients are held by soil colloids through several mechanisms e.g. acting as bridges between clay and organic matter colloids, held by mineral surfaces, and making complexes with organic matter (Ashworth and Alloway, 2007; Koopmans and Groenberge, 2011). There is some evidence suggesting that rewetting of dried soils increases extractability of metal micronutrients (e.g. Peltovuori and Soinne, 2005; Koopmans and Groenenberg, 2011). Peltovuori and Soinne (2005), for example, showed that air-drying increased extractability of Fe and Mn in mineral soils. Koopmans and Groenenberg (2011) also reported drying induced significant increases in the total dissolve concentrations Cu and Ni in weak salt solution extracts. One of the underlying mechanisms involved is that drying of soil causes breakdown

of organo-metallic complexes due to drying-induced breakdown of hydrogen bond (Raveh and Avnimelech, 1978). Consequently, when a dried soil is rewetted the availability of moisture and freshly exposed labile organic matter causes a burst of microbial activity with elevated mineralisation rate. Microbial decomposition of high molecular weight organic matter releases low molecular weight DOC and metals into soil solution. Once in soil solution, DOC can make soluble complexes with metals in potentially leachable forms (Guo et al., 2016). Although, DOC in leachate samples was not measured in this study, it seems likely that the formation of soluble DOC-metal complexes, which later would have leached out, might also have partly contributed to the increased micronutrients leachate concentrations.

Drying-induced increase in overall soil porosity as a result of aggregate shrinkage provides new surfaces for nutrients desorption/sorption, alongside exposing freshly labile organic matter for microbial decomposition (Blackwell et al., 2009). These factors may also have influenced micronutrients leachate concentrations.

Furthermore, it is anticipated that rapid rewetting of soil might have caused the developments of short-term anoxic conditions (at least in saturated soil pockets) likely due to slow water percolation, and subsequent solubilisation of redox sensitive Fe/Me bearing minerals. However, it must be noted here that unlike long-term flooding with the continuous prevalence of anoxic conditions, here in contrary, only short-term reducing conditions might have developed in some saturated soil compartments, whilst water would be percolating down through the soil strata. Metal oxides are important sorbents of metals (Tack et al., 2006). Increased solubilisation of Fe and Mn following the first rewetting cycle in this experiment (Figure 6.1) and their strong positive correlations (Table 6.3) with Cu, Co, Ni and Zn may at least partly explain their influence on increasing micronutrient leaching. However, the strong positive correlation among Fe, Mn, Cu, Co, Ni and Zn (Table 6.3) may also be an indication that all these trace metals might have originated from the same sources upon rewetting of dried soils, perhaps soil drying-induced breakdown of organo-metallic complexes and subsequent solubilisation of previously sorbed micronutrient metals may explain this assumption. Nevertheless, based on the current data, it is hard to decide which one of these possibilities had significantly influenced micronutrients leachate concentrations. It is clear from the results that the leaching of micronutrients following rewetting is influenced by soil drying intensity and duration. Drying soil at different intensities causes moisture reduction to a specific threshold

Table 6.2
Summary statistics of univariate ANOVA. Assessing the effects of three independent factors (drying intensity, drying duration and the rewetting frequency) on dissolved concentrations of Mn, Fe, Co, Ni, Cu and Zn in leachates.

Tests of between subjects' effects		**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**
Source	**DF**	**F**	**F**	**F**	**F**	**F**	**F**
Drying intensity	2	956^{***}	4073^{***}	1538^{***}	271^{***}	11387^{***}	13832^{***}
Drying duration	1	62^{***}	3686^{***}	196^{***}	62^{***}	1179^{***}	154^{***}
Rewetting frequency	2	520^{***}	5579^{***}	1466^{***}	287^{***}	5714^{***}	5106^{***}
Drying intensity* Drying duration	2	40^{***}	922^{***}	52^{***}	17^{***}	407^{***}	190^{***}
Drying intensity* Rewetting frequency	4	26^{***}	1783^{***}	52^{***}	58^{***}	2657^{***}	831^{***}
Drying duration* Rewetting frequency	2	24^{***}	3354^{***}	21^{***}	85^{***}	1917^{***}	2665^{***}
Drying intensity* Drying duration* Rewetting frequency	4	6^{**}	880^{***}	20^{***}	29^{***}	518^{***}	774^{***}

****** Significant at $p < 0.01$ probability level, *******Significant at $p < 0.001$ probability level.

Table 6.3
Pearson's correlation coefficients among micronutrient metals in the leachate samples

	Mn	Fe	Co	Ni	Cu
Fe	0.545^{***}				
Co	0.812^{***}	0.780^{***}			
Ni	0.781^{***}	0.850^{***}	0.919^{***}		
Cu	0.709^{***}	0.871^{***}	0.825^{***}	0.924^{***}	
Zn	0.574^{***}	0.791^{***}	0.697^{***}	0.737^{***}	0.739^{***}

*** Significant at $p < 0.001$

e.g. the average moisture contents after 2-days drying were 3.7% and 1.1% at 30°C and 40°C, respectively which possibly have influenced the extent of micronutrient leaching.

6.3.2 Duration of soil drying

Overall, soil drying duration had a significant ($p < 0.001$) effect on the leachate dissolved micronutrient concentrations (Table 6.2). Consequently, soil drying for 14-days at 30°C or 40°C followed by the first rewetting cycle increased the dissolved concentrations of Fe, Mn, Cu, Co, Ni, and Zn significantly ($p < 0.001$) relative to the 2-days drying counterparts (Figures 6.1a-f). For instance, 14-days soil drying at 40°C followed by the first rewetting cycle significantly increased leachate dissolved Fe concentration by 9 times relative to its 2-days counterparts (Figure 6.1a). The extent of increases in other micronutrients varied from 3.8 for Zn (Figure 6.1f) to 0.6 times for Cu (Figure 6.1e). Soil drying for a longer period may have detrimental effects on soil microbial biomass and may thus cause a substantial reduction in microbial biomass due to unavailability of moisture dependent activities during an extended period of drying (Borken and Matzner, 2009). Extended drying and a subsequent reduction in microbial activity could reduce mineralisation of soil organic matter but may increase nutrients losses upon rewetting due to accumulated dead microbial cells (Borken and Matzner, 2009). Some microorganisms produce extracellular polymeric substances (EPS) to increase their survival by retaining moisture under drying conditions (Schimel et al., 2018) and most of the microorganisms are known to produce higher concentrations of EPS at temperature ranges between 25ºC to 30ºC (More et al., 2014). Also, rewetting of soils after prolonged drying tends to release both macro- and micro-nutrients (metals) from these structures since EPS are bound with cells through ion bridging by multi-valent metals (More et al., 2014); thus, may trigger release of bound-metals from these structures upon drying induced cell disruption. Beside these mechanisms and factors, drying-induced increased crystallinity of metal oxides and subsequent reduction in sorption capacity (Schonbrunner et al., 2012; Dieter et al., 2015) could also be a contributory factor in increasing micronutrients concentrations in the leachates following rewetting of dried soil. Crystalline metal oxides become more dehydrated and less strongly charged with time during drying-induced oxidation in a process known as ‘mineral aging’ (Alloway, 2013; Schulz-Zunkel et al., 2015). Subsequently, when a dried soil is rewetted, loss of sorption capacity due to increased mineral crystallinity may prevent the adsorption of micronutrients originated from microbial and non-microbial sources at least in the initial stages of rewetting. Thus, increased soil solution metal concentration in this experiment could have

caused increased metal leaching as has been noticed in other studies (e.g. Tack et al., 2006; Guo et al., 2016). Although, metal oxides become more crystalline over time following drying, rewetting/flooding of dried soil appears to cause transformation of crystalline metal oxides to non-crystalline forms which have larger sorption capacity than crystalline forms due to their larger surface area (Darke and Walbridge, 2000; Bruland and Richardson, 2006). Thus, some metals solubilised can leached out whilst other may be re-adsorbed to soil particles.

6.3.3 Frequency of succeeding rewetting events

Overall, rewetting frequency had a significant ($p < 0.001$) effect on the total dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn (Table 6.2). Although, in this experiment soil was oven dried just once before subjecting to the first rewetting cycle, the influence of soil drying on micronutrients leaching remained in the successive rewetting cycles though mostly limited to the second rewetting cycle (Figure 6.2a-f). As for instance, in the second rewetting cycle, the dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn leached from the soil previously dried for either 2-days or 14-days at 30°C or 40°C were significantly ($p > 0.05$) greater than the control moist soil (Figure 6.2a-f). However, in the second rewetting cycle, the dissolved concentration of Cu leached from the soil dried for 2-days at 30°C was not significantly different relative to the control moist soil (Figure 6.2e). The third rewetting cycle showed a varied trend with leachate dissolved metal concentrations either significantly increased (Mn and Co; Figure 6.3b-c), remained same, or showed a declining trend relative to the control moist soil (Figure 6.3).

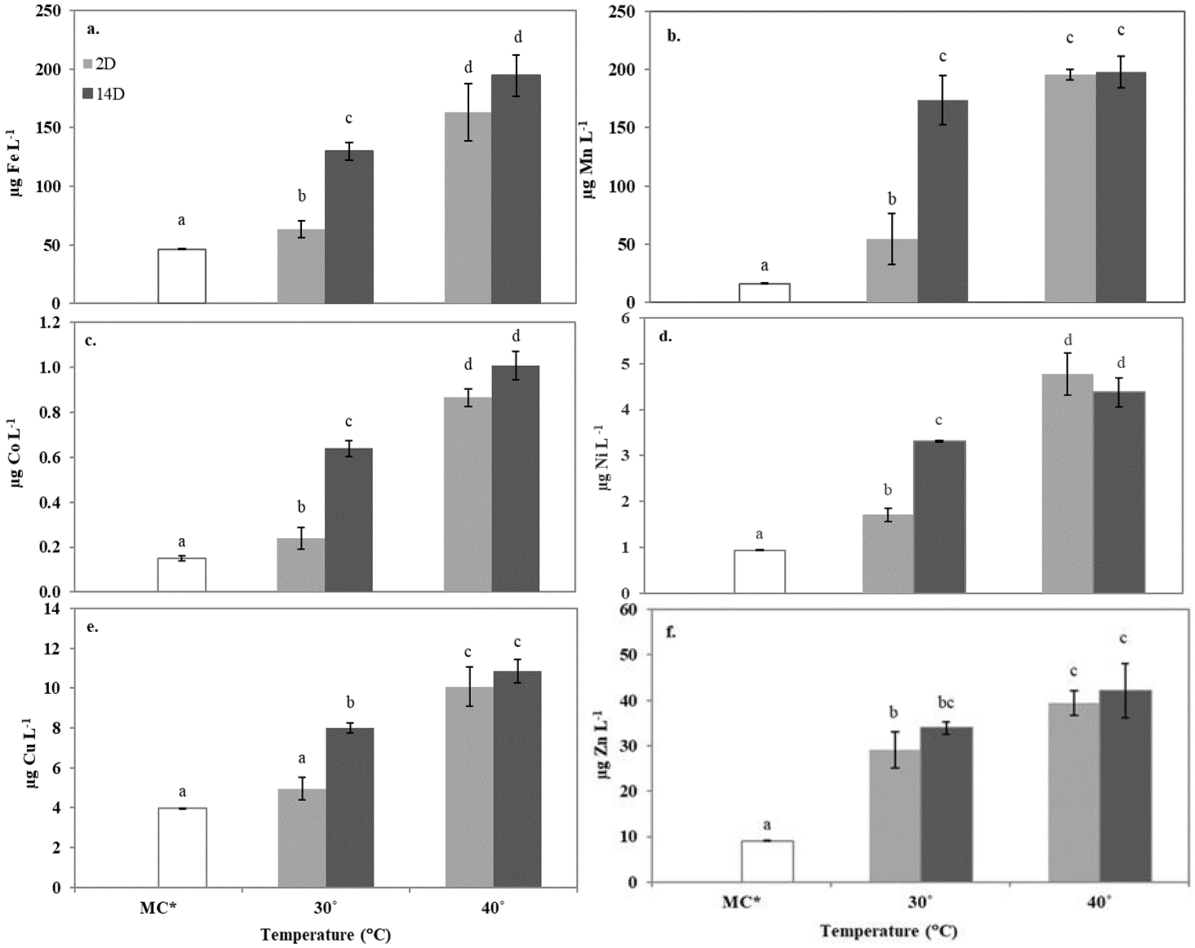

Figure 6.2a-f: Metals (µg L^{-1}) leached in 2-days (2 D) and 14-days (14 D) drying, second rewetting cycle (**a.** Iron (Fe), **b.** Manganese (Mn), **c.** Cobalt (Co), **d.** Nickel (Ni), **e.** Copper (Cu), **f.** Zinc (Zn)). Error bars represent standard deviation, n = 2. Bars labelled with the same or coinciding letters are not significantly ($p > 0.05$) different from each other e.g. **bc** is not statistically different from either **b or c**. * MC is the moist control soil.

Drying duration seems to have a long-term impact on micronutrients leaching that even after the normal soil moisture and temperature conditions were returned following the first rewetting cycle, drying duration influence was clearly seen in the second rewetting cycle, though it was largely limited to treatments where the soil was dried at 30°C, i.e. the second rewetting cycle leached significantly ($p < 0.05$) greater concentrations of Fe, Mn, Cu, Co and Ni from the soil previously dried at 30°C for 14-days relative to the 2-days drying counterparts (Figures 6.2a-e). Greater micronutrient leaching in the second rewetting cycle from the soil previously dried for a longer duration could be due to greater lethal and sub-lethal damages of long-term drying on microbial biomass and cellular structures, as for example prolong drying will not only kill microbial biomass, but can slow down growth and respiration rate of the surviving biomass upon rewetting (Rahman et al., 2018; Meisner et al., 2015).

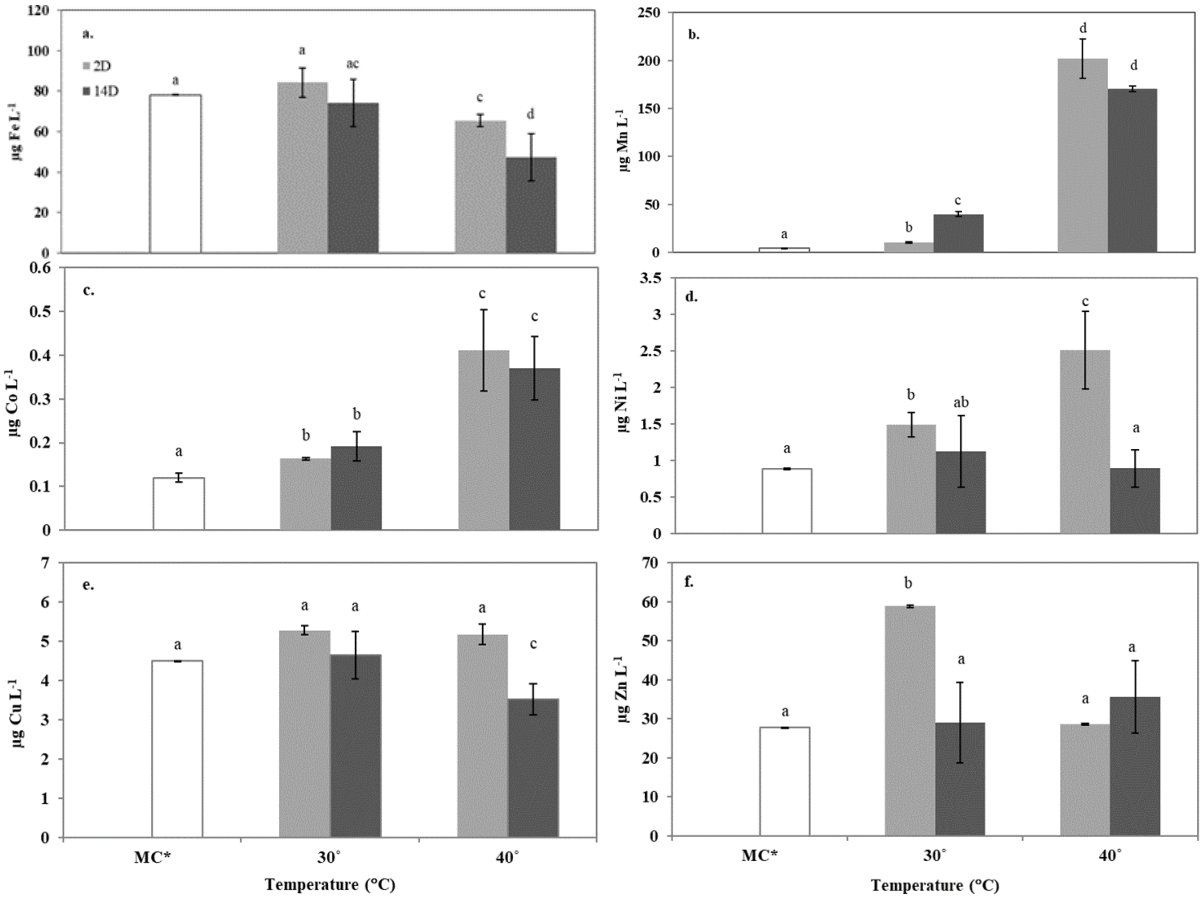

Figure 6.3a-f: Metals (µg L^{-1}) leached in 2-days (2 D) and 14-days (14 D) drying, third rewetting cycle (**a.** Iron (Fe), **b.** Manganese (Mn), **c.** Cobalt (Co), **d.** Nickel (Ni), **e**. Copper (Cu), **f.** Zinc (Zn)). Error bars represent standard deviation, n = 2. Bars labelled with the same or coinciding letters are not significantly ($p > 0.05$) different from each other e.g. **ac** is not statistically different from either **a** or **c**. * MC is the moist control soil.

In treatments where the soil was dried for 2-days followed by the second and the third rewetting cycles, the leachate dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn showed varied trends with concentrations increased, remained same or declined relative to the leachate concentrations from the first rewetting cycle (6.4a-f). However, in treatments where the soil was previously dried for 14-days, the dissolved concentrations of Fe, Mn, Co, Ni, Cu, and Zn leached in the second and the third rewetting cycles significantly ($p < 0.05$) declined relative to the leachate concentrations from the first rewetting cycle (Figure 6.5a-f).

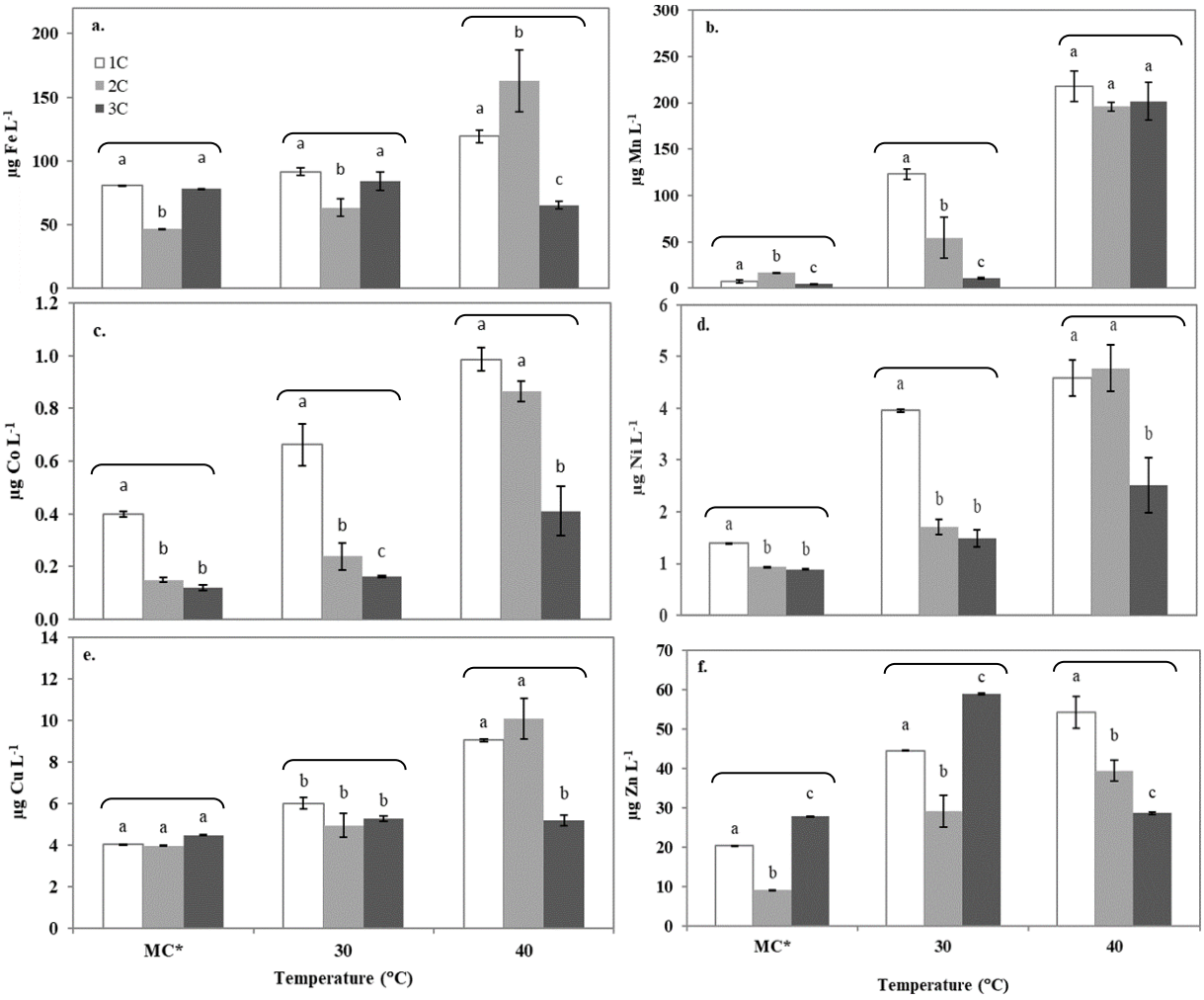

Figure 6.4a-f: Metals (µg L^{-1}) leached in 2-days drying followed by the first, second, and third rewetting cycles at different imposed temperatures: (**a.** Iron (Fe), **b.** Manganese (Mn), **c.** Cobalt (Co), **d.** Nickel (Ni), **e.** Copper (Cu) and **f.** Zinc (Zn)). Error bars represent standard deviation, n = 2. The letter 'C' represents the number of cycles e.g. the first (1C), second (2C), and the third (3 C) rewetting cycle. Bars labelled with the same letters are not significantly ($p > 0.05$) different from each other. The bracket signs indicate the significance differences (as indicated by the letters) among the means of 1C, 2C, and the 3C at each particular temperature e.g. Significance differences among the means of 1C, 2C, and the 3C at 40°C. Note: * MC is the moist control soil.

The declining trend in the micronutrients leaching in the third rewetting cycle could be caused by several possible factors e.g. limitation in labile organic matter pool (Fierer et al., 2003; Butterly et al., 2009), resistance of aggregates to slaking (Denef et al., 2001a), and microbial community shift (Butterly et al., 2009; Pezzolla et al., 2019).

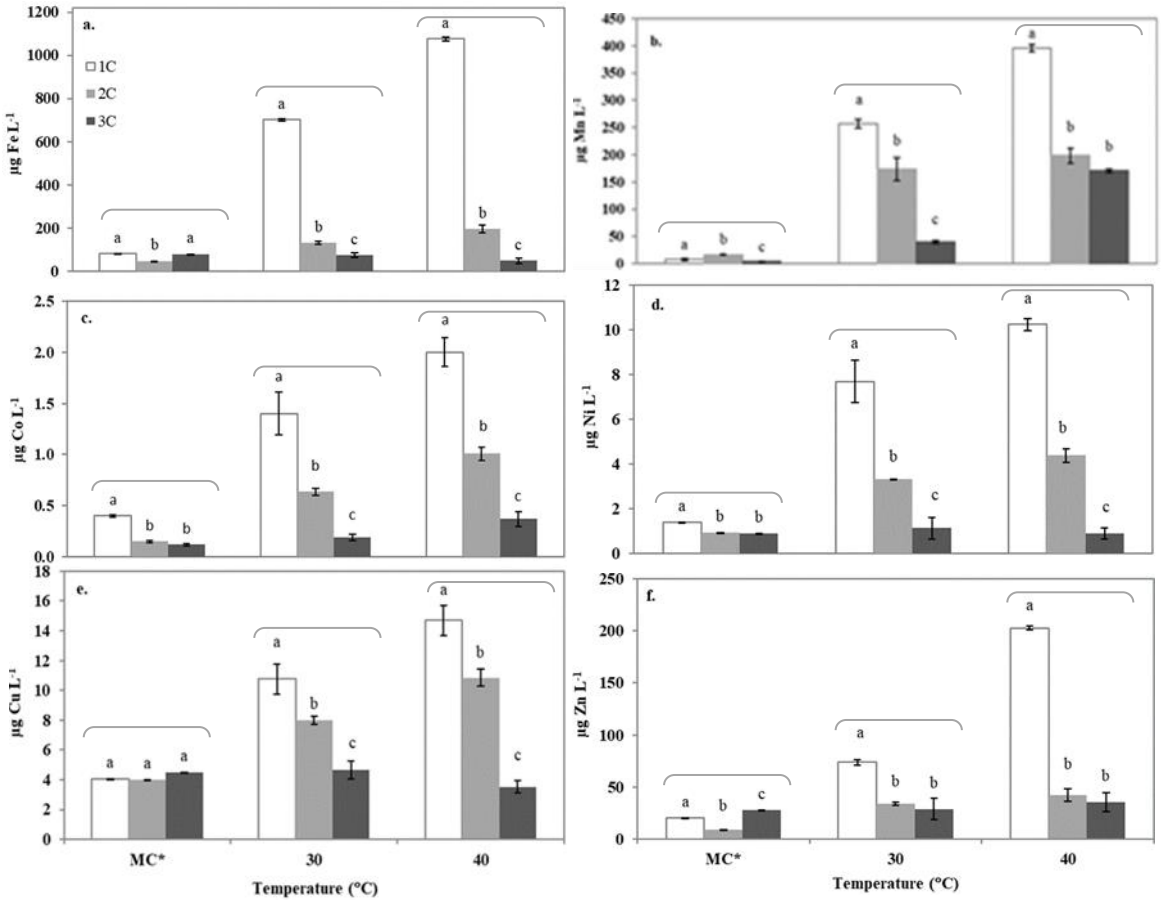

Figure 6.5a-f: Metals (µg L^{-1}) leached in 14-days drying followed by first, second, and the third rewetting cycles at different imposed temperatures: (**a.** Iron (Fe), **b.** Manganese (Mn), **c.** Cobalt (Co), **d.** Nickel (Ni), **e.** Copper (Cu) and **f.** Zinc (Zn)). Error bars represent standard deviation, n = 2. The letter 'C' represents the number of cycles e.g. the first (1C), second (2C), and the third (3 C) rewetting cycle. Bars labelled with the same letters are not significantly ($p > 0.05$) different from each other. The bracket signs indicate the significance differences (as indicated by the letters) among the means of 1C, 2C, and the 3C at each particular temperature e.g. Significance differences among the means of 1C, 2C, and the 3C at 40°C. Note: * MC is the moist control soil.

Microbial community shift could be one of the possible reasons that leaching of the micronutrients decreased in the successive rewetting cycles perhaps due to greater tolerance of adapted microbial species to drying-rewetting stresses. Microbial species surviving the first rewetting event are more capable to increase their biomass by assimilating available substrates. This causes a shift in microbial community structure which is better able to withstand stresses (Butterly et al., 2009). Another factor contributing to the reduced micronutrients leaching in successive rewetting cycles can be aggregates resistance to slaking (Hentschel et al., 2007; Zhou et al., 2011), since slake resistance of aggregates prevents exposure of labile organic matter from microbial decomposition. Depletion in the quantity of labile organic matter held within micro-aggregates could also be another reason causing reduced nutrients release with repeated rewetting cycles as in this controlled study no organic amendments/organic matter

was added so it is possible that soluble/easily leachable forms of nutrients leached during the first rewetting, and the nutrients remaining in the successive rewetting were not in readily leachable forms. Furthermore, the time gap between the two rewetting cycles was relatively short (7 days), that means soil was not really dry before the next rewetting, and hence there would be little or no aggregate shrinkage exposing new mineral surfaces and organic matter for microbial mineralization to replenish the leached nutrients.

Microbial community composition and function are both susceptible to climate change mediated variations in rainfall and temperature patterns. It appears that soils which are rarely exposed to physical stresses are more likely to be affected by climate change induced increased frequency of such physical stresses since microbial biomass is not yet adapted to tolerate such stresses and even a mild single episode of these physical stresses can be extremely detrimental to microbial biomass and may increase nutrients mobilisation driven from microbial sources (Bunemann et al., 2013). However, it is possible that microbial communities will subsequently adapt to such changes in magnitude and timings of rainfall and/or extended periods of dry weather. Nevertheless, micronutrients leaching is likely to be increased particularly in the initial stages of soils suffering drying-rewetting stresses prior to microbial communities acquire adaptation to such climate change mediated stresses (Evans et al., 2014).

Drying-rewetting is a common soil stress in summers, however in winters, nutrients dynamics are more susceptible to soil freezing and thawing. Both processes share some similar mechanisms e.g. promote soil weathering (Li et al., 2018), reduce aggregate stability, cause cell dehydration and potential cell death (Blackwell et al., 2010). However, the effect of drying-rewetting is reported to be more pronounced in terms of nutrients (e.g. P) extractability/mobilisation and transformation from occluded forms to more labile fractions (Peltovuori and Soinne, 2005; Xu et al., 2011). For example, Xu et al. (2011) reported that freezing caused few changes on phosphorus fraction but air-drying significantly changed the distribution of phosphorus fraction by increasing the transformation of occluded-P forms to labile-P and organic- P from.

6.4 Conclusion

Overall, drying intensity had a significant ($p < 0.001$) effect on the leaching of micronutrients. Soil drying at 30°C and 40°C followed by the first rewetting cycle, caused greater leaching of Fe, Mn, Cu, Co, Ni and Zn relative to the control moist soil. Also, drying soil at 40°C leached considerably greater concentrations of micronutrients relative to their 30°C drying counterparts.

Overall, soil drying duration had a significant ($p < 0.001$) effect on the leachate dissolved micronutrient concentrations. Consequently, the dissolved concentrations of Fe, Mn, Cu, Co, Ni, and Zn in the leachates collected from the soils dried for 14-days at 30ºC or 40ºC were significantly ($p < 0.001$) greater relative to those counterparts dried for 2-days in the first rewetting cycle. The observed increase in micronutrients leaching upon rewetting of dried soil is likely to be caused by drying-rewetting induced aggregate destruction, breakdown of organo-metallic complexes, enhanced mineralisation of organic matter upon availability of moisture, and possibly microbial death.

In the second and the third rewetting cycles, the leachate dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn from the soil dried for 2-days, showed varied trends with concentrations increased, remained similar or declined relative to the first rewetting cycle. However, in treatments where soil was previously dried for 14-days, the leachate dissolved concentrations of Fe, Mn, Co, Ni, Cu, and Zn significantly ($p < 0.05$) declined in the second and third rewetting cycles relative to the first rewetting cycle. The declining trend in micronutrients leaching in the successive rewetting cycles could be because soluble forms of micronutrients would have been leached and those that remained were not in readily leachable forms. The results suggest that longer-drier periods proceeded by rainy events will render enhanced leaching/solubilisation of soil micronutrients. The quantities of nutrients mobilised would depend upon the extent of these perturbations, soil type, soil texture and microbial adaptation to withstand those stresses. These findings clearly show the influence of climate-change mediated soil drying and rewetting, but these results do not truly represent actual field conditions where nutrients mobilised in runoff may be retained in the sub-soil or elsewhere along the pathway of runoff.

Soil preparation (sieving) - often requires performing laboratory microcosm studies - can significantly alter microbial community structure of the homogenised, dried and rewetted soil compared to the intact soil cores. To avoid any such change in the microbial community structure, in this study sieving of field moist soil was performed as this method has been

reported to least disturb the bacterial diversity and functioning relative to homogenised dried-rewetted soil (Thomson et al., 2010). However, drying-rewetting experiments should better be performed using intact soil cores to least disturb the soil structure, aggregation, and microbial community structure, and thus to better understand how soils under natural-field conditions may respond to climate change-driven drying rewetting processes.

Chapter 7 - Influence of soil drying and flooding on mobilization of phosphorus

7.1 Introduction

Flooding induced mobilisation of nutrients (e.g. P, N, C) has long been known (e.g. Baldwin et al., 2000; Yan et al., 2015; Bai et al., 2017; Rapin et al., 2019). However, studies investigating the effect of flooding on phosphorus dynamics are mainly confined to paddy soils (Yan et al., 2015; Li et al., 2017), wetlands (Surridge et al., 2007; Lai and Lam, 2008; Bai et al., 2017) and floodplains (Loeb et al., 2008; Schonbrunner et al., 2012) where soils remain flooded part of the year and therefore may have microbial population well adapted to the seasonal fluctuation in soil moisture content. Moreover, in soils, under going through repeated drying-wetting/flooding cycles, aggregates may become slake resistant (Denef et al., 2001a) thus lowering the risk of nutrients release due to breakdown of aggregates and exposure of new labile organic matter. Phosphorus dynamics in soils, generally not prone to seasonal flooding but could be flooded due to climate change, have not been investigated. Arable soils which are not normally flooded may have a microbial population which is not adapted to sudden changes in moisture. In such soils microbial biomass may contain large quantities of phosphorus embedded in their cellular structure (Zhang et al., 2013), which may be released following flooding of dried soils as a result of osmotic shock and cellular lysis due to sudden change in moisture content if microbial biomass is not adapted to tolerate sudden change in the moisture content.

Climate change predictions (IPCC, 2014) suggest that more intense episodic rains can cause soil saturation or flash floods as is evident by increased incidence and extent of flooding in the UK (Marsh, 2007). The phosphorus dynamics (retention and mobilisation) in flooded soil across soil-water interface have been suggested to be mediated by a combination of biological, e.g. microbial cell lysis, activity of facultative bacteria (Wright et al., 2001; Maranguit et al., 2017) and non-biological mechanisms, e.g. reductive dissolution of Fe/Mn oxides, hydrolysis or non-reductive dissolution of Fe and Al phosphate (Maranguit et al., 2017; Tian et al., 2017). Here, phosphorus is particularly important as mobilisation and transportation of phosphorus from seasonally flooded soils is a concern since phosphorus is eutrophication-limiting nutrient (Dodds and Smith, 2016).

Soil drying-rewetting is known to increase extractability of phosphorus (Turner and Haygarth, 2001; Turner et al., 2003; Sun et al, 2017; Forber et al., 2017; Homberg and Matzner., 2018). However, it is still not clear to what extent a combination of extended period

of soil drying followed by flooding can mobilise phosphorus under reducing conditions. To the best of our knowledge, no study has yet explored how climate-change predicted (Bates et al., 2008) soil-drying followed by heavy rains with high risks of flash flooding, can potentially alter phosphorus dynamics in soils which are generally not dried-flooded. Climate change predictions suggest that many areas will become significantly drier than they currently are (Bates et al., 2008). This is evidenced by increased incidence of summer heat waves in the UK as well as wider Europe, e.g. the highest temperature recorded in the UK (38.7°C on 29 Jul 2019) (www. metoffice.gov.uk). Extended period of drought proceeded with flash flooding can impair catchment water quality whilst compromising soil fertility by mobilising and transporting nutrients from land to surface waters. Despite the large number of studies on paddy soils (e.g. Yan et al., 2015; Li et al., 2017) and soil drying-rewetting influences on phosphorus mobilisation (e.g. Blackwell et al., 2013; Forber et al., 2017; Homberg and Matzner., 2018), it is unclear to what extent flooding of dried soils would influence soil-phosphorus mobilisation as compared to flooding of soils which are not dried. Moreover, it is still not clear how soils with different texture, organic matter and concentration of microbial biomass may respond to drying-flooding situations by mobilising phosphorus originated from microbial and non-microbial sources. The objectives of this study therefore were to assess: (1) how soil pre-condition (moist or dried soil) alter soil-P dynamics upon flooding. (2) How soils with different organic matter, ammonium oxalate extractable Fe-/Al-oxide and microbial biomass phosphorus concentrations respond to soil drying-flooding.

7.2 Materials and Methods

7.2.1 Site description, sample collection and preparation

Bulk samples of Hallsworth-II and Crediton soils were collected from sheep and beef cattle grazed permanent grassland fields located in North Wyke, Devon, UK. See Section 3.1 for the site and soil description. Soil was prepared as described in Section 3.2.

7.2.2 Flooding experiment design

Moist Hallsworth-II and Crediton soils were oven-dried for 10-days at 40°C. The soils were weighed before and after drying to measure the moisture loss, subsequently 84 replicates, 3 for each sampling day, with each replicate comprising 150 g dry-weight equivalent (DWE) of the soils were placed in 500 ml polypropylene, translucent, wide neck bottles (Figure 7.2 a-d). The control counterparts of these soils were kept stored at 3°C and maintained at room temperature

(21°C) for one day (at 25% of maximum WHC moisture content) before subjecting to flooding. Soil was gently compacted in the mesocosm and flooded with a given amount of water, as appropriate, so that 150 g dry-weight equivalent soil received 400 ml of water. Deionised water (18MΩ) was applied on the soil surface and water columns were maintained to a water-column depth of approximately 10 cm throughout the flooding duration. There were 4 types of treatments (see Table 7.1), each type of treatment had 3 replications for each monitoring day (n = 7). Surface water samples (supernatant) were collected using syringes on day 1, 3, 7, 10, 15, 22 and 31 of flooding by sacrificing 3 replicates of each treatment on each sampling day, and analysed for total phosphorus (TP), dissolve reactive phosphorus (DRP), total dissolve phosphorus (TDP) and total dissolved concentrations of Fe, Mn, Al, Cu, Co, Ni and Zn (see section 7.2.3 for the laboratory analysis). To compensate for any water loss by evaporation during flooding period (31-days), the level in the mesocosm was maintained by replenishing with DI water. The Table 7.1 shows further information about the treatments.

Table 7.1:
Types of treatments imposed (storage conditions, dry flooded vs. moist flooded)

Treatments	
Crediton moist flooded (CMF)	Control moist Crediton soil, stored at 3°C and maintained at 25% WHC moisture content before subjecting to flooding for 31-days at room temperature (21 - 22°C).
Crediton dry flooded (CDF)	Dried at 40°C for 10-days followed by flooding for 31-days at room temperature (21 - 22°C).
Hallsworth-II moist flooded (HMF)	Control moist Hallsworth-II soil stored at 3°C and maintained at 25% WHC moisture content before subjecting to flooding for 31-days at room temperature (21 -22°C).
Hallsworth-II dry flooded (HDF)	Dried at 40°C for 10-days followed by flooding for 31-days at room temperature (21 - 22°C).

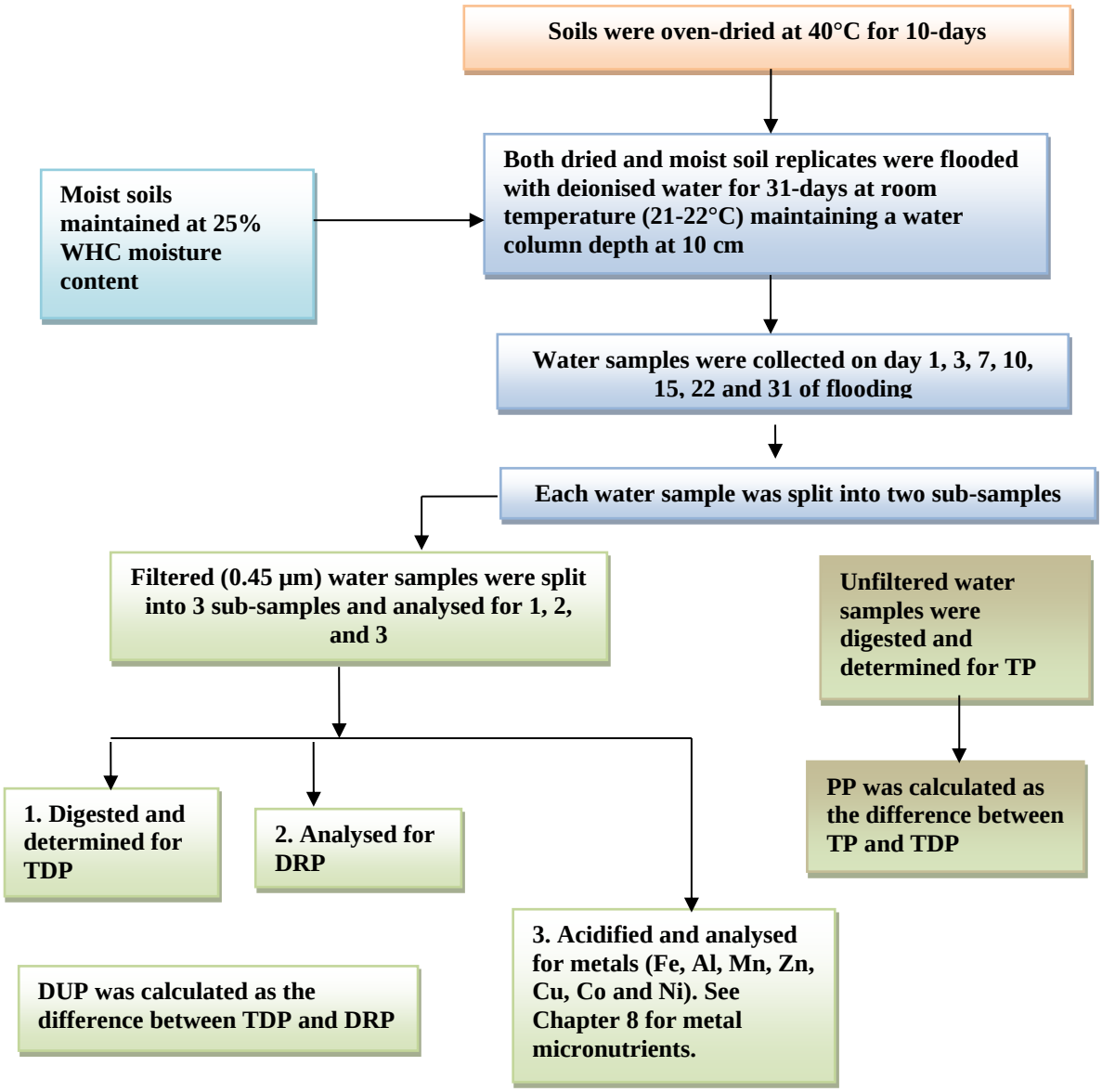

Figure 7.1 Flooding experiment design flowchart**.**

Figure 7.2a-d: Experimental set-up for flooding – Soil samples were placed in polypropylene, translucent 500 ml HDPE wide neck bottles as shown, flooded with deionised water for 31-days maintaining a water column depth of approx. 10 cm as shown in figure 7d.

7.2.3 Laboratory analysis

Redox potential (Eh) and pH of the soil-water column were measured periodically throughout the flooding duration (31-days) using a redox/ORP electrode (Thermo scientific Orion metallic combination electrode) and a pre-calibrated pH meter (Oakton™ pH 700 Benchtop Meter) respectively. Water samples (supernatant) were collected using syringes on day 1, 3, 7, 10, 15, 22 and 31 of flooding. Subsequently, each water sample was split into two sub-samples, with one sub-sample filtered (Whatman 0.45 µm cellulose nitrate membrane) and analysed for dissolve reactive phosphorus (DRP) using the Murphy and Riley (1962) method. Both filtered (0.45 µm) and unfiltered water samples were digested by the persulfate digestion method (O'Dell, 1993) using a mixture of H_2SO_4 (11 N) and ammonium persulfate (Section 3.4.1) for the measurement of TDP and TP, respectively. Dissolve unreactive phosphorus (DUP) was calculated as the difference between TDP and DRP. Particulate phosphorus (PP) was calculated as the difference between TP and TDP (See Appendix 1 for PP and TP data). For the

determination of total dissolved concentrations of Fe, Mn, Al, Cu, Co, Ni and Zn water samples were filtered (Whatman 0.45 µm cellulose nitrate membrane filters), acidified (Aristar HNO_3; 2-3 drops per 50 ml) and analysed by mass spectrometry using ICP-MS (Agilent 7700 series) following a standard protocol (see section 8.2.3 for quality control). See Figures 7.1 and 7.2 for experimental design flow chart and experimental set-up respectively.

Soils were characterised in detail (Table 7.2) using the standard procedures and quality controls. In all cases triplicate soil samples (n = 3) were analysed. Soil pH was measured in 1:2.5 (w/v) soil-water suspensions, using a pre-calibrated pH meter (Oakton™ pH 700 Benchtop Meter). Soil moisture content (%) was determined gravimetrically by oven-drying soil samples at 105°C for 16 hours. Soil texture was determined by hydrometer method. Soil organic carbon was determined by Walkley and Black oxidation method as described in Carter and Gregorich (2008). Cation exchange capacity of the soil was determined by sodium saturation (sodium acetate) and displaced sodium (using ammonium acetate) was then analysed by flame emission spectrometry. Soil total phosphorus was determined by digesting finely ground soil in perchloric acid ($HClO_4$) on hot plate for about 40 min at 203°C (boiling point of $HClO_4$) until the dark colour of organic matter disappeared. Digests were filtered (Whatman 541), diluted to 250 ml with de-ionised water and P in the digestates determined by ICP-AES. For the measurement of soil organic P, soil was ignited at 550°C for 2 hours. Both ignited and unignited samples were extracted by 0.5 M sulphuric acid (H_2SO_4) before analysing them on ICP-AES (Carter and Gregorich, 2008). Total organic P was then measured as the difference between ignited and unignited samples.

Water extractable phosphorus was determined as described by Carter and Gregorich (2007). Briefly, 20 ml of DI water was added to 2 g DWE (dry weight equivalent) soil and the mixture was then shaken for 1 hour at 10 rpm, followed by centrifugation at 3000 g, filtration (Whatman no. 42) and determination of P colorimetrically (Murphy and Riley, 1962).

Bicarbonate extractable phosphorus (Olsen-P) was measured based on the method described by Olsen et al. (1954) as outlined in Carter and Gregorich (2008). Briefly, 5 g DWE of soil was extracted in 100 ml of 0.5 M $NaHCO_3$ solution adjusted to pH 8.5 along with 1g acid washed charcoal on a shaker for 1h. Extracts were then filtered (Whatman No. 42), acidified (0.25 M H_2SO_4), and determined colorimetrically (Murphy and Riley, 1962) method. Microbial biomass P was measured in both moist and dry soil (40°C for 10 days) replicates using chloroform fumigation extraction method as described by Brookes et al. (1982). Metal oxides (amorphous Fe, Mn and Al) in the soil were extracted by the acid ammonium oxalate

method (Carter and Gregorich, 2008) and the metals (Fe, Mn, and Al) were analysed by ICP-AES (Jobin Yvon Horiba – ULTIMA 2C / 2CE). Soil total metal content was determined by digesting in hot concentrated nitric acid (Aristar grade) (EPA 3050 B) and analysing by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Jobin Yvon Horiba – ULTIMA 2C / 2CE). Certified reference material (CRM005) was included in the digestion as a check for quality control.

7.2.4 Statistical analysis

The nature of data distribution was assessed using Shapiro-Wilk's test, Skewness and kurtosis, and a visual inspection of their histograms and normal Q-Q plots showed that data were normally distributed for pH and redox. However, for all other dependant variables (DRP, DUP and TDP) data were normalised using $\log_{10}$ transformation. The significance of difference between individual means was determined by one-way ANOVA (significance reported at $p < 0.05$). Fisher's Least Significant Difference (LSD) post-hoc test was employed for multiple comparisons. Three-way ANOVA was performed to find out the effect of interaction of independent variables (flooding duration, soil pre-condition and soil type) on dependent measured parameters in water samples (Table 7.3). The data are reported as means ($n = 3$) ± standard deviation. Pearson's correlation analysis was performed to explore relationship among studied parameters (Table 7.4). Analysis of variance and correlation analysis were performed using SPSS (IBM SPSS statistics 24). Means and standard deviation were assessed using Microsoft Excel 2007.

Table 7.2
Initial soil properties (mean ± standard deviation, n = 3) of the Hallsworth-II and Crediton soils

Soil Properties	Hallsworth-II	Crediton
pH	5.13 ± 0.01	5.41 ± 0.01
Cation exchange capacity, c.molkg^{-1}	22.12 ± 1.08	36.77 ± 1.95
% Organic carbon	3.65 ± 0.10	1.08 ± 0.21
Water extractable DRP, mg P kg^{-1}	13.45 ± 2.33	21.63 ± 0.68
Sodium bicarbonate extractable P, mg P kg^{-1}	26 ± 2.0	42 ± 0.5
Total soil P, mg P kg^{-1}	1283 ± 37	883 ± 48
Total organic P, mg P kg^{-1}	725 ± 56	396 ± 28
Sand, %	35	77
Silt, %	46	12
Clay, %	19	11
Moist soil microbial biomass P, mg P kg^{-1}	68 ± 1	14 ± 4
Dry soil (40°C. 10 days) microbial biomass P, mg P kg^{-1}	11.43 ± 1.73 (-83%)	7.45 ± 1.58 (-47%)
Fe-ox[a],%	1.06	0.47
Mn-ox[a], %	0.04	0.09
Al-ox[a], %	0.02	0.01
Fe[b], mg Fe kg^{-1}	35859	39359
Mn[b], mg Mn kg^{-1}	1169	647
Al[b], mg Al kg^{-1}	1963	2067
Cu[b], mg Cu kg^{-1}	17	26
Co[b], mg Co kg^{-1}	14	8
Ni[b], mg Ni kg^{-1}	16	13
Zn[b], mg Zn kg^{-1}	57	99

[a]Acid ammonium oxalate extractable metal (Fe/Mn/Al) oxides
[b]Total soil metal content

7.3 Results and discussion

7.3.1 Soil pH and redox potential

Overall, soil flooding duration caused a significant reduction in redox potential ($p < 0.001$; Table 7.3), ranging from 390 mV to -57 mV and 246 mV to -230 mV for the CMF and CDF treatments, and from 281 to -114 and from -9 mV to -282 mV for HMF and HDF respectively. Both Hallsworth-II dry-flooded (HDF) and Crediton dry-flooded (CDF) soils attained their minimum redox potentials rapidly relative to their moist-flooded counterparts, most likely due to greater availability of labile organic matter from drying induced destruction/mineralisation of organic matter and dead microbial biomass, since redox potential of soils well supplied with organic matter drops rapidly due to increase in the availability of oxidizable organic carbon for microbial decomposition and concomitant O_2 depletion (Kogel-Knabner et al., 2010; Jayalath et al., 2016). The decrease in redox potential was greater in

Hallsworth-II dry-flooded soil than in Crediton dry-flooded soil (Figure 7.3 a), which is reflective of greater organic matter and microbial biomass of this soil.

The onset of flooding induced reducing conditions caused an increase in soil pH, with a slight variation in the first few days (Figure 7.3b). The pH of the HMF and HDF ranged from 5.8 to 6.4 and from 5.8 to 7.3 respectively, while the pH of CMF and CDF ranged from 5.2 to 6.1 and 5.7 and 7.1 respectively. The pH increase upon flooding was greater in the dry-flooded soils relative to the moist-flooded counterparts. The increase in pH was greater in dry-flooded Hallsworth-II soil than in dry-flooded Crediton soil (Figure 7.3b). The increase in pH of soils upon flooding is caused by consumption of protons under reducing conditions (Kogel-Knabner et al., 2010; Jayalath et al., 2016). The rapid increase in pH of the dry-flooded soil is caused by increased availability of labile organic matter when the dried soil is flooded due to detrimental effects of soil drying on organic matter and microbial biomass since the availability of oxidizable organic carbon stimulate pH increase by increased proton consumption under reducing condition as many anaerobes are heterotrophic (Jayalath et al., 2016).

7.3.2 Effect of soil pre-condition on solubilisation of phosphorus in flooded soils

Overall, soil condition (moist or dry) prior to flooding had highly significant effect ($p < 0.001$) on mobilisation of all forms of P (DRP, DUP and TDP) in water column (Table 7.3). The Hallsworth-II dry-flooded (HDF) soil released significantly ($p < 0.001$) greater concentrations of DRP than their moist-flooded counterparts (Figure 7.4b). The Crediton dry-flooded soil (CDF) generally released greater concentrations of DRP relative to the Crediton moist-flooded soil until day-10 of flooding beyond which DRP showed a declining trend (Figure 7.4a). The DUP released from Crediton dry-flooded soil was significantly ($p < 0.01$) greater than the moist-flooded counterparts except on day 15 and 22 of flooding where the differences between means of the moist and dry soils were not significant ($p > 0.05$) (Figure 7.5a). Similarly, DUP released from the Hallsworth-II dry-flooded soil was significantly ($p < 0.001$) greater than the Hallsworth-II moist-flooded soil with the exception of day-31 of flooding where the difference between means of moist- and dry-flooded soils was not significant ($p > 0.05$) (Figure 7.5b). TDP released from the Crediton dry-flooded soil was significantly ($p < 0.05$) greater than the Crediton moist-flooded soil until day-10 of flooding beyond which TDP showed a declining trend (Figure 7.6a-b). TDP released from the Hallsworth-II dry-flooded soil (HDF) was significantly ($p < 0.001$) greater than its corresponding moist-flooded counterpart except on day-31 of flooding when the difference between them was not significant (Figures 7.7 a-b). Greater concentrations of all forms of P

from flooded-soils previously dried could be due to greater destruction of organic matter and microbial biomass during drying induced dehydration (Turner et al., 2003; Sun et al., 2017; Brodlin et al., 2019). Drying induced loss of sorption capacity in previously dried soils could also be the possible cause of elevated dissolved P in water column upon flooding, since reduction in phosphorus sorption capacity as a result of increased minerals crystallisation (mineral-aging) prevents sorption of P originated from microbial cell lyses or organic matter mineralisation (Schonbrunner et al., 2012; Dieter et al., 2015; Attygalla et al., 2016; Sun et al., 2017). Alongside these factors the pace of redox development may also have played a role (see Section 7.3.3).

On the third day of flooding, DRP was not detected, i.e. was less than the limit of detection (LOD) in the water samples collected from the Hallsworth-II dry-flooded (HDF) soil (Figure 7.4b). While it is difficult to explain this, it is possible that Hallsworth-II dry-flooded soil with its greater organic matter had a population of microbial biomass more adapted to tolerate variation in moisture and survived the drying-flooding-stress by assimilating phosphorus released in the water column from other less adapted members of microbial communities during cell bursting and breakdown of organic matter. In this regard Zhao et al. (2010) state that soil with high organic carbon content provide better capacity to maintain its original biomass and enzymatic activity following drying-rewetting stresses, likely due to better adaptability of soil microbes to cope with these perturbations. Secondly, phosphorus sorption on soil minerals could also have played a role in DRP removal from the water column on the third day of flooding.

Furthermore, there is some evidence suggesting that flooding can increase sorption of inorganic phosphorus by causing transformation of crystalline Fe-oxide to amorphous oxide forms which have larger sorption capacity than more crystalline forms due to their larger surface area (cf., Darke and Walbridge, 2000). These results suggest that if the soil is dried it is likely to release greater quantities of nutrients during initial stages of flooding which in the field may be transported to the nearby surface waterbody. However, if the soil is moist the slow pace of redox development and thus the mobilisation of nutrients is slower. It is also possible that under natural field conditions the moist flooded soil may not cause substantial transportation of nutrients as the water drains-off and mobilised nutrients are re-precipitated/sorbed in the crystal lattice of metal oxides, reducing their transportation. However, if the dry soil is flooded the faster pace of reducing conditions development and thus the mobilisation of nutrients is likely to be greater especially in the first few days of flooding.

7.3.3 Phosphorus solubilisation under flooding induced anaerobic conditions

Overall, flooding duration had highly significant effect ($p < 0.001$) on mobilisation of all forms of P (DRP, DUP and TDP) (Table 7.3). The increase in TDP showed the same trend as the increase in total dissolved Fe and Mn (Figures 7.6 a-b and 7.7 a-b). The increase in the water column P concentrations is thus reflective of reductive dissolution of metals (Fe/Mn) oxide as is evident by highly significant ($p < 0.001$) positive correlation between total dissolved P and metals (Fe/Mn) (Table 7.4). However, in case of Crediton dry flooded (CDF) soil Mn showed an irregular trend which was not very similar to the pattern followed by TDP (Figure 7.6b). Metal oxyhydroxides are well recognised as P sorbents (Bruland and Dement, 2009; Schonbrunner et al., 2012; Amarawansha et al., 2015; Sun et al., 2017). Iron (Fe^{+3}) is redox sensitive and reduces to Fe^{+2} with the development of reducing conditions, subsequently releasing previously sorbed phosphorus into soil solution. Phosphorus release as a result of reductive dissolution of P bearing minerals has been reported in studies on flooded soils from wetlands (Lai and Lam, 2008; Liu et al, 2012; Maranguit et al., 2017), paddy soils (Yan et al., 2015; Rakotoson et al., 2016; Li et al., 2017) and floodplains (Loeb et al., 2008; Schonbrunner et al., 2012). Although, Al is not redox sensitive, flooding induced increase in pH can solubilise Al-OM-PO_4 complexes (Darke and Walbridge et al., 2000). The significant positive correlation ($p < 0.05$) (Table 7.4) between total dissolved concentrations of Al and P indicates that non-reductive dissolution of P bearing Al minerals (Al-OM-phosphate) may have also partly caused release of P in the water column. However, for Al the Pearson's correlative between TDP and Al was less robust (Table 7.4) compared to the correlations with Fe and Mn, which perhaps is reflective of their predominant role in P solubilisation due to reductive dissolution.

The increase in TDP was also concurrent with increase in pH in all treatments as indicated by highly significant positive correlation between TDP and pH ($p < 0.01$) (Table 7.4). This could be because the rise in pH under anaerobic conditions decreases P binding capacity of iron and aluminium minerals primarily due to ion-exchange reactions in which hydro-oxide (OH-) replaces orthophosphate (PO_4^{-3}) (Sun et al., 2017).

Another factor contributing to P release into water column could be the release of microbial biomass P either through microbial decay under flooding induced anaerobic condition (Fungi for instance are more sensitive to flooding induced anaerobic conditions relative to bacterial biomass; Drenovsky et al., 2004; Mentzer et al., 2006; Voroney, 2007), or as a protective mechanism while shifting between oxic-anoxic environments (Wright et al., 2001; Hupfer and Lewandowski., 2008). Under oxic environment and adequate nutrients supply, microorganisms assimilate dissolve P. However, as conditions become anaerobic, this

previously stored P is released back into soil solution (Wright et al., 2001; Khoshmanesh et al., 2002).

The Crediton dry-flooded soil released greater concentrations of TDP during initial stages of flooding (up to day-7 of flooding). However, from day-7 onwards the Hallsworth-II dry-flooded soil with its greater oxalate-extractable Fe/Al and organic matter contents released greater concentrations of TDP (Figure 7.7 b). Phosphorus sorption capacities have been known to be significantly positively correlated with concentration of oxalate-extractable Fe and Al (amorphous) oxide (Darke and Walbridge, 2000). Metal oxy/hydroxides are important in retaining phosphorus. However, in flooded soils, with the development of reducing conditions, one of the strongest phosphate sorbent Fe^{+3} oxy/hydroxide becomes unstable and results in reductive dissolution of Fe^{+3} and release of previously sorbed phosphorus (Rapin et al., 2019). Nevertheless, the oxidation state of aluminium is not affected by variation in redox potential but flooding induced increase in pH favours solubilisation of humic-Al complexes (Darke and Walbridge, 2000).

Soils with greater quantities of organic matter seem to be more susceptible in releasing phosphorus upon flooding through a number of mechanisms - blocking or occluding phosphorus binding sites on mineral surfaces (Dieter et al., 2015), competing with phosphate anion for adsorption sites on mineral surfaces, and dissolution of organo-metallic-P complexes (Abit et al., 2013). In this study, DOC was not measured; however, the role of organic carbon in contributing to dissolved phosphorus by these mechanisms cannot be ruled out.

Table 7.3
Summary statistics of three-way ANOVA
Assessing the effects of independent factors (flooding duration, soil pre-condition and soil type) on pH, redox, DRP, DUP, TDP, Fe, Mn, Al, Cu, Co, Ni and Zn in water column

Tests of between subjects effects	**pH**	**Redox**	**Fe**	**Mn**	**DUP**	**DRP**	**Al**	**Cu**	**Co**	**Ni**	**Zn**	**TDP**
Source	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**
Flooding duration	492***	2466***	757***	935***	173***	142***	105***	178***	162***	222***	135***	100***
Soil pre-condition	2573***	14572***	1755***	16337***	1011***	213***	810***	281***	3331***	1150***	1 NS	449***
Soil type	763***	1743***	4028***	53***	0.004 NS	2020***	459***	225***	444***	108***	7**	95***
Flooding duration * Soil pre-condition	52***	102***	174***	209***	16***	56***	335***	257***	76***	83***	18***	24***
Flooding duration * Soil type	6***	78***	502***	193***	148***	48***	76***	18***	58***	27***	29***	76***
Soil pre-condition * Soil type	16***	27***	2109***	2557***	120***	722***	1194***	55***	977***	34***	18***	165***
Flooding duration *soil pre-condition * Soil type	5**	52***	245***	219***	62***	276***	199***	50***	68***	14***	37***	50***

The means difference is significant at $p < 0.05$ level.

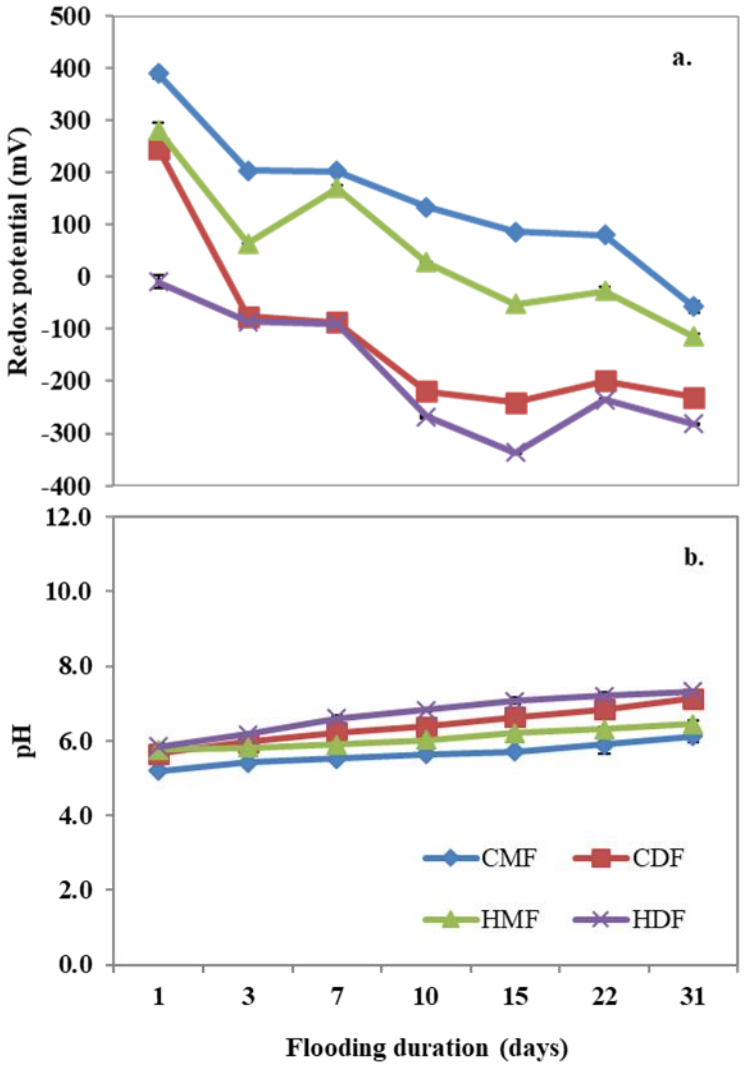

Figure 7.3a-b: Flooding induced variations in soil redox potential and pH (a. variations in redox potential of Crediton moist-flooded (CMF), Crediton dry-flooded (CDF), Hallsworth-II moist-flooded (HMF) and Hallsworth-II dry-flooded (HDF) soil, b. Variation in pH of CMF, CDF, HMF and HDF soil). Error bars represent standard deviation, n = 3. In some cases, error bars are too small to be visible.

7.3.4 Flooding induced variations in phosphorus forms in water column

The various forms of P released upon flooding from all flooded treatments were compared. The results show that overall the Crediton moist and dry-flooded soil released greater concentrations of DRP into water column relative to that released from Hallsworth-II moist – and dry-flooded soils which was perhaps due to greater water and $NaHCO_3$ extractable phosphorus fraction of this soil, indicative of easily bio-available (soluble) phosphorus upon rewetting (Table 7.2). Another factor which presumably may have caused relatively less mobilisation of DRP from the Hallsworth-II moist- and dry-flooded soils could be due to its greater organic matter and clay contents (Table 7.2), which may have resorbed some of the DRP released from microbial cell lysis and reductive dissolution of P-bearing minerals. Also soil high in organic matter content have reduced wettability due to hydrophobic characteristics of organic compounds and have a higher resistance to disaggregation upon availability of moisture (Zhao et al., 2010). Soil hydrophobicity provides microbial communities enough time to equilibrate with surroundings by releasing compatible solutes upon rewetting, while the high resistance to disaggregation protects organic matter from microbial decomposition. Most of the P in the water column from dry-flooded soils was unreactive with Hallsworth-II dry-flooded soil released greater concentrations of DUP relative to the concentrations released from Crediton dry-flooded soil (Figure 7.5 a-b) perhaps due to greater organic matter and microbial biomass phosphorus of Hallsworth-II soil relative to that of Crediton soil (Table 7.2), since unreactive phosphorus mostly comprises of organic forms of phosphorus, usually derived from microbial lysis (Blackwell et al., 2013; Gu et al., 2018). It is further supported by a reduction in microbial biomass P due to soil drying (40°C. 10 days) which was greater in case of Hallsworth-II dry soil (-83%) relative to that of Crediton dry soil (-47%) (Table 7.2).

Nevertheless, in both CDF and HDF soils concentrations of DRP and DUP declined after attaining their maximum levels (Figures 7.4 and 7.5) with exception of CDF soil where DUP concentrations gradually declined up to day-22 of flooding followed by an increase (Figure 7.5a). Studies demonstrating initial increase in P release upon flooding have also reported decrease in P pulses at later stages (Chacon et al., 2005; Amarawansha et al., 2015; Tian et al., 2017) presumably due to re-sorption by soil minerals, microbial uptake or/and re-precipitation of Fe-/Mn -oxide as it moves up in the water column across anoxic-oxic zone and sorption of P on freshly precipitated Fe-oxide (Peltovuori and Soinne, 2005).

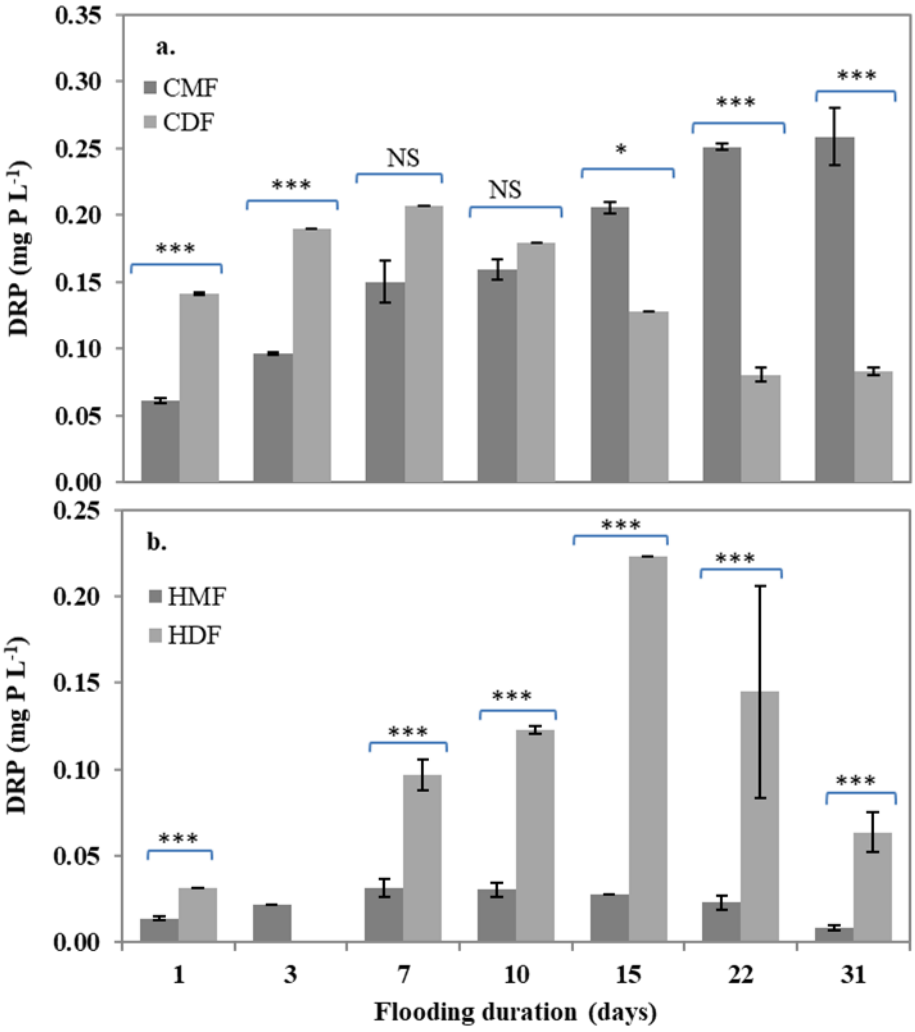

Figure 7.4a-b: Changes in concentrations of DRP (mg P L^{-1}) during 31-days of flooding period (**a**. DRP released from Crediton moist-flooded (CMF) and Crediton dry-flooded (CDF) soils and **b**. DRP released from Hallsworth-II moist-flooded (HMF) and Hallsworth-II dry-flooded (HDF) soils. Error bars represent standard deviation, n = 3. In some cases, error bars are too small to be visible. The means difference is significant at $p < 0.05$ as determined using LSD post-hoc test (***** Significant at $p < 0.05$ probability level, ****** Significant at $p < 0.01$ probability level, *******Significant at $p < 0.001$ probability level). Where 'NS' means not significant. DRP was not detected in the water samples collected from HDF on the third day of flooding.

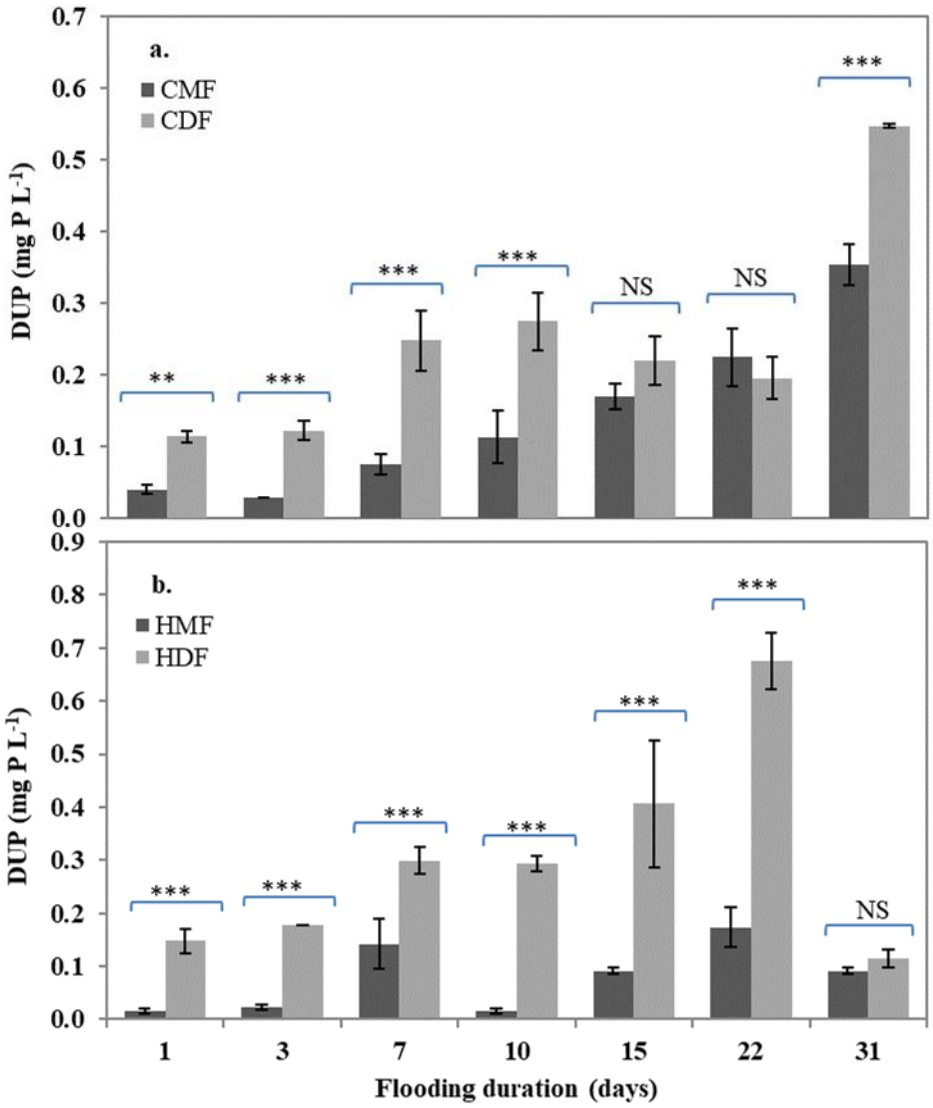

Figure 7.5 a-b: Changes in concentrations of DUP (mg P L^{-1}) during 31- days of flooding period (**a**. DUP released from Crediton moist-flooded (CMF) and Crediton dry-flooded (CDF) soils, **b**. DUP released from Hallsworth-II moist-flooded (HMF) and Hallsworth-II dry-flooded (HDF) soils. Error bars represent standard deviation, n = 3. In some cases, error bars are too small to be visible. Where 'NS' means not significant. The means difference is significant at $p < 0.05$ as determined using LSD post-hoc test (***** Significant at $p < 0.05$ probability level, ****** Significant at $p < 0.01$ probability level, *******Significant at $p < 0.001$ probability level).

Table 7.4: Pearson's correlation coefficients among studied parameters in water samples collected from the Hallsworth-II and Crediton moist and dry-flooded soils

	pH	Redox	DUP	TDP	Fe	Mn	Al	DRP	Co	Zn	Ni
Redox	-.586***										
DUP	.369**	-.660***									
TDP	.330**	-.542***	.886***								
Fe	.541**	-.565***	.491***	.430***							
Mn	.511**	-.684***	.678***	.622***	.755***						
Al	NS	NS	NS	.218*	NS	NS					
DRP	NS	NS	.527**	.728***	NS	.306**	.348**				
Co	.533***	-.712***	.661***	.600***	.763***	.909***	NS	.322**			
Zn	NS	.331**	-.278*	-.229*	NS	NS	NS	NS	NS		
Ni	.577***	-.784***	.770***	.685***	.714***	.811***	NS	.287**	.794***	NS	
Cu	NS	-.362**	.531***	.541***	NS	NS	NS	.351**	NS	-.375***	.423***

*** Significant at $p < 0.001$
**Significant at $p < 0.01$
*Significant at $p < 0.05$
NS, not significant ($p > 0.05$)

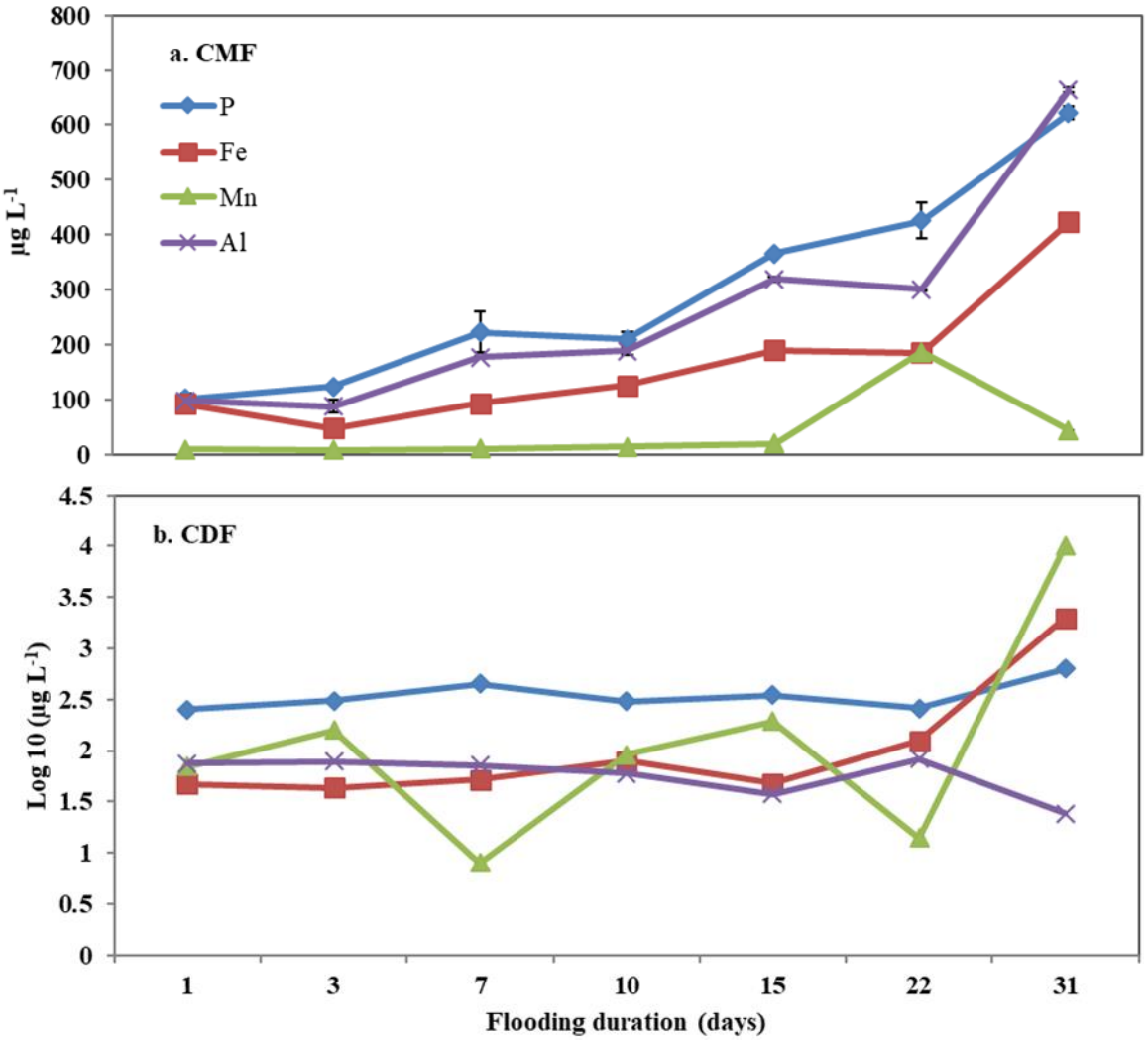

Figure 7.6a-b*:* Changes in total dissolved concentrations (µg L^{-1}) of P, Fe, Mn and Al during 31-days flooding period (a. Crediton moist-flooded (CMF) and b. Crediton dry-flooded (CDF) soils). Error bars represent standard deviation, n = 3. In some cases, error bars are too small to be seen. Note: in case of chart 'b' data was log transformed (Log 10) as some of the concentrations were too small while others too large.

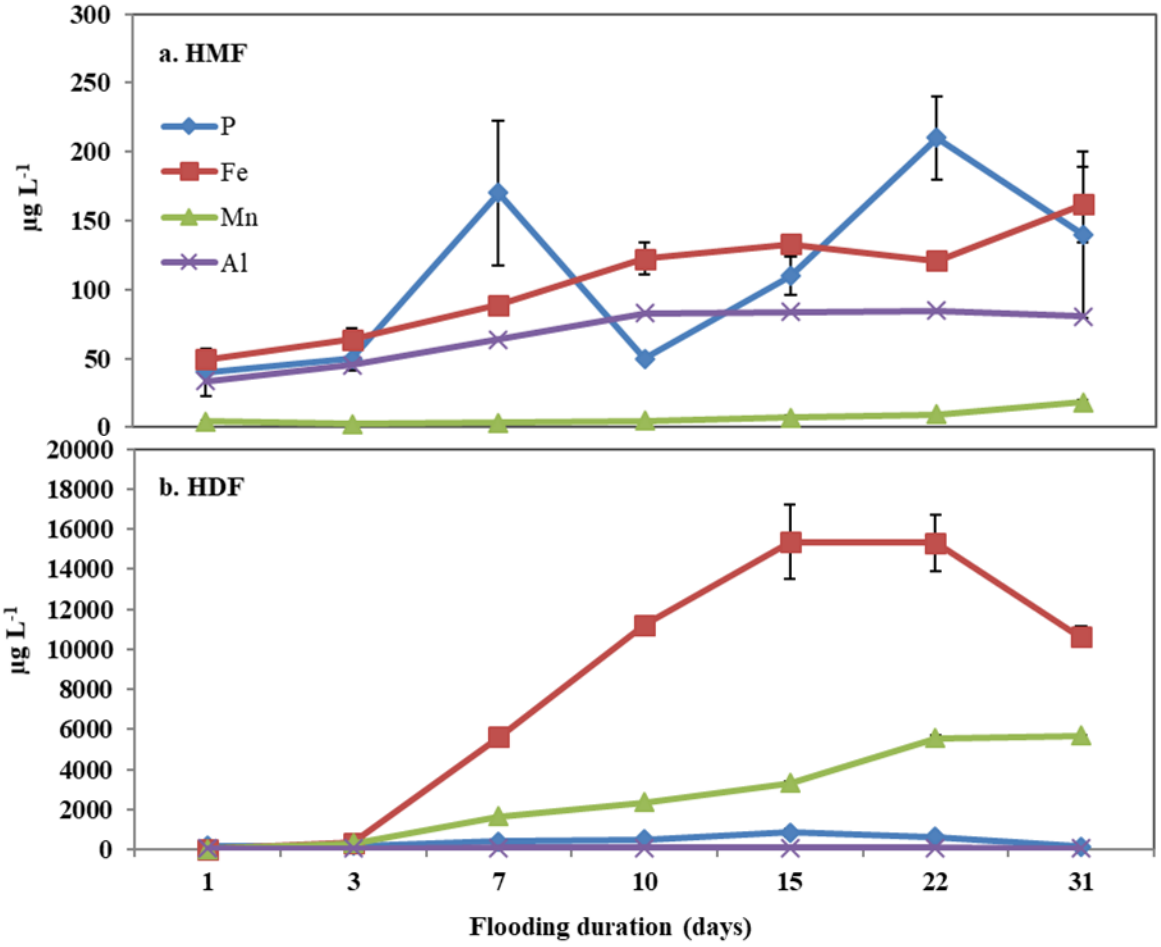

Figure 7.7a-b*:* Changes in total dissolved concentrations of P, Fe, Mn, and Al during 31-days flooding period (a. Hallsworth-II moist-flooded (HMF) and Hallsworth-II dry-flooded (HDF) soil. Error bars represent standard deviation, n = 3. In some cases, error bars are too small to be seen.

7.4 Conclusion

The onset of flooding increased dissolved concentrations of all forms of P. The release of phosphorus coincided with a reduction in redox potential, suggesting reductive dissolution of metal oxides (Fe/Mn oxide) and desorption of previously sorbed P could be linked with observed elevated dissolved concentrations of P. This was further supported by a strong significant positive correlation ($p < 0.001$) between TDP and Fe ($r = 0.430$, $p < 0.001$); TDP and Mn ($r = 0.622$, $p < 0.001$). Upon flooding the soils which were previously dried released significantly greater ($p < 0.05$) concentrations of P and metals in water column relative to the soils which were moist prior to flooding, likely due to a combination of soil drying (e.g. microbial death, increased crystallinity of metal-oxides) and flooding (reductive dissolution of metal oxides) associated factors. The Hallsworth-II dry-flooded soil released greater concentrations of DUP, possibly due to its greater organic matter (OM) and microbial biomass phosphorus concentrations. However, the Crediton dry-flooded soil released greater concentrations of DRP possibly due to greater water and bicarbonate extractable P, indicative of easily soluble P upon rewetting/flooding. Once in soil solution, phosphorus can either be re-sorbed by non-redox sensitive minerals/elements (e.g. Al, Ca, Mg) or transported to surface water through run-off. The results suggest that soil drying followed by flooding have the potential to promote much greater mobilisation of soil-P compared to the flooding of non-dried soil and thus have implications for soil fertility and surface water quality. However further studies are needed to investigate microbial colonial structure in detail with the focus on species more susceptible to drying-flooding processes. For this purpose, it would be worth examining different soils with varying amounts of organic matter, clay metal-oxide content, cation exchange capacity and microbial biomass concentrations. Nutrients mobilised from a site upon moist-/dry-flooding, however, may be retained elsewhere in the landscape and hence to assess the potential of phosphorus release field scale studies are needed.

Chapter 8 - Impact of soil drying and flooding on mobilization of Micronutrients

8.1 Introduction

Flooding is known to cause mobilisation of nutrients which is mostly controlled by the dynamics of redox, pH, iron (Fe) and manganese (Mn). In soils metal oxides (Fe/Mn oxides) are important in retaining macro and micronutrients through chemosorption (Alloway, 2013; Schulz-Zunkel et al., 2015). When soils are flooded, the development of anaerobic conditions causes the dissolution of Fe-/Mn- oxides as microorganisms use Mn^{+4} and Fe^{+3} as terminal electron acceptors for anaerobic organic matter decomposition. The reductive dissolution of metal Fe-/Mn- oxides causes release of Mn^{+2} and Fe^{+2} ions along with other sorbed or occluded metals (Lin et al., 2018).

Most of the recent work on metals mobilisation as a result of reductive dissolution of metals oxides has been reported on soils from wetlands (Grybos et al., 2007), paddy soils (Kashem and Singh, 2011; Pan et al., 2016; Rinklebe et al., 2016), and floodplains (Shaheen et al., 2014a,b; Frohne et al., 2014; Antic-Mladenovic et al., 2017) where soils remain flooded part of the year. However, it is still unknown if climate-change mediated prolonged period of droughts followed by flash flooding could increase mobilisation of micronutrients metals under reducing conditions. Here metals mobilisation can be affected by both drying (e.g. aggregate breakdown, microbial biomass destruction) and redox sensitive (reductive dissolution of metal oxides) processes. Although, the role of soil drying-rewetting in mobilising enhanced quantities of metals has been studied in drying-rewetting extraction experiments (Peltovuori and Soinne, 2005; Koopmans and Groenenberg, 2011), it is still unclear if prolonged drying followed by flooding-induced reduced conditions could cause greater mobilisation of metals than their mobilisation from flooding of moist soil or drying-rewetting processes.

Understanding the mechanisms and factors involved in controlling the dynamics of micronutrient metals in soils that undergo a period of drying before flooding are needed to better understand how metals mobilisation is affected by drying-flooding. This will have potential implications for climate change mediated droughts-flooding processes. The metals e.g. Fe, Mn, Cu, Zn, Ni and Co are important micronutrients required for the growth and development of all living organisms, including crop plants; their availability in soils is important for sustaining a healthy ecosystem (Alloway, 2013). Increased leaching loss of these nutrients can render soil systems deficient in these essential micronutrients, depleting their supply to crop plants, and can also increase their input to surface waters with potential

consequences for aquatic life and human health (Zhao et al., 2012; El-Moselhy et al., 2014). In the above context, the objectives of this study were to assess: (1) the effects of extended periods of soil drying followed by flooding on dynamics of dissolved concentrations of Fe, Mn, Cu, Co, Zn and Ni, 2) the effects of flooding induced variations in pH and redox potentials on mobilisation of micronutrient metals as governed by reductive dissolution of metal (Fe-/Mn-) oxides.

8.2 Material and Methods

8.2.1 Site description, sample collection and preparation

The work presented in this chapter is part of the same study described in the chapter 7 using Hallsworth-II and Crediton soils. See Section 3.1 for the site description and Section 3.2 for the soil sample preparation.

8.2.2 Flooding experiment design

As pointed above this is the same experimental set-up described in the chapter 7 for phosphorus mobilisation under the influence of drying-flooding process (see section 7.2.2). But here in this chapter the data on the micronutrient's mobilisation under the influence of drying-flooding processes are presented. See Figure 7.1 and 7.2 for experimental design flow chart and experimental set-up respectively. The Table 7.1 shows further information about the treatments.

8.2.3 Laboratory analysis

Soil characterisation

Soils were characterised in detail (see Table 7.2 for physical and chemical properties of Hallsworth-II and Crediton soil) using the standard procedures and quality controls (see section 7.2.3 for a brief methodology of initial soil properties (e.g. pH, texture, percent organic carbon, cation exchange capacity, easily reducible metal oxide content) and chapter 3 for detailed methods).

Flooding Experiment

Redox potential (Eh) and pH of the soil-water column were measured periodically throughout the flooding duration (31-days) as described in section 7.2.3. Filtered (Whatman 0.45 µm cellulose nitrate membrane filters) and acidified (Aristar HNO_3; 2-3 drops per 50 ml)

water samples were determined for total dissolved concentrations of Fe, Mn, Al, Cu, Co, Ni and Zn as described in section 7.2.3 (however, the data on Al are presented only in chapter 7 for non-reductive dissolution of P bearing Al minerals; section 7.3.3). For quality control following approaches were followed – (a) blanks were run to estimate levels of procedural contamination, (b) samples were spiked at two levels with known concentrations (10 mg L^{-1} and 20 mg L^{-1} for Fe and Mn, and 30 µg L^{-1} and 60 µg L^{-1} for Cu, Zn, Co, and Ni, and subsequently percent recoveries were calculated (Table 8.1).

Table 8.1
Percent recoveries of Fe, Mn, Co, Zn, Ni and Cu in flooded samples.

Sampling date	Recovery (%)	Fe[b]	Mn[b]	Co[c]	Zn[c]	Ni[c]	Cu[c]
D1[a]	Low*	107	99	107	88	104	97
	High**	108	99	117	87	112	105
D3	Low	120	111	115	102	110	103
	High	108	92	107	92	104	98
D7	Low	103	96	109	104	107	100
	High	108	92	108	97	103	98
D10	Low	99	92	124	116	119	114
	High	108	91	100	92	96	90
D15	Low	100	94	109	100	107	101
	High	101	91	111	104	108	101
D22	Low	100	92	125	88	127	121
	High	100	92	104	112	103	98
D31	Low	101	93	102	87	97	91

[a] Letter D followed by a number indicates flooding days.
[b] Samples were spiked to two known concentrations - 10 mg L^{-1} (*low level spike) and 20 mg L^{-1} (**high level spike).
[c] Samples were spiked to two known concentrations - 30 µg L^{-1} (*low level spike) and 60 µg L^{-1} (**high level spike)

8.3 Results and discussion

8.3.1 Soil Characterisation

Initial soil properties of the two grassland soils used in the flooding experiment are presented in Table 7.2. Both soils were acidic. The organic carbon and clay contents of the Hallsworth-II soil were higher than the Crediton soil (Table 7.2), reflecting its better ability to hold nutrients. The higher Fe-ox (ammonium oxalate extractable Fe-oxide) content of Hallsworth-II soil shows that this soil is more susceptible to mobilise nutrients upon flooding due to reductive dissolution of Fe- oxides (Table 7.2). Soil low in organic matter is often known to maintain positive redox potential for longer time after submergence (Ponnamperuma, 1972). This could be one of the reasons that Crediton moist soil maintained positive redox throughout

the flooding duration and negative redox value (-57 mV) was recorded only on the last day of imposed flooding (day-31 of flooding) (Figure 7.3a).

8.3.2 Effect of soil pre-condition on solubilisation of metals

Overall soil pre-condition (moist or dry) had highly significant effect ($p < 0.001$) on the total dissolved concentrations of all studied metals in water columns with the exception for Zn where the effect was not significant (Table 7.3). The onset of flooding caused the release of significantly ($p < 0.05$) greater concentrations of Mn, Co, Ni and Cu in the water column of the Hallsworth-II dry-flooded soil relative to its control moist counterpart (moist-flooded Hallsworth-II soil) (Figure 8.1). For instance, on the day-1 of flooding, the dissolved Mn concentration measured in the water column of HDF (Hallsworth-II dried-flood) soil was 9.1 times higher than its control moist-flooded counterpart (Figure 8.1b). The concentration increases relative to the control moist-flooded soil for other metals ranged from (3.5 times for Cu; Figure 8.1f) to (2.6 times for Ni; Figure 8.1e). Same as with Hallsworth-II dry-flooded soil, the commencement of flooding caused the release of significantly greater concentrations of Mn, Ni and Cu in the water column of Crediton dry-flooded (CDF) soil relative to the moist-flooded counterparts (Figure 8.2); however, on the day-1 of flooding, the dissolved concentrations of Zn and Co released in the water columns of CDF soil were not statistically different from those concentrations released from their moist-flooded counterparts (CMF soil) (Figure 8.2c-d). Soil drying is known to increase solubility of organic matter due to several plausible factors e.g. drying induced breakdown of organo-mineral complexes due to breakage of hydrogen bond (Soinne et al., 2010), aggregate breakdown and increased mineralisation of organic matter upon availability of moisture (Wu and Brookes, 2005). Metals e.g. Cu, Co and Ni are known to make organo-metallic complexes with organic matter (Koopmans and Groenenberg, 2011). The increased metal solubilisation from flooded soils which were previously dried might be caused by drying-flooding induced increased solubilisation of organic matter and formation of metal-DOM complexes as dissolved organic matter is known to cause metal solubilisation by making complexes with metals previously bound to soil minerals (Koopmans and Groenenberg, 2011). Although, soil microbial biomass comprises only a small percentage (3%) of soil organic matter, they may immobilise large quantities of nutrients (Brookes et al., 1984). Microbial biomass accumulates metals (e.g. Cu, Fe, Zn, Co and Mn) by several mechanisms: 1) bio-sorption to cell wall, pigments and extracellular polysaccharides; 2) metabolism dependent uptake and intracellular accumulation and

sequestration, and 3) precipitation of metal compounds around hypae (Ledin, 2000; Gadd, 2007; Khan et al., 2009). Drying induced cellular-dehydration and the absence of moisture dependant cellular activities cause microbial biomass death and upon flooding of dried soil the lysis of dead biomass due to osmotic shock release nutrients into soil solution (Flemming et al., 1990; 2010). This could be another reason that higher concentrations of metals solubilised in the water column of dry-flooded soils. Nevertheless, the dissolved Fe concentrations in the water column of both HDF and CDF soils on the day-1 of flooding decreased by 20% and 50%, respectively relative to their moist-flooded counterparts (Figures 8.1a and 8.2a). Drying induced increased crystallinity of metal-oxides (Koopmans and Groenenberg, 2011) and reduction in metal solubility upon flooding might explain the significant reduction in total dissolved metal concentrations in this study.

Hallsworth-II dry-flooded (HDF) soil generally released greater total dissolved metal concentrations of Fe, Mn, Co, and Ni in most of the sampling days relative to all other treatments (HMF, CMF and CDF) (Figures 8.1-8.2) probably due to high organic carbon (% OC = 3.65 ± 0.10) and microbial biomass content of this soil relative to Crediton soil (% OC = 1.08 ± 0.21) (Table 7.2). Since soils rich in organic matter and microbial biomass have better ability to hold nutrients but are susceptible to release greater concentrations of nutrients following a drying rewetting stress from both microbial and non-microbial pools (Achat et al., 2012a,b). Though in the current study microbial biomass was not measured, greater concentrations of microbial biomass phosphorus (Table 7.2) of Hallsworth-II soil is an indicative of high level of microbial biomass of this soil relative to Credition soil (Table 7.2).

In case of HDF (Hallsworth-II dry-flooded) soil after the initial pulse, the total dissolved concentrations of metals (Fe, Cu, Co, and Ni) decreased during later stages of the flooding (Figure 8.1). The reduced metals solubility has been noticed previously (Shaheen et al., 2014b) during long-term flooding of contaminated flood plain soil at low redox. Formation of insoluble metal sulphides at very low redox (< -250 mV; Figure 7.3a) seems to be a plausible reason of reduced metal solubility (Kashem and Singh, 2001; Shaheen et al., 2014b). Drying induced oxidation of soil minerals accelerates formation of sulphate. However, when the dry soil is flooded, sulphate (SO_4^{-2}) is reduced to sulfide (S^{-2}) at significantly lower redox potential, which may react with metals to give their insoluble sulphides e.g. FeS, ZnS, CuS (Pan et al., 2016). Shaheen et al. (2014b) for instance linked low concentrations of Cu at low redox (-85mV) to the formation of insoluble sulphides. Though sulphate was not measured in the water column, the development of extreme reducing conditions as indicated by significantly low

redox (e.g. -337 mV for Hallsworth-II dry-flooded soil; Figure 7.3a) suggests that the formation of insoluble metal sulphides might have controlled low metal solubility in water column.

An additional factor other than the formation of insoluble metal sulphides, which might have controlled low metals solubility in the water column, was assumed to be the re-precipitation of Fe (II) to Fe (III) along with sorbed metals as it diffuses upward in the water column across anoxic-oxic zone. Since freshly precipitated or amorphous iron-oxide are known to provide greater reactive surface area and porosity for trace metals chemo-sorption relative to the more crystalline forms (Alloway, 2013). This is supported by the similar declining trend followed by total dissolve concentrations of Fe, Co, and Ni (Figure 8.1) and strong positive correlations ($p < 0.001$) between Fe and metals Co ($r = 0.763$, $p < 0.001$) and Ni ($r = 0.714$, $p < 0.001$) (Table 7.4). Furthermore, metals adsorption on soil minerals and organic colloids can also cause low metals solubility in water column.

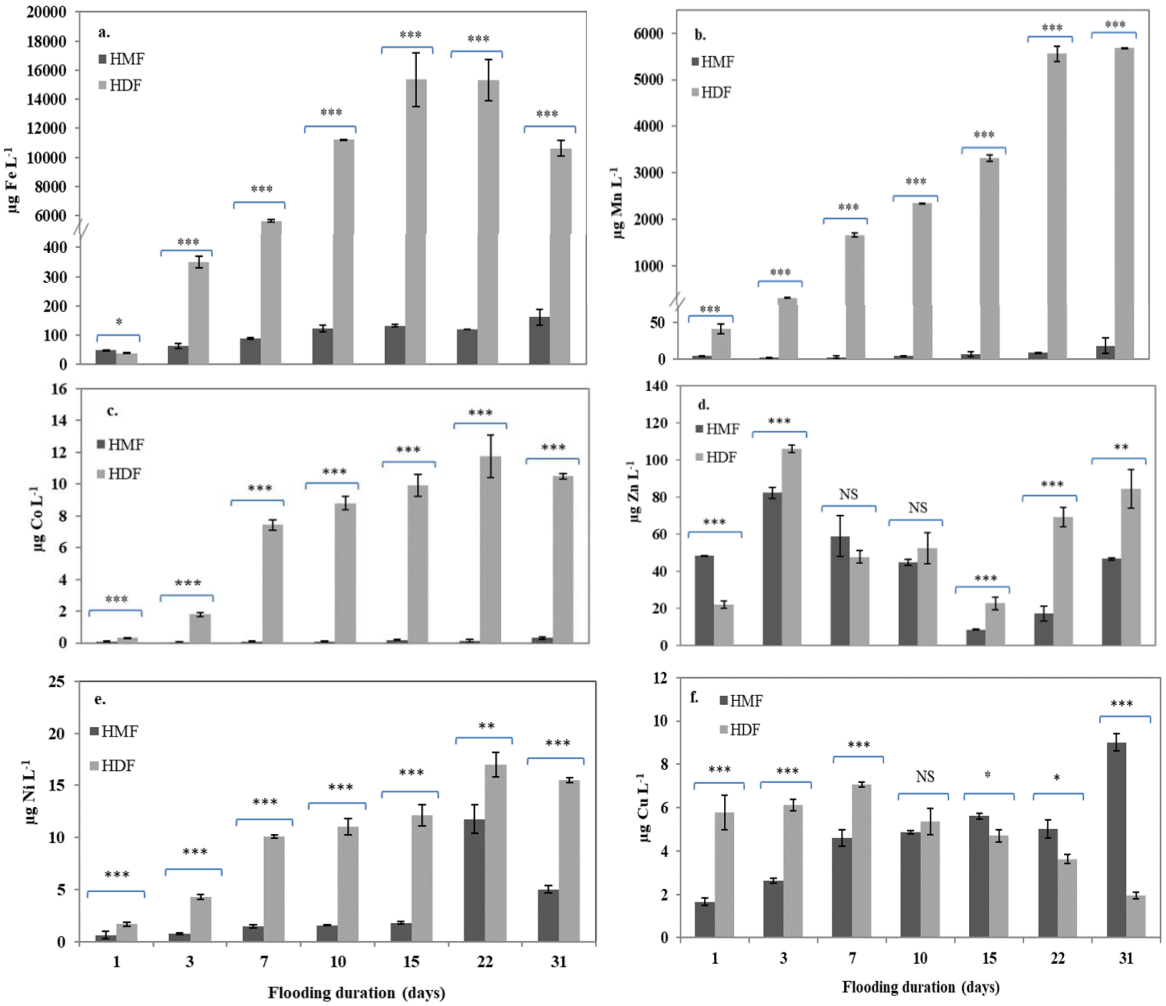

Figure 8.1a-f: Changes in total dissolved concentrations of metals (µg L^{-1}) in the water columns of moist and dry-flooded Hallsworth-II soil during 31-days flooding period (**a**. Iron (Fe), **b**. Manganese (Mn), **c**. Cobalt (Co), **d**. Zinc (Zn), **e**. Nickel (Ni) and **f**. Copper (Cu)). Error bars represent standard deviation, n = 3. In some cases, error bars are too small to be seen. The means difference between each moist and dry-flooded treatment as indicated by the bracket sign is significant at $p < 0.05$ as determined using LSD post-hoc test (***** Significant at $p < 0.05$ probability level, ****** Significant at $p < 0.01$ probability level, *******Significant at $p < 0.001$ probability level. 'NS' indicates not significant ($p > 0.05$). Note: The two parallel lines on the y-axis of **a** and **b** indicate an axis break as some of the concentrations were too low whilst others too high.

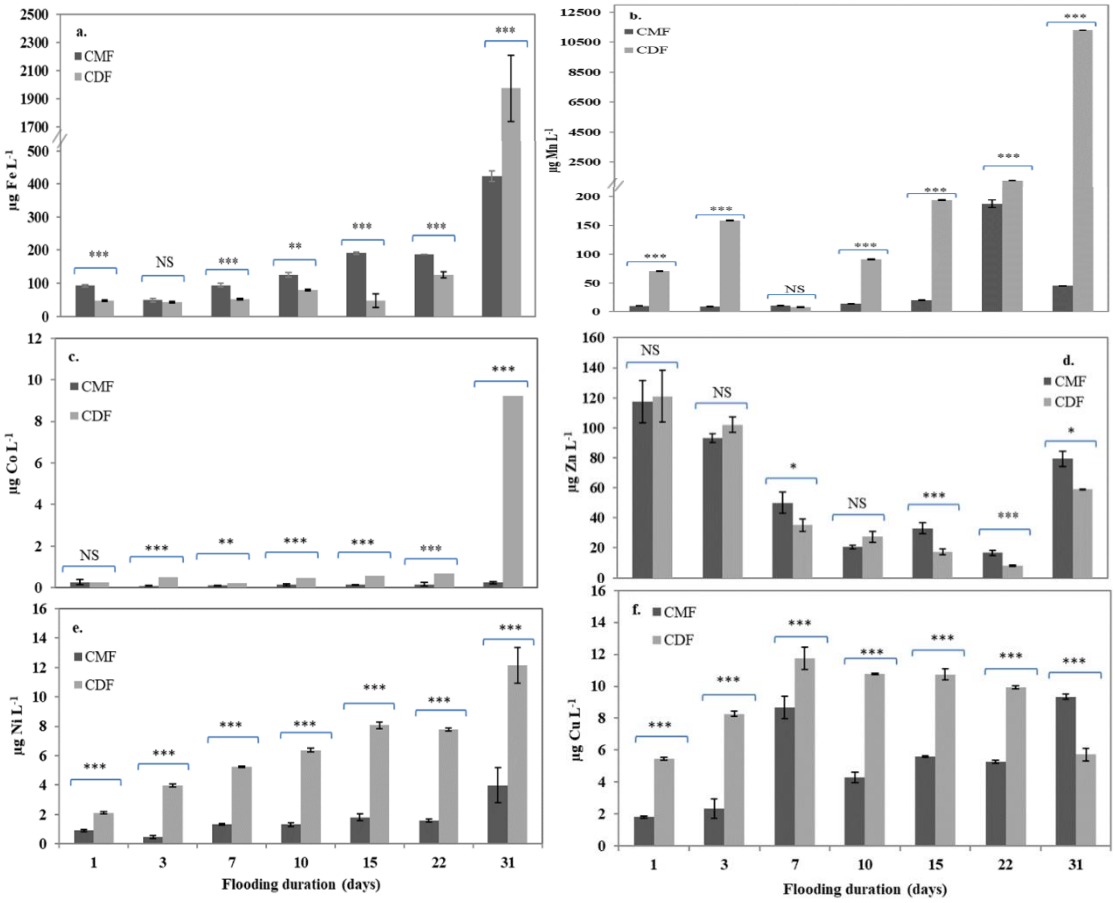

Figure 8.2a-f: Changes in total dissolved concentrations of metals (µg L^{-1}) in the water columns of moist and dry-flooded Crediton soil during 31-days flooding period (**a**. Iron (Fe), **b**. Manganese (Mn), **c**. Cobalt (Co), **d**. Zinc (Zn), **e**. Nickel (Ni) and **f**. Copper (Cu)). Error bars represent standard deviation, n = 3. In some cases, error bars are too small to be seen. The means difference between each moist and dry-flooded treatment as indicated by the bracket sign is significant at $p < 0.05$ as determined using LSD post-hoc test (***** Significant at $p < 0.05$ probability level, ****** Significant at $p < 0.01$ probability level, *******Significant at $p < 0.001$ probability level. 'NS' indicates not significant ($p > 0.05$). Note: The two parallel lines on the y-axis of **a** and **b** indicate an axis break as some of the concentrations were too low whilst others too high.

8.3.3 Solubilisation of micronutrients under flooding induced anaerobic conditions

Overall flooding-duration (31-days) had highly significant effect ($p < 0.001$) (Table 7.3) on total dissolved concentrations of all studied metals in the water column. The similar increasing trend in the total dissolved concentrations of Co and Ni (Figure 8.1c and 8.1e) and that followed by Fe and Mn (Figures 8.1a and b) shows that the reductive dissolution of Fe- and Mn- oxides was most likely be linked to the increase in the total dissolved concentrations of Co and Ni in the water column of HDF (Hallsworth-II dried flooded soil). This is further supported by highly significant positive correlation between total dissolve concentrations of Fe and total dissolved concentrations of Co ($r = 0.763$, $p < 0.001$) and Ni ($r = 0.714$, $p < 0.001$) (Table 7.4). Similarly, highly significant positive correlations were obtained between total dissolve concentrations of Mn and total dissolved concentrations of Co ($r = 0.909$, $p < 0.001$) and Ni ($r = 0.811$, $p < 0.001$)

(Table 7.4). Metals like Co, Fe, Ni, Cu and Mn have high adsorption affinities for metal oxides (Ashworth and Alloway, 2004). The reductive dissolution of Fe^{+3} causes solubilisation of previously sorbed trace metals. These results are in good agreement with Shaheen et al. (2014a; b), Frohne et al. (2014) and Rinklebe et al. (2016) who have reported mobilisation of trace metals in flood plain and paddy soils as a result of reductive dissolution of metal oxides. Shaheen et al. (2014b), for instance, studied the impact of soil redox potentials on mobilisation of Cu, Co and Ni in contaminated flood plain soils. Rinklebe et al. (2016) studied release dynamic of dissolved Cu in paddy soils under controlled redox conditions. Frohne et al. (2014) studied impact of pre-definite redox condition on dynamics of Co, Cu, Ni and Zn in acid flood plain soils. These studies indicate that redox processes can cause transfer of metals from soil minerals to soil solution.

The absence of significant correlation ($p > 0.05$) of Cu and Zn with metals Fe or Mn (Table 7.4) remained unclear. It was assumed that factors other than reductive dissolution of metal (Fe/Mn) oxides had caused possible solubilisation of Cu and Zn. The increase of dissolved organic matter (DOM) under reducing conditions and formation of organo-metallic complexes might explain the observed increased solubilisation of Cu and Zn since Cu is known to have strong binding affinity with DOM (Koopmans and Groenenberg; 2011; Shaheen et al., 2016). Nevertheless, in the current study DOM was not measured, the development of strong reducing conditions, increase in pH and concurrent increase in total dissolve concentrations of Cu support this assumption. The strong significant negative correlation ($r = -0.362$ and $p < 0.001$) between Cu and redox indicates its possible mobilisation under reducing conditions but here the correlation between Cu and metals (Fe/Mn) was either negatively non-significant (in case of Fe) or positively non-significant (in case of Mn). The non-significant positive relation between Cu and Mn gives a clue that Cu might be indirectly associated with Mn through OM-complexes and reductive dissolution of Mn might explain these results. High association of Cu with Mn-oxides in soils used in this study might be one of the reasons that Crediton dry-flooded soil with high easily reducible Mn-oxide content (0.09 %) (Table 7.2) released higher concentrations of Cu in water columns almost throughout the flooding durations relative to the Hallsworth-II dry-flooded soil with lower easily reducible Mn-oxide content (0.04 %).

Pearson's correlation between Zn and redox was significant and positively correlated ($r = 0.331$, $p < 0.01$) (Table 7.4) indicating its possible mobilisation before the development of strong reducing conditions. Zn mobilisation does not seem to be linked with the chemistry of Fe-/Mn-oxides as the relation between them was not significantly correlated. It is possible that

in this study Zn was presumably associated with DOM. Increase in DOM and the formation of soluble Zn-DOM may explain this behaviour when Fe/Mn oxides were not yet reduced.

The greater solubilisation of total dissolved concentrations of metals Fe, Mn, Co, and Ni was observed in water columns of Hallsworth-II dry-flooded (HDF) soils (Figure 8.1) with greater content of ammonium oxalate extractable Fe-oxides and organic matter (Table 7.2). In contrary, Credition dry-flooded (CDF) soil released generally less concentrations of metals (Fe, Mn, Co, and Ni) in the water columns almost throughout the flooding duration relative to HDF (Hallsworth-II dry-flooded) soil (Figure 8.2). However, after day-22 or day-31 of flooding a peak in metal concentrations in the water column of CDF soil was observed e.g.125 µg Fe L^{-1} to 1975 µg Fe L^{-1}, 1271 µg Mn L^{-1} to 11310 µg Mn L^{-1}, 0.7 µg Co L^{-1} to 9.2 µg Co L^{-1}, and 7.8 µg Ni L^{-1} to 12 µg Ni L^{-1} (Figure 8.2). A sudden increase in the dissolved metal concentrations in the water column of Crediton dry-flooded soil was perhaps surprising as this soil had already attained low redox potential and thus strong reducing conditions. Presumably this sudden increase in metals concentrations could be partly linked with dissolution of metal (Fe-/Mn-) oxides as was indicated by highly significant positive correlation between Fe and metals: Co ($r = 0.763$, $p < 0.001$) and Ni ($r = 0.714$, $p < 0.001$) and also between Mn and metals: Co ($r = 0.909$, $p < 0.001$) and Ni ($r = 0.811$, $p < 0.001$) (Table 7.4). This is further supported by highly significant negative relation between redox and metals: Co ($r = -0.712$, $p < 0.001$), Ni ($r = -0.784$, $p < 0.001$), Fe ($r = -0.565$, $p < 0.001$), and Mn ($r = -0.684$, $p < 0.001$) (Table 7.4). These correlations indicate that there might be a delay in development of strong anaerobic conditions in Crediton dry-flooded soil (CDF) required for reductive dissolution of Fe^{+3} to Fe^{+2} most likely due to relatively low content of organic matter and ammonium oxalate extractable Fe-oxide in this soil.

8.4 Conclusion

Two UK grassland soils with different organic matter and easily reducible metal (Fe/Mn) oxide content were used to investigate the impact of soil drying followed by flooding on the mobilisation of Co, Cu, Zn and Ni as governed by pH, redox and the chemistry of Fe and Mn. The results demonstrate that the flooding induced variations in pH and redox potential influence mobilisation of metals in the water column.

The mobilisation of Co and Ni seems to be controlled by redox driven reductive dissolution of Fe-/Mn-oxides. This is supported by significantly ($p < 0.001$) strong negative correlation between redox and metals – Co ($r = -0.712$) and Ni ($r = -0.784$) and their strong positive correlations ($p < 0.001$) with Fe/Mn. However, the non-significant correlation of Zn and Cu with Fe/Mn was unclear. Presumably the mobilisation of Zn and Cu might be associated with organic matter and the formation of soluble Cu- or Zn–DOM complexes, which might explain the increase in the total dissolve concentrations of Zn and Cu in the water column.

Overall, soil pre-condition (moist or dry) had highly significant effect ($p < 0.001$) on the total dissolved concentrations of all studied metals in the water column with the exception of Zn where the effect was not significant. The onset of flooding caused the release of greater concentrations of Mn, Co, Ni and Cu from soils which were previously dried relative to the moist non-dried counterparts. This could be due to a combination of soil drying (e.g. microbial death, increased crystallinity of metal oxides) and flooding (reductive dissolution of metal oxides) associated factors. Hallsworth-II dry-flooded (HDF) soil generally released greater total dissolved metal concentrations of Fe, Mn, Co and Ni in most of the sampling days relative to all other treatments (HMF, CMF and CDF) perhaps due to its greater organic matter (OM) and easily reducible ammonium oxalate extractable Fe-oxide content.

These results suggest that the soil drying followed by flooding have the potential to promote greater mobilisation of soil micronutrients than the soil flooding when it is not dried and thus have implications for soil nutrients mobilisation, soil fertility and surface water quality. This is particularly relevant under changing climate when the intensity and frequency of these severe climatic extremities (droughts followed by heavy rains causing flash floods) is predicted to increase.

These findings contribute to a better understanding of the processes and mechanisms involved in the mobilisation of metals under redox driven anaerobic conditions and might be useful in providing critical knowledge for interpreting potential risks associated with climate change induced increased mobilisation of soil micronutrients.

Detailed knowledge of how dried soils are likely to respond under saturated anaerobic conditions could help understand the extent of nutrients mobilisation in response to climate-change induced flash floods proceeded by drought. However, this was a controlled laboratory experiments, in natural environments many other factors are involved so in future further studies are needed under nature field conditions considering other interlinked factors e.g. continuous input of organic matter in the form of dead plants and micro/macro-organisms, water percolation/evaporation etc. These studies will give a better understanding of nutrients dynamics under natural field conditions in response to climate change. A wider area with a broad spectrum of soil texture, percent organic matter, microbial community structure and geogenic/anthropogenic metal content may help understand how soils from wider geographical locations may respond to climate extremities e.g. droughts proceeded by floods.

Chapter 9 - Summary, conclusions and future research directions

The intensity and frequency of naturally occurring soil processes e.g. drying-rewetting, drying-flooding and freezing-thawing will increase due to changing pattern of climate with the increase in ambient temperature and variations in rain-fall pattern. Longer periods of drought followed by intense rain-fall events can potentially mobilise significantly greater quantities of soil-borne macro- and micro-nutrients from soil solid-phase to solution-phase, which can eventually be transported to catchment waters, with the potential implications for soil fertility and catchment water quality. A series of experiments were designed to understand the influence of soil drying-rewetting and drying-flooding processes under controlled laboratory conditions with the following research objectives:

1. To examine the influence of soil drying and rewetting cycles on the leaching of DRP.
2. To examine the influence of soil drying and rewetting cycles on the leaching of micronutrients (Fe, Mn, Cu, Zn, Co and Ni).
3. To evaluate the role of soil microbial biomass-P to the leaching of DRP following drying and rewetting.
4. To examine if soil drying followed by extended period of flooding increases mobilisation of phosphorus and micronutrients (Fe, Mn, Cu, Zn, Co and Ni) in the overlying water column under the influence of flooding induced variations in redox potential.

The key findings are summarised as follows:

9.1 Microbial biomass response to soil drying-rewetting and phosphorus leaching

Increase in the intensity of soil (Hallsworth-I) drying (from 30°C to 40°C) and duration of soil drying (from 2-days to 14-days) caused greater DRP leaching relative to the control moist soil. The drying induced reduction in microbial biomass phosphorus as indicated from the results of $NaHCO_3$ extraction experiments partly contributed to the enhanced observed DRP leachate concentrations. The results show that soil drying at higher intensity and for prolonged duration affect the microbial biomass phosphorus to a greater extent than low intensity-short duration drying, and subsequently cause the leaching of higher concentrations of DRP following rewetting of dried soils. Fumigating soil samples before or after drying exhibit similar trends with reduction in microbial biomass phosphorus concurrent with the drying intensity and duration. However, the effect of chloroform fumigation was more

pronounced in terms of microbial biomass phosphorus reduction in the DF (drying followed by fumigation) treatments.

9.2 Influence of soil drying and rewetting cycles on leaching of phosphorus

The results demonstrated that the intensity and duration of soil (Hallsworth-I) drying, rate of soil rewetting and frequency of rewetting cycles play important roles in determining the extent of phosphorus leaching following rewetting of the dried soil. The dissolved phosphorus concentrations in the leachates collected from the soil dried for either 2-days or 14-days at 25ºC, 30ºC, 35ºC or 40ºC followed by the first rapid or slow rewetting cycle were significantly greater than the control moist counterparts. Although, there was a tendency of increasing DRP concentrations parallel to the drying temperature, the effect of drying intensity on the DRP leaching was more pronounced in the treatment where the soil was dried for 14-days at 40°C. Drying duration increased the leachate DRP concentrations, with significantly higher concentrations resulted from 14-days drying at 25ºC, 30ºC, 35ºC and 40ºC followed by the first rapid or slow rewetting cycle compared to 2-days drying counterparts. The rate at which the dried soil was rewetted generally affected the leachate dissolved concentrations of phosphorus, with the DRP concentrations tended to be higher in the leachates collected from the soil rewetted at slow rate relative to their rapid rewetting counterparts. The frequency of rewetting cycles also influenced the leachate DRP concentrations, with the concentrations showing some trends (increasing, decreasing or no significant change) in the second rewetting cycle relative to the first rewetting counterparts, though the concentrations remained higher than the control moist counterparts. Nevertheless, in the third rewetting cycle, there was a tendency of DRP concentrations declining in most of the treatments in particularly those where the soil was dried for 14-days. The tendency of decreasing DRP leaching in the succeeding rewetting cycles is perhaps due to depletion in the easily leachable phosphorus. The results suggest that the longer-drier periods proceeded with rainy events will mobilise soil-borne P, which could potentially be transported to the catchment waters through run-off. It may have potential implications for soil fertility and catchment water quality.

9.3 Influence of soil drying and rewetting cycles on micronutrients leaching

The results showed that the soil (Hallsworth-I) drying at 30°C or 40°C followed by the first rewetting cycle leached greater concentrations of Fe, Mn, Cu, Co, Ni and Zn relative to the control moist soil. Also, drying soil at 40°C leached considerably greater concentrations of micronutrients relative to their 30°C drying counterparts. The soil dried for 14-days (either at 30ºC or 40ºC) followed by the first rewetting cycle, leached significantly greater dissolved concentrations of Fe, Mn, Cu, Co, Ni and Zn relative to their 2-days drying counterparts. The frequency of rewetting cycles also influenced leachate dissolved metal concentrations, with the concentrations showing varied trends (increased, remained similar or decreased) in the second and third rewetting cycles for those treatments where the soil was dried for 2-days. However, in the treatments where the soil was dried for 14-days, the dissolved leachate concentrations of Fe, Mn, Co, Ni, Cu and Zn significantly decreased in the second and third rewetting cycle relative to the first rewetting counterparts, as observed for DRP. The declining trend in micronutrients leaching in the successive rewetting cycles could possibly because soluble forms of micronutrients would have been leached and those that remained were not in readily leachable forms. The results suggest that longer-drier periods proceeded by rainy events will render enhanced leaching/solubilisation of micronutrients (Fe, Mn, Cu, Co, Ni and Zn).

9.4 Influence of soil drying-flooding on mobilization of phosphorus and micronutrients

The onset of flooding increased dissolved phosphorus concentration. The increase in TDP (total dissolved phosphorus) concentration coincided with a reduction in redox potential, suggesting reductive dissolution of phosphorus bearing Fe/Mn minerals, and the observed increase in TDP was found to have a significant positive correlation with metals (Fe and Mn), supporting reductive dissolution of P. The significant positive correlation between total dissolved concentrations of Al and P indicates that non-reductive dissolution of Al-organic matter-P complexes may have also been partly responsible for phosphorus release to the water column.

The mobilisation of Co and Ni seems to be controlled by redox driven reductive dissolution of Fe/Mn minerals. This is supported by significantly strong negative correlation between redox and metals (Co and Ni) and their strong positive correlation with Fe and Mn. However, the non-significant correlation of Zn and Cu with Fe and Mn was unclear. Presumably, the mobilisation of Zn and Cu might be associated with organic matter and the

formation of soluble Cu- or Zn–dissolved organic matter complexes, which might explain the increase in total dissolve concentrations of Zn and Cu in the water column.

The flooding of dried soils generally caused greater solubilisation of dissolved concentrations of phosphorus (total dissolved phosphorus, dissolved reactive phosphorus and dissolved unreactive phosphorus) and metals (Mn, Co, Ni and Cu) in the water columns relative to their moist-flooded counterparts. The Crediton dry-flooded (CDF) soils released higher concentrations of dissolved reactive phosphorus (DRP) upon flooding than the Hallsworth-II dry-flooded (HDF) soil. However, most of the phosphorus in the water column of dry-flooded soils was unreactive, with the HDF soil releasing higher concentrations of dissolved unreactive phosphorus (DUP).

Hallsworth-II dry-flooded soil generally released greater total dissolved metal concentrations of Fe, Mn, Co and Ni in most of the sampling days relative to all other treatments (Hallsworth-II moist-flooded, Crediton moist-flooded and Crediton dried-flooded). These results suggest that extended periods of soil drying followed by flooding have the potential to promote greater mobilisation of soil macro- (e.g. P) and micro-nutrients (e.g. Mn, Co, Ni and Cu) compared to flooding of moist soils. It may have implications for soil fertility and catchment water quality, particularly under changing climate when the intensity and frequency of these severe climatic extremities (droughts and flash floods) will increase.

9.5 Limitations, recommendations and future research directions

The findings from drying-rewetting (DRW) experiments indicate that climate change triggered variations in the pattern of naturally occurring soil drying-rewetting processes could have significant impacts on nutrients (e.g. dissolved reactive phosphorus and studied micronutrients) leaching from the soil (Hallsworth-I series) used in this study. However, in this study leachate dissolved organic phosphorus (DOP) was not measured. Measuring dissolved organic phosphorus would have allowed a better understanding of the underlying processes, particularly the role of microbial biomass-P and transformation between inorganic and organic P. Furthermore, these findings cannot be precisely replicated under natural-field conditions where nutrients mobilised in runoff may be retained in the sub-soil and/or elsewhere in the landscape along the pathway of runoff before they reach catchment waters. There remains a need to evaluate soil drying-rewetting impacts on nutrients leaching on a wider scale and in a range of soil types, as well as under natural-field conditions, to obtain a more realistic assessment of nutrients leaching. So far majority of the work has been done to address the

effects of soil drying-rewetting on nutrients extraction, whilst only a few studies have considered the effects of repeated drying-rewetting cycles on nutrient dynamics (mobilisation and sequestration), with some contradictory results – some are reporting increase while other are reporting decrease in nutrient concentrations with the increase in the frequency of drying-rewetting cycles. There is much need to study the factors which influence leachate nutrient concentrations under field conditions, following a drying-rewetting stress in detail, especially the rate (at which the dried soil is rewetted) and duration of soil drying. However, field-scale studies are difficult due to natural variations in the temperature, perhaps farm-scale platform such as that at North Wyke could capture such influences of climate change. Furthermore, laboratory leaching trials using homogenised sieved soils could also overestimate the quantity of nutrient that is leached as soil is not well-structured/aggregated. Thus, for future studies, performing laboratory drying-rewetting leaching experiments using intact soil cores could be a more realistic approach.

The findings from drying-flooding experiments contribute to a better understanding of how dried soils are likely to respond under reducing conditions when they get suddenly flooded and might be useful in evaluating the extent of nutrients mobilisation in response to climate-change mediated flash flooding after prolonged drought conditions. However, nutrients mobilised from a site upon moist-/dry-flooding may be retained in the sub-soil or elsewhere in the landscape. Hence, to assess the potential of nutrients release, field-scale studies are needed, perhaps, on a wider scale using contrasting soils with varying amounts of organic matter, clay, metal-oxide content, cation exchange capacity and microbial biomass concentrations.

References

Abit, S. M., Vepraskas, M. J., Duckworth, O. W. and Amoozegar, A., 2013. Dissolution of phosphorus into pore-water flowing through an organic soil. Geoderma 197, 51-58.

Achat, D. L., Augusto, L., Bakker,M. R., Gallet-Budynek, A. and Morel, C., 2012a. Microbial processes controlling P availability in forest spodosols as affected by soil depth and soil properties. Soil Biology and Biochemistry 44(1), 39-48.

Achat, D. L., Augusto, L., Gallet-Budynek, A. and Bakker, M. R., 2012b. Drying-induced changes in phosphorus status of soils with contrasting soil organic matter contents–Implications for laboratory approaches. Geoderma 187, 41-48.

Achat, D. L., Bakker, M. R., Zeller, B., Pellerin, S., Bienaimé, S. and Morel, C., 2010. Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. Soil Biology and Biochemistry 42 (9), 1479-1490.

Aldous, A. R., Craft, C. B., Stevens, C. J., Barry, M. J. and Bach, L. B., 2007. Soil phosphorus release from a restoration wetland, Upper Klamath Lake, Oregon. Wetlands 27(4), 1025-1035.

Alloway, B. J., 2013. Sources of heavy metals and metalloids in soils. In Heavy metals in soils (11-50). Springer, Dordrecht.

Amarawansha, E., Kumaragamage, D., Flaten, D., Zvomuya, F. and Tenuta, M., 2015. Phosphorus mobilization from manure-amended and unamended alkaline soils to overlying water during Simulated flooding. Journal of Environmental Quality 44 (4), 1252-1262.

Antic-Mladenovic, S., Frohne, T., Kresović, M., Stärk, H. J., Tomić, Z., Ličina, V. and Rinklebe, J., 2017. Biogeochemistry of Ni and Pb in a periodically flooded arable soil: fractionation and redox-induced (im) mobilization. Journal of environmental management 186, 141-150.

Ashworth, D. and Alloway, B., 2004. Soil mobility of sewage sludge-derived dissolved organic matter, copper, nickel and zinc. Environmental Pollution 127 (1), 137-144.

Ashworth, D. and Alloway, B., 2007. Complexation of copper by sewage sludge-derived dissolved organic matter: Effects on soil sorption behaviour and plant uptake. Water, Air, and Soil Pollution 182 (1-4), 187-196.

Attygalla, N. W., Baldwin, D. S., Silvester, E., Kappen, P. and Whitworth, K. L., 2016. The severity of sediment desiccation affects the adsorption characteristics and speciation of phosphorus. Environmental Science: Processes and Impacts 18(1), 64-71.

Austin, A. T., Yahdjian, L., Stark, J. M., Belnap, J., Porporato, A., Norton, U., Ravetta, D. A. and Schaeffer, S. M., 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologi*a* 141 (2), 221-235.

Bai, J., Ye, X., Jia, J., Zhang, G., Zhao, Q., Cui, B. and Liu, X., 2017. Phosphorus sorption-desorption and effects of temperature, pH and salinity on phosphorus sorption in marsh soils from coastal wetlands with different flooding conditions. Chemosphere 188, 677-688.

Baldwin, D. S., Mitchell, A. M. and Rees, G. N., 2000. The effects of in situ drying on sediment–phosphate interactions in sediments from an old wetland. Hydrobiologia 431(1), 3-12.

Bates, B.C., Kundzewicz, Z.W., Wu.S. and Palutikof.J.P., Eds., 2008: Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change, IPCC Secretariat, Geneva, 210.

Beare, M., Hendrix, P. and Coleman, D., 1994. Water-stable aggregates and organic matter fractions in conventional-and no-tillage soils. Soil Science Society of America Journal 58 (3), 777-786.

Bechmann, M. E., Kleinman, P. J., Sharpley, A. N. and Saporito, L. S., 2005. Freeze–thaw effects on phosphorus loss in runoff from manured and catch-cropped soils. Journal of Environmental Quality 34 (6), 2301-2309.

Berryman, E. M., Venterea, R. T., Baker, J. M., Bloom, P. R. and Elf, B., 2009. Phosphorus and greenhouse gas dynamics in a drained calcareous wetland soil in Minnesota. Journal of environmental quality 38(5), 2147-2158.

Blackwell, M. S., Carswell, A. M. and Bol, R., 2013. Variations in concentrations of N and P forms in leachates from dried soils rewetted at different rates, Biology and Fertility of Soils 49 (1), 79-87.

Blackwell, M., Brookes, P., de La Fuente-Martinez, N., Gordon, H., Murray, P., Snars, K., Williams, J., Bol, R. and Haygarth, P., 2010. Phosphorus solubilization and potential transfer to surface waters from the soil microbial biomass following drying–rewetting and freezing–thawing. Advances in Agronomy 106, 1-35.

Blackwell, M., Brookes, P., De la Fuente-Martinez, N., Murray, P., Snars, K., Williams, J. and Haygarth, P., 2009. Effects of soil drying and rate of re-wetting on concentrations and forms of phosphorus in leachate. Biology and Fertility of Soils 45 (6), 635-643.

Borken, W. and Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology 15 (4), 808-824.

Bostrom, B., Andersen, J. M., Fleischer, S. and Jansson, M., 1988. Exchange of phosphorus across the sediment-water interface. In Phosphorus in freshwater ecosystems (229-244). Springer, Dordrecht.

Bottner, P., 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with 14 C-and 15 N-labelled plant material, Soil Biology and Biochemistry 17 (3), 329-337.

Brodlin, D., Kaiser, K., Kessler, A. and Hagedorn, F., 2019. Drying and rewetting foster phosphorus depletion of forest soils. Soil Biology and Biochemistry 128, 22-34.

Brookes, P. C., Powlson, D. and Jenkinson, D., 1984. Phosphorus in the soil microbial biomass. Soil Biology and Biochemistry 16 (2), 169-175.

Brookes, P., Powlson, D. and Jenkinson, D., 1982. Measurement of microbial biomass phosphorus in soil, Soil Biology and Biochemistry 14 (4), 319-329.

Brooks, P. D., Williams, M. W. and Schmidt, S. K., 1998. Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. Biogeochemistry 43 (1), 1-15.

Brubaker, S. C., Jones, A. J., Lewis, D. T. and Frank, K., 1993. Soil properties associated with landscape position. Soil Science Society of America Journal *57*(1), 235-239.

Bruland, G. L. and DeMent, G., 2009. Phosphorus sorption dynamics of Hawaii's coastal wetlands. Estuaries and Coasts 32(5), 844-854.

Bruland, G. L. and Richardson, C. J., 2006. An assessment of the phosphorus retention capacity of wetlands in the Painter Creek Watershed, Minnesota, USA. Water, Air, and Soil Pollution *171*(1-4), 169-184.

Bunemann, E., Keller, B., Hoop, D., Jud, K., Boivin, P. and Frossard, E., 2013. Increased availability of phosphorus after drying and rewetting of a grassland soil: processes and plant use. Plant and Soil 370 (1-2), 511-526.

Butterly, C. R., McNeill, A. M., Baldock, J. A. and Marschner, P., 2011. Rapid changes in carbon and phosphorus after rewetting of dry soil, Biology and Fertility of Soils. 47 (1), 41-50.

Butterly, C., Bünemann, E., McNeill, A., Baldock, J. and Marschner, P., 2009. Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biology and Biochemistry 41 (7) 1406-1416.

Caetano, M., Bernárdez, P., Santos-Echeandia, J., Prego, R. and Vale, C., 2012. Tidally driven N, P, Fe and Mn exchanges in salt marsh sediments of Tagus estuary (SW Europe). Environmental monitoring and assessment 184(11), 6541-6552.

Carpenter, S. R., 2008. Phosphorus control is critical to mitigating eutrophication. Proceedings of the National Academy of Sciences 105(32), 11039-11040.

Carter, M. R. and Gregorich, E. G. (Eds.)., 2008. Soil sampling and methods of analysis.

Chacon, N., Dezzeo, N., Munoz, B. and Rodríguez, J. M., 2005. Implications of soil organic carbon and the biogeochemistry of iron and aluminum on soil phosphorus distribution in flooded forests of the lower Orinoco River, Venezuela. Biogeochemistry 73(3), 555-566.

Chapman, H., 1965. Cation-exchange capacity, Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties 9, 891-901.

Chen, H., Lai, L., Zhao, X., Li, G. and Lin, Q., 2016. Soil microbial biomass carbon and phosphorus as affected by frequent drying–rewetting. Soil Research 54(3), 321-327.

Chen, Y. C., Liu, J. H., Kuo, J. T. and Lin, C. F., 2013. Estimation of phosphorus flux in rivers during flooding. Environmental monitoring and assessment 185(7), 5653-5672.

Chenu, C., Le Bissonnais, Y. and Arrouays, D., 2000. Organic matter influence on clay wettability and soil aggregate stability. Soil Science Society of America Journal 64 (4), 1479-1486.

Chepkwony, C., Haynes, R., Swift, R. and Harrison, R., 2001. Mineralization of soil organic P induced by drying and rewetting as a source of plant-available P in limed and unlimed samples of an acid soil. Plant and Soil 234 (1), 83-90.

Cisneros-Dozal, L. M.,Trumbore, S. E. and Hanson, P. J., 2007. Effect of moisture on leaf litter decomposition and its contribution to soil respiration in a temperate forest. Journal of Geophysical Research: Biogeosciences (2005–2012), 112 (G1).

Daly, K., Jeffrey, D. and Tunney, H., 2001. The effect of soil type on phosphorus sorption capacity and desorption dynamics in Irish grassland soils. Soil use and management 17(1), 12-20.

Darke, A. K. and Walbridge, M. R., 2000. Al and Fe biogeochemistry in a floodplain forest: implications for P retention. Biogeochemistry 51(1), 1-32.

DeLuca, T., Keeney, D. and McCarty, G., 1992. Effect of freeze-thaw events on mineralization of soil nitrogen. Biology and Fertility of Soils 14 (2), 116-120.

Denef, K., Six, J., Bossuyt, H., Frey, S. D., Elliott, E. T., Merckx, R. and Paustian, K., 2001a. Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics, Soil Biology and Biochemistry 33 (12), 1599-1611.

Denef, K., Six, J., Paustian, K. and Merckx, R., 2001b. Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry–wet cycles. Soil Biology and Biochemistry 33(15), 2145-2153.

Diaz, O. A., Reddy, K. R. and Moore Jr, P. A., 1994. Solubility of inorganic phosphorus in stream water as influenced by pH and calcium concentration. Water Research 28(8), 1755-1763.

Dieter, D., Herzog, C. and Hupfer, M., 2015. Effects of drying on phosphorus uptake in re-flooded lake sediments. Environmental Science and Pollution Research 22(21), 17065-17081.

Dinh, M. V., Guhr, A., Spohn, M. and Matzner, E., 2017. Release of phosphorus from soil bacterial and fungal biomass following drying/rewetting. Soil Biology and Biochemistry 110, 1-7.

Dinh, M., Guhr, A., Weig, A. R. and Matzner, E., 2018. Drying and rewetting of forest floors: dynamics of soluble phosphorus, microbial biomass-phosphorus, and the composition of microbial communities. Biology and Fertility of Soils, 54 (6), pp. 761-768.

Dodds, W. K. and Smith, V. H. (2016). Nitrogen, phosphorus, and eutrophication in streams. Inland Waters 6(2), 155-164.

Dong, D. M., Yang, F., Li, Y., Hua, X. Y., Lu, X. J. and Zhang, J. J., 2005. Adsorption of Pb, Cd to Fe, Mn oxides in natural freshwater surface coatings developed in different seasons, Journal of Environmental Sciences (China) 17 (1), 30-36.

Drenovsky, R. E., Vo, D., Graham, K. J. and Scow, K. M., 2004. Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microbial ecology 48(3), 424-430.

Drewry, J., Newham, L. and Croke, B., 2009. Suspended sediment, nitrogen and phosphorus concentrations and exports during storm-events to the Tuross estuary, Australia. Journal of Environmental Management 90 (2), 879-887.

El-Moselhy, K. M., Othman, A. I., El-Azem, H. A. and El-Metwally, M. E. A., 2014. Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. Egyptian Journal of Basic and Applied Sciences 1(2), 97-105.

Emmett, B. A., Beier, C.,Estiarte, M., Tietema, A., Kristensen, H. L., Williams, D., Penuelas, J., Schmidt, I. and Sowerby, A., 2004. The response of soil processes to climate change: results from manipulation studies of shrublands across an environmental gradient. Ecosystems 7 (6), 625-637.

Erich, M. S., Fitzgerald, C. B. and Porter, G. A., 2002. The effect of organic amendments on phosphorus chemistry in a potato cropping system. Agriculture, Ecosystems and Environment 88(1), 79-88.

Evans, S. E., Wallenstein, M. D. and Burke, I. C., 2014. Is bacterial moisture niche a good predictor of shifts in community composition under long-term drought? Ecology 95(1), 110-122.

Fierer, N. and Schimel, J. P., 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations, Soil Biology and Biochemistry 34 (6), 777-787.

Fierer, N., Schimel, J. and Holden, P., 2003. Influence of drying–rewetting frequency on soil bacterial community structure, Microbial Ecology 45 (1), 63-71.

Flemming, C. A., Ferris, F. G., Beveridge, T. J. and Bailey, G. W., 1990. Remobilization of toxic heavy metals adsorbed to bacterial wall-clay composites, Applied and Environmental Microbiology 56 (10), 3191-3203.

Flemming, H. and Wingender, J., 2010. The biofilm matrix. Nature Reviews Microbiology 8 (9), 623.

Fonte, S. J., Nesper, M., Hegglin, D., Velásquez, J. E., Ramirez, B., Rao, I. M, Bernasconi, S.M, Bünemann, E. K, Frossard, E. and Oberson, A., 2014. Pasture degradation impacts soil phosphorus storage via changes to aggregate-associated soil organic matter in highly weathered tropical soils. Soil Biology and Biochemistry, 68, 150-157.

Forber, K. J., Ockenden, M. C., Wearing, C., Hollaway, M. J., Falloon, P. D., Kahana, R., Villamizar, M. L., Zhou, J. G., Withers, P. J. and Beven, K. J., 2017. Determining the effect of drying time on phosphorus solubilization from three agricultural soils under climate change scenarios, Journal of Environmental Quality 46 (5), 1131-1136.

Fowler, H. and Kilsby, C., 2007. Using regional climate model data to simulate historical and future river flows in northwest England. Climatic Change 80 (3-4), 337-367.

Freppaz, M., Williams, B. L., Edwards, A. C., Scalenghe, R. and Zanini, E., 2007. Simulating soil freeze/thaw cycles typical of winter alpine conditions: implications for N and P availability, Applied Soil Ecology 35 (1), 247-255.

Frohne, T., Rinklebe, J. and Diaz-Bone, R. A., 2014. Contamination of floodplain soils along the Wupper River, Germany, with As, Co, Cu, Ni, Sb, and Zn and the impact of pre-definite redox variations on the mobility of these elements. Soil and Sediment Contamination: An International Journal 23 (7), 779-799.

Gadd, G. M., 2007. Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation, Mycological Research 111 (1), 3-49.

Gilbert, J. D., Guerrero, F. and de Vicente, I., 2014. Sediment desiccation as a driver of phosphate availability in the water column of Mediterranean wetlands. Science of the Total Environment 466, 965-975.

Glendell, M., Granger, S. J., Bol, R. and Brazier, R. E., 2014. Quantifying the spatial variability of soil physical and chemical properties in relation to mitigation of diffuse water pollution. Geoderma 214, 25-41.

Goebel, M., Woche, S. K., Bachmann, J.,Lamparter, A. and Fischer, W. R., 2007. Significance of wettability-induced changes in microscopic water distribution for soil organic matter decomposition, Soil Science Society of America Journal 71 (5), 1593-1599.

Gordon, H., Haygarth, P. M. and Bardgett, R. D., 2008. Drying and rewetting effects on soil microbial community composition and nutrient leaching. Soil Biology and Biochemistry 40(2), 302-311.

Gotoh, S. and Patrick, W., 1974. Transformation of iron in a waterlogged soil as influenced by redox potential and pH. Soil Science Society of America Journal 38 (1), 66-71.

Grogan, P., Michelsen, A., Ambus, P. and Jonasson, S., 2004. Freeze–thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. Soil Biology and Biochemistry 36 (4), 641-654.

Grybos, M., Davranche, M., Gruau, G. and Petitjean, P., 2007. Is trace metal release in wetland soils controlled by organic matter mobility or Fe-oxyhydroxides reduction? Journal of Colloid and Interface Science 314 (2), 490-501.

Grybos, M., Davranche, M., Gruau, G., Petitjean, P. and Pédrot, M., 2009. Increasing pH drives organic matter solubilization from wetland soils under reducing conditions. Geoderma 154(1-2), 13-19.

Gu, S., Gruau, G., Malique, F., Dupas, R., Petitjean, P. and Gascuel-Odoux, C., 2018. Drying/rewetting cycles stimulate release of colloidal-bound phosphorus in riparian soils. Geoderma 321, 32-41.

Guo, S., Liu, Z., Li, Q., Yang, P., Wang, L., He, B., Xu, Z., Ye, J. and Zeng, E. Y., 2016. Leaching heavy metals from the surface soil of reclaimed tidal flat by alternating seawater inundation and air drying, Chemosphere 157, 262-270.

Hagg, H. E., Lyon, S. W., Wällstedt, T., Mörth, C. M., Claremar, B. and Humborg, C., 2014. Future nutrient load scenarios for the Baltic Sea due to climate and lifestyle changes. Ambio 43(3), 337-351.

Halverson, L. J., Jones, T. M. and Firestone, M. K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal, 64 (5), 1630-1637.

Harrod, T. and Hogan, D., 2008. The soils of North Wyke and Rowden. Revised edition of Harrod TR (1981) Soil in Devon IV: Sheet SS61 (Chulmleigh). Soil Survey Rec No 70.

Haynes, R. and Swift, R., 1990. Stability of soil aggregates in relation to organic constituents and soil water content, Journal of Soil Science 41 (1), 73-83.

Haynes, R. and Swift, R., 1991. Concentrations of extractable Cu, Zn, Fe and Mn in a group of soils as influenced by air-and oven drying and rewetting, Geoderma 49 (3-4), 319-333.

Hentschel, K., Borken, W. and Matzner, E., 2007. Leaching losses of inorganic N and DOC following repeated drying and wetting of a spruce forest soil, Plant and Soil 300 (1-2), 21-34.

Herrmann, A. and Witter, E., 2002. Sources of C and N contributing to the flush in mineralization upon freeze–thaw cycles in soils, Soil Biology and Biochemistry 34 (10), 1495-1505.

Homberg, A. and Matzner, E., 2018. Effects of drying and rewetting on soluble phosphorus and nitrogen in forest floors: An experiment with undisturbed columns, Journal of Plant Nutrition and Soil Science 181 (2), 177-184.

Hooda, P., Moynagh, M., Svoboda, I., Edwards, A., Anderson, H. and Sym, G., 1999. Phosphorus loss in drain flow from intensively managed grassland soils. Journal of Environmental Quality 28 (4), 1235-1242.

Hook, P. B. and Burke, I. C., 2000. Biogeochemistry in a shortgrass landscape: control by topography, soil texture, and microclimate. Ecology 81(10), 2686-2703.

Hulme, M., Jenkins, G. J., Lu, X., Turnpenny, J. R., Mitchell, T. D., Jones, R. G., Lowe, J., Murphy, J. M., Hassell, D., Boorman, P., McDonald, R. and Hill, S., 2002. Climate change scenarios for the 21st century for the UK. Scientific and Tech. Report for UKCIP, Tyndall Centre, University of East Anglia, Norwich, UK

Hunt, J. F., Ohno, T., He, Z., Honeycutt, C. W. and Dail, D. B., 2007. Inhibition of phosphorus sorption to goethite, gibbsite, and kaolin by fresh and decomposed organic matter. Biology and fertility of soils 44(2), 277-288.

Huntington, T. G., 2006. Evidence for intensification of the global water cycle: review and synthesis, Journal of Hydrology 319 (1) 83-95.

Hupfer, M. and Lewandowski, J., 2008. Oxygen controls the phosphorus release from lake sediments – a long-lasting paradigm in limnology. International Review of Hydrobiology 93(4-5), 415-432.

Huser, B. J. and Rydin, E., 2005. Phosphorus inactivation by aluminum in Lakes Gårdsjön and Härsvatten sediment during the industrial acidification period in Sweden, Canadian Journal of Fisheries and Aquatic Sciences 62 (8), 1702-1709.

Hutchison, K. J. and Hesterberg, D., 2004. Dissolution of phosphate in a phosphorus-enriched ultisol as affected by microbial reduction. Journal of environmental quality 33(5), 1793-1802.

Huttunen, I., Lehtonen, H., Huttunen, M., Piirainen, V., Korppoo, M., Veijalainen, N., Viitasalo, M. and Vehviläinen, B., 2015. Effects of climate change and agricultural adaptation on nutrient loading from Finnish catchments to the Baltic Sea. Science of the Total Environment 529, 168-181.

IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151.

Jayalath, N., Mosley, L. M., Fitzpatrick, R. W. and Marschner, P., 2016. Addition of organic matter influences pH changes in reduced and oxidised acid sulfate soils. Geoderma 262, 125-132.

Kaiser, M., Kleber, M. and Berhe, A. A., 2015. How air-drying and rewetting modify soil organic matter characteristics: an assessment to improve data interpretation and inference. Soil Biology and Biochemistry 80, 324-340.

Kashem, M. A. and Singh, B. R., 2001. Metal availability in contaminated soils: I. Effects of flooding and organic matter on changes in Eh, pH and solubility of Cd, Ni and Zn. Nutrient Cycling in Agroecosystems 61(3), 247-255.

Khan, K. S., Heinze, S. and Joergensen, R. G., 2009. Simultaneous measurement of S, macronutrients, and heavy metals in the soil microbial biomass with $CHCl_3$ fumigation and NH_4NO_3 extraction, Soil Biology and Biochemistry 41 (2), 309-314.

Khan, S. U., Hooda, P. S., Blackwell, M. S., and Busquets, R., 2019. Microbial biomass responses to soil drying-rewetting and phosphorus leaching. Frontiers in Environmental Science *7*, 133.

Khoshmanesh, A., Hart, B. T., Duncan, A., and Beckett, R., 1999. Biotic uptake and release of phosphorus by a wetland sediment. Environmental Technology 20(1), 85-91.

Khoshmanesh, A., Hart, B. T., Duncan, A., and Beckett, R., 2002. Luxury uptake of phosphorus by sediment bacteria. Water Research 36(3), 774-778.

Kieft, T. L., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted, Soil Biology and Biochemistry 19 (2), 119-126.

Kogel-Knabner, I., Amelung, W., Cao, Z., Fiedler, S., Frenzel, P., Jahn, R., Kalbitz, K., Kölbl, A. and Schloter, M., 2010. Biogeochemistry of paddy soils, Geoderma 157 (1), 1-14.

Kolahchi, Z. and Jalali, M., 2013. Phosphorus movement and retention by two calcareous soils. Soil and Sediment Contamination: An International Journal 22(1), 21-38.

Koopmans, G. F. and Groenenberg, J. E., 2011. Effects of soil oven-drying on concentrations and speciation of trace metals and dissolved organic matter in soil solution extracts of sandy soils. Geoderma 161 (3-4), 147-158.

Koopmans, G. F., Chardon, W. J., Dekker, P. H. M., Römkens, P. F. A. M. and Schoumans, O. F., 2006. Comparing different extraction methods for estimating phosphorus solubility in various soil types. Soil science 171(2), 103-116.

Kopacek, J., Borovec, J., Hejzlar, J., Ulrich, K. U., Norton, S. A. and Amirbahman, A., 2005. Aluminum control of phosphorus sorption by lake sediments. Environmental Science and Technology 39(22), 8784-8789.

Kopacek, J., Hejzlar, J., Borovec, J., Porcal, P. and Kotorová, I., 2000. Phosphorus inactivation by aluminum in the water column and sediments: Lowering of in-lake phosphorus availability in an acidified watershed-lake ecosystem, Limnology and Oceanography 45 (1), 212-225.

Kostaschuk, R., Terry, J. and Raj, R., 2003. Suspended sediment transport during tropical-cyclone floods in Fiji, Hydrological Processes 17 (6), 1149-1164.

Kundzewicz, Z., Mata, L., Arnell, N., Döll, P., Kabat, P., Jiménez, B., Miller, K., Oki, T., Şen, Z. and Shiklomanov, I., 2007. Freshwater resources and their management. [W:] Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, (red. Parry, ML, Canziani, OF, Palutikof, JP, Hanson, CE and van der Linden, PJ).

Lai, D. Y. F. and Lam, K. C., 2008. Phosphorus retention and release by sediments in the eutrophic Mai Po Marshes, Hong Kong. Marine Pollution Bulletin 57(6-12), 349-356.

Larsen, K. S., Grogan, P., Jonasson, S. and Michelsen, A., 2007. Respiration and microbial dynamics in two subarctic ecosystems during winter and spring thaw: effects of increased snow depth. Arctic, Antarctic, and Alpine Research 39(2), 268-276.

Ledin, M., 2000. Accumulation of metals by microorganisms—processes and importance for soil systems. Earth-Science Reviews 51 (1-4), 1-31.

Lee, S. B., Lee, C. H., Lee, Y. B. and Kim, P. J., 2013. Impacts of Molybdate Unreactive Phosphorus on Phosphate Release from Intensively Fertilized Soil. Communications in soil science and plant analysis 44(18), 2746-2756.

Lewis Jr, W. M., Wurtsbaugh, W. A. and Paerl, H. W., 2011. Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. Environmental science and technology 45(24), 10300-10305.

Lewis, D. B., Brown, J. A. and Jimenez, K. L., 2014. Effects of flooding and warming on soil organic matter mineralization in Avicenniagerminans mangrove forests and Juncusroemerianus salt marshes, Estuarine, Coastal and Shelf Science 139, 11-19.

Li, G., Wang, F., Ma, W., Fortier, R., Mu, Y., Mao, Y. and Hou, X., 2018. Variations in strength and deformation of compacted loess exposed to wetting-drying and freeze-thaw cycles. Cold Regions Science and Technology 151, 159-167.

Li, L., Qu, Z., Jia, R., Wang, B., Wang, Y. and Qu, D., 2017. Excessive input of phosphorus significantly affects microbial Fe (III) reduction in flooded paddy soils by changing the abundances and community structures of Clostridium and Geobacteraceae, Science of the Total Environment 607, 982-991.

Li, M., Hu, Z., Zhu, X. and Zhou, G., 2015. Risk of phosphorus leaching from phosphorus-enriched soils in the Dianchi catchment, Southwestern China. Environmental Science and Pollution Research 22(11), 8460-8470.

Lin, Y., Bhattacharyya, A., Campbell, A. N., Nico, P. S., Pett-Ridge, J. and Silver, W. L., 2018. Phosphorus fractionation responds to dynamic redox conditions in a humid tropical forest soil, Journal of Geophysical Research: Biogeosciences 123 (9), 3016-3027.

Lipson, D. A., Schmidt, S. K. and Monson, R. K., 2000. Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass, Soil Biology and Biochemistry 32 (4), 441-448.

Liu, J., Aronsson, H., Bergström, L. and Sharpley, A., 2012. Phosphorus leaching from loamy sand and clay loam top soils after application of pig slurry. Springer Plus 1(1), 53.

Loeb, R., Lamers, L. P. and Roelofs, J. G., 2008. Prediction of phosphorus mobilisation in inundated floodplain soils. Environmental Pollution 156(2), 325-331.
Magid, J., Kjærgaard, C., Gorissen, A. and Kuikman, P., 1999. Drying and rewetting of a loamy sand soil did not increase the turnover of native organic matter, but retarded the decomposition of added 14 C-labelled plant material, Soil Biology and Biochemistry 31 (4), 595-602.
Maranguit, D., Guillaume, T. and Kuzyakov, Y., 2017. Effects of flooding on phosphorus and iron mobilization in highly weathered soils under different land-use types: Short-term effects and mechanisms, Catena 158, 161-170.
Marsh, T., 2007. The summer 2007 floods in England and Wales: a hydrological appraisal. Wallingford: Centre for Ecology and Hydrology.
Meisner, A., Rousk, J. and Bååth, E., 2015. Prolonged drought changes the bacterial growth response to rewetting, Soil Biology and Biochemistry 88, 314-322.
Mentzer, J. L., Goodman, R. M. and Balser, T. C., 2006. Microbial response over time to hydrologic and fertilization treatments in a simulated wet prairie, Plant and Soil 284 (1-2), 85-100.
Miyata, S., Kosugi, K. I., Gomi, T., Onda, Y. and Mizuyama, T., 2007. Surface runoff as affected by soil water repellency in a Japanese cypress forest. Hydrological Processes: An International Journal 21(17), 2365-2376.
Mondini, C., Contin, M., Leita, L. and De Nobili, M., 2002. Response of microbial biomass to air-drying and rewetting in soils and compost. Geoderma 105(1-2), 111-124.
More, T. T., Yadav, J. S. S., Yan, S., Tyagi, R. D. and Surampalli, R. Y. (2014). Extracellular polymeric substances of bacteria and their potential environmental applications. Journal of environmental management 144, 1-25.
Murphy, J. A. M. E. S. and Riley, J. P., 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica chimica acta 27, 31-36.
Nesper, M., Bünemann, E. K., Fonte, S. J., Rao, I. M., Velásquez, J. E., Ramirez, B., Hegglin, D., Frossard, E. and Oberson, A., 2015. Pasture degradation decreases organic P content of tropical soils due to soil structural decline, Geoderma 257, 123-133.
Newman, S. and Pietro, K., 2001. Phosphorus storage and release in response to flooding: implications for Everglades stormwater treatment areas. Ecological Engineering 18(1), 23-38.
Nguyen, B. T. and Marschner, P., 2005. Effect of drying and rewetting on phosphorus transformations in red brown soils with different soil organic matter content. Soil Biology and Biochemistry 37(8), 1573-1576.
Nguyen, L. T., Osanai, Y., Anderson, I. C., Bange, M. P., Tissue, D. T. and Singh, B. K., 2018. Flooding and prolonged drought have differential legacy impacts on soil nitrogen cycling, microbial communities and plant productivity. Plant and soil 431(1-2), 371-387.
Novak, J. M. and Watts, D. W., 2006. Phosphorus sorption by sediments in a southeastern coastal plain in-stream wetland. Journal of environmental quality 35(6), 1975-1982.
OECD Guidelines for the Testing of Chemicals. (1993) and Addenda 6-14 to OECD Guidelines for the Testing of Chemicals. (1994-2002). Paris
O'Dell, J. W. (1993). Method 365.1, Revision 2.0: determination of phosphorus by semi-automated colorimetry. EPA-United States Environ Protection Agency, 1-15.
Olsen, S. R., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture; Washington.
Oztas, T. and Fayetorbay, F. (2003). Effect of freezing and thawing processes on soil aggregate stability. Catena 52(1), 1-8.
Page, T., Haygarth, P. M., Beven, K. J., Joynes, A., Butler, T., Keeler, C., Freer, J., Owens, P. N. and Wood, G. A., 2005. Spatial variability of soil phosphorus in relation to the topographic index and critical source areas. Journal of Environmental Quality 34 (6), 2263-2277.

Pan, Y., Bonten, L. T., Koopmans, G. F., Song, J., Luo, Y., Temminghoff, E. J. and Comans, R. N., 2016. Solubility of trace metals in two contaminated paddy soils exposed to alternating flooding and drainage, Geoderma 261, 59-69.

Pant, H. K. and Reddy, K. R., 2003. Potential internal loading of phosphorus in a wetland constructed in agricultural land. Water Research 37(5), 965-972.

Peltovuori, T. and Soinne, H., 2005. Phosphorus solubility and sorption in frozen, air-dried and field-moist soil. European Journal of Soil Science 56(6), 821-826.

Peng, X., Horn, R. and Smucker, A., 2007. Pore shrinkage dependency of inorganic and organic soils on wetting and drying cycles, Soil Science Society of America Journal 71 (4), 1095-1104.

Pezzolla, D., Cardenas, L. M., Mian, I. A., Carswell, A., Donovan, N., Dhanoa, M. S. and Blackwell, M. S., 2019. Responses of carbon, nitrogen and phosphorus to two consecutive drying–rewetting cycles in soils. Journal of Plant Nutrition and Soil Science 182(2), 217-228.

Ponnamperuma, F. N., 1972. The chemistry of submerged soils. In Advances in agronomy 24, 29-96. Academic Press.

Provin, T. L., Wright, A. L., Hons, F. M., Zuberer, D. A. and White, R. H., 2008. Seasonal dynamics of soil micronutrients in compost-amended bermudagrass turf. Bioresource technology 99(7), 2672-2679.

Qiu, S. and McComb, A. J., 1994. Effects of oxygen concentration on phosphorus release from reflooded air-dried wetland sediments. Marine and Freshwater Research 45(7), 1319-1328.

Qiu, S. and McComb, A. J., 2002. Interrelations between iron extractability and phosphate sorption in reflooded air-dried sediments. Hydrobiologia 472(1-3), 39-44.

Rahman, M. M., Hicks, L. C., Verheyen, K., Rousk, J. and Carnol, M., 2018. Effects of drought legacy and tree species admixing on bacterial growth and respiration in a young forest soil upon drying and rewetting, Soil Biology and Biochemistry 127,148-155.

Rakotoson, T., Rabeharisoa, L. and Smolders, E., 2016. Effects of soil flooding and organic matter addition on plant accessible phosphorus in a tropical paddy soil: an isotope dilution study. Journal of Plant Nutrition and Soil Science 179(6), 765-774.

Rapin, A., Grybos, M., Rabiet, M., Mourier, B. and Deluchat, V., 2019. Phosphorus mobility in dam reservoir affected by redox oscillations: An experimental study. Journal of Environmental Sciences 77, 250-263.

Raveh, A. and Avnimelech, Y., 1978. The effect of drying on the colloidal properties and stability of humic compounds. Plant and Soil, 50(1-3), 545-552.

Reddy, K. R., Kadlec, R. H., Flaig, E. and Gale, P. M., 1999. Phosphorus retention in streams and wetlands: a review. Critical reviews in environmental science and technology 29(1), 83-146.

Rehana, S. and Mujumdar, P., 2012. Climate change induced risk in water quality control problems, Journal of Hydrology 444, 63-77.

Rinklebe, J., Shaheen, S. M. and Yu, K., 2016. Release of As, Ba, Cd, Cu, Pb, and Sr under pre-definite redox conditions in different rice paddy soils originating from the USA and Asia, Geoderma 270, 21-32.

Rowell, R.L., 1994. Soil Science: Methods and Applications, Longman, 350.

Sardans, J. and Penuelas, J., 2005. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. Soil Biology and Biochemistry 37 (3), 455-461.

Sardans, J. and Peñuelas, J., 2007. Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest. Functional Ecology 21(2), 191-201.

Schimel, J. P., 2018. Life in dry soils: effects of drought on soil microbial communities and processes. Annual review of ecology, evolution, and systematics 49, 409-432.

Schimel, J. P., Gulledge, J. M., Clein-Curley, J. S., Lindstrom, J. E. and Braddock, J. F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga, Soil Biology and Biochemistry 31 (6), 831-838.

Schimel, J., Balser, T. C. and Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function, Ecology 88 (6), 1386-1394.

Schonbrunner, I. M., Preiner, S. and Hein, T., 2012. Impact of drying and re-flooding of sediment on phosphorus dynamics of river-floodplain systems. Science of the total environment 432, 329-337.

Schulz-Zunkel, C., Rinklebe, J. and Bork, H. R., 2015. Trace element release patterns from three floodplain soils under simulated oxidized–reduced cycles. Ecological Engineering 83, 485-495.

Shaheen, S. M., Frohne, T., White, J. R., DeLaune, R. D. and Rinklebe, J., 2017. Redox-induced mobilization of copper, selenium, and zinc in deltaic soils originating from Mississippi (USA) and Nile (Egypt) River Deltas: A better understanding of biogeochemical processes for safe environmental management. Journal of Environmental Management 186, 131-140.

Shaheen, S. M., Rinklebe, J., Frohne, T., White, J. R. and DeLaune, R. D., 2016. Redox effects on release kinetics of arsenic, cadmium, cobalt, and vanadium in Wax Lake Deltaic freshwater marsh soils, Chemosphere 150, 740-748.

Shaheen, S. M., Rinklebe, J., Rupp, H. and Meissner, R.., 2014a. Lysimeter trials to assess the impact of different flood–dry-cycles on the dynamics of pore water concentrations of As, Cr, Mo and V in a contaminated floodplain soil, Geoderma 228. 5-13.

Shaheen, S. M., Rinklebe, J., Rupp, H., & Meissner, R., 2014b. Temporal dynamics of pore water concentrations of Cd, Co, Cu, Ni, and Zn and their controlling factors in a contaminated floodplain soil assessed by undisturbed groundwater lysimeters. Environmental Pollution, 191, 223-231.

Shenker, M., Seitelbach, S., Brand, S., Haim, A. and Litaor, M. I., 2005. Redox reactions and phosphorus release in re-flooded soils of an altered wetland. European Journal of Soil Science 56(4), 515-525.

Shi, Y., Lalande, R., Hamel, C., Ziadi, N., Gagnon, B. and Hu, Z., 2013. Seasonal variation of microbial biomass, activity, and community structure in soil under different tillage and phosphorus management practices. Biology and fertility of soils 49(7), 803-818.

Six, J. A. E. T., Elliott, E. T. and Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32(14), 2099-2103.

Six, J., Conant, R., Paul, E. and Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils, Plant and Soil 241 (2), 155-176.

Six, J., Elliott, E. T., Paustian, K. and Doran, J. W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62(5), 1367-1377.

Soinne, H., Räty, M. and Hartikainen, H., 2010. Effect of air-drying on phosphorus fractions in clay soil, Journal of Plant Nutrition and Soil Science 173 (3), 332-336

Sondergaard, M., Jensen, P. J. and Jeppesen, E., 2001. Retention and internal loading of phosphorus in shallow, eutrophic lakes. The Scientific World Journal 1, 427-442.

Stocker, T.F., D. Qin, G.-K. Plattner, L.V. Alexander, S.K. Allen, N.L. Bindoff, F.-M. Bréon, J.A. Church, U. Cubasch, S. Emori, P. Forster, P. Friedlingstein, N. Gillett, J.M. Gregory, D.L. Hartmann, E. Jansen, B. Kirtman, R. Knutti, K. Krishna Kumar, P. Lemke, J. Marotzke, V. Masson-Delmotte, G.A. Meehl, I.I. Mokhov, S. Piao, V. Ramaswamy, D.Randall, M. Rhein, M. Rojas, C. Sabine, D. Shindell, L.D. Talley, D.G. Vaughan and S.-P. Xie, 2013: Technical Summary. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change

[Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Storey, K. and Storey, J., 2005. Freeze tolerance, Extremophiles. Eolss Publishers, Oxford, UK, 1-24.

Styles, D. and Coxon, C., 2006. Laboratory drying of organic-matter rich soils: Phosphorus solubility effects, influence of soil characteristics, and consequences for environmental interpretation. Geoderma 136(1), 120-135.

Suda, A., Makino, T., Maejima, Y., Akahane, I. and Higashi, T., 2009. Effects of sterilization on the chemical forms of heavy metals in soils. Soil science and plant nutrition 55(5), 623-626.

Sun, D., Bi, Q., Xu, H., Li, K., Liu, X., Zhu, J., Zhang, Q., Jin, C., Lu, L. and Lin, X., 2017. Degree of short-term drying before rewetting regulates the bicarbonate-extractable and enzymatically hydrolyzable soil phosphorus fractions. Geoderma, 305, 136-143.

Surridge, B. W., Heathwaite, A. L. and Baird, A. J., 2007. The release of phosphorus to porewater and surface water from river riparian sediments. Journal of Environmental Quality 36(5), 1534-1544.

Tack, F., Van Ranst, E., Lievens, C. and Vandenberghe, R., 2006. Soil solution Cd, Cu and Zn concentrations as affected by short-time drying or wetting: The role of hydrous oxides of Fe and Mn, Geoderma 137 (1), 83-89.

Thomson, B. C., Ostle, N. J., McNamara, N. P., Whiteley, A. S. and Griffiths, R. I., 2010. Effects of sieving, drying and rewetting upon soil bacterial community structure and respiration rates. Journal of Microbiological Methods 83(1), 69-73.

Tian, J., Dong, G., Karthikeyan, R., Li, L. and Harmel, R., 2017. Phosphorus dynamics in long-term flooded, drained, and reflooded soils. Water, 9 (7), 531.

Toor, G. S., Condron, L. M., Di, H. J. and Cameron, K. C., 2004. Seasonal fluctuations in phosphorus loss by leaching from a grassland soil. Soil Science Society of America Journal 68(4), 1429-1436.

Turner, B. L.and Haygarth, P. M., 2001. Biogeochemistry: phosphorus solubilization in rewetted soils. Nature 411(6835), 258.

Turner, B. L. and Haygarth, P. M., 2003. Changes in bicarbonate-extractable inorganic and organic phosphorus by drying pasture soils. Soil Science Society of America Journal 67(1), 344-350.

Turner, B. L. and Romero, T. E., 2010. Stability of hydrolytic enzyme activity and microbial phosphorus during storage of tropical rain forest soils. Soil Biology and Biochemistry 42(3), 459-465.

Turner, B. L., Driessen, J. P., Haygarth, P. M. and Mckelvie, I. D., 2003. Potential contribution of lysed bacterial cells to phosphorus solubilisation in two rewetted Australian pasture soils. Soil Biology and Biochemistry 35(1), 187-189.

Unger, I. M., Kennedy, A. C. and Muzika, R. M., 2009. Flooding effects on soil microbial communities. Applied Soil Ecology 42(1), 1-8.

Van Gestel, M., Merckx, R. and Vlassak, K., 1993a. Microbial biomass responses to soil drying and rewetting: the fate of fast-and slow-growing microorganisms in soils from different climates. Soil Biology and Biochemistry 25 (1), 109-123.

Van Gestel, M., Merckx, R. and Vlassak, K., 1993b. Microbial biomass and activity in soils with fluctuating water contents. Geoderma 56 (1), 617-626.

Van Vliet, M. and Zwolsman, J., 2008. Impact of summer droughts on the water quality of the Meuse river, Journal of Hydrology 353 (1), 1-17.

Vance, C. P., Uhde-Stone, C. and Allan, D. L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a non renewable resource. New phytologist 157(3), 423-447.

Vaz, M. R., Edwards, A., Shand, C. and Cresser, M., 1994. Changes in the chemistry of soil solution and acetic-acid extractable P following different types of freeze/thaw episodes, European Journal of Soil Science 45 (3), 353-359.

Voroney, R., 2007. The soil habitat. Soil Microbiology, Ecology, and Biochemistry. 4th Ed. UK: Academic Press.

Walkley, A. and Black, I. A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science 37 (1), 29-38.

Wang, Y., Jensen, C. R. and Liu, F., 2017. Nutritional responses to soil drying and rewetting cycles under partial root-zone drying irrigation. Agricultural Water Management 179, 254-259.

Water Environment (Water Framework Directive) (England and Wales) (Amendment) Regulations 2015. Available online at:http://www.legislation.gov.uk/uksi/2015/1623/pdfs/uksi_20151623_en.pdf (accessed August 7, 2019).)

Wilby, R., Whitehead, P., Wade, A., Butterfield, D., Davis, R. and Watts, G., 2006. Integrated modelling of climate change impacts on water resources and quality in a lowland catchment: River Kennet, UK, Journal of Hydrology 330 (1), 204-220.

Wilson, J. S. and Baldwin, D. S., 2008. Exploring the 'Birch effect'in reservoir sediments: influence of inundation history on aerobic nutrient release. Chemistry and Ecology 24(6), 379-386.

Wright, R. B., Lockaby, B. G. and Walbridge, M. R., 2001. Phosphorus availability in an artificially flooded southeastern floodplain forest soil. Soil Science Society of America Journal 65(4), 1293-1302.

Wu, J. and Brookes, P., 2005. The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil, Soil Biology and Biochemistry 37 (3), 507-515.

Xiang, S., Doyle, A., Holden, P. A. and Schimel, J. P., 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biology and Biochemistry 40 (9), 2281-2289.

Xu, G., Sun, J., Xu, R., Lv, Y., Shao, H., Yan, K., Zhang, L. and Blackwell, M., 2011. Effects of air-drying and freezing on phosphorus fractions in soils with different organic matter contents. Plant, Soil and Environment 57 (5), 228-234.

Yan, X., Wang, D., Zhang, H., Zhang, G., & Wei, Z., 2013. Organic amendments affect phosphorus sorption characteristics in a paddy soil. Agriculture, ecosystems and environment 175, 47-53.

Yan, X., Wei, Z., Wang, D., Zhang, G. and Wang, J., 2015. Phosphorus status and its sorption-associated soil properties in a paddy soil as affected by organic amendments. Journal of soils and sediments 15(9), 1882-1888.

Yanai, Y., Toyota, K. and Okazaki, M., 2004. Effects of successive soil freeze-thaw cycles on soil microbial biomass and organic matter decomposition potential of soils. Soil science and plant nutrition 50(6), 821-829.

Yevdokimov, I., Larionova, A. and Blagodatskaya, E., 2016. Microbial immobilisation of phosphorus in soils exposed to drying-rewetting and freeze-thawing cycles. Biology and Fertility of Soils 52(5), 685-696.

Zhang, B., Yao, S. H. and Hu, F., 2007. Microbial biomass dynamics and soil wettability as affected by the intensity and frequency of wetting and drying during straw decomposition. European Journal of Soil Science 58(6), 1482-1492.

Zhang, H. L., Fang, W., Wang, Y. P., Sheng, G. P., Zeng, R. J., Li, W. W. and Yu, H. Q. (2013). Phosphorus removal in an enhanced biological phosphorus removal process: roles of extracellular polymeric substances. Environmental science and technology 47(20), 11482-11489.

Zhang, W., Faulkner, J. W., Giri, S. K., Geohring, L. D. and Steenhuis, T. S. (2010). Effect of soil reduction on phosphorus sorption of an organic-rich silt loam. Soil Science Society of America Journal 74(1), 240-249.

Zhang, X. and Nearing, M., 2005. Impact of climate change on soil erosion, runoff, and wheat productivity in central Oklahoma, Catena 61 (2), 185-195.

Zhang, Y. and Marschner, P., 2018. Respiration, microbial biomass and nutrient availability are influenced by previous and current soil water content in plant residue amended soil. Journal of soil science and plant nutrition 18(1), 173-187.

Zhao, B., Chen, J., Zhang, J. and Qin, S., 2010. Soil microbial biomass and activity response to repeated drying–rewetting cycles along a soil fertility gradient modified by long-term fertilization management practices. Geoderma 160(2), 218-224.

Zhao, L. Y., Schulin, R., Weng, L. and Nowack, B., 2007. Coupled mobilization of dissolved organic matter and metals (Cu and Zn) in soil columns. Geochimica et Cosmochimica Acta 71(14), 3407-3418.

Zhao, S., Feng, C., Quan, W., Chen, X., Niu, J. and Shen, Z., 2012. Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. Marine pollution bulletin 64(6), 1163-1171.

Zhou, W., Chen, H., Zhou, L., Lewis, B. J., Ye, Y., Tian, J., Li, G. and Dai, L., 2011. Effect of freezing-thawing on nitrogen mineralization in vegetation soils of four landscape zones of Changbai Mountain, Annals of Forest Science 68 (5), 943-951.

Appendix -1

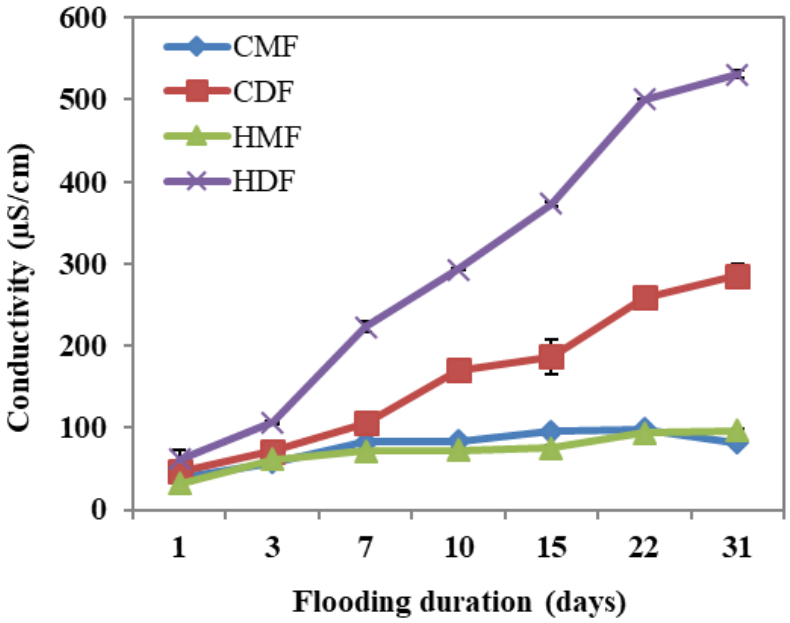

Figure 1: Changes in conductivity (µS/cm) in the water columns of Crediton moist-flooded (CMF), Crediton dry-flooded (CDF), Hallsworth-II moist-flooded (HMF) and Hallsworth-II dry-flooded soils during 31-days flooding period. Error bars represent standard deviation, n = 3. In some cases, error bars are too small to be visible.

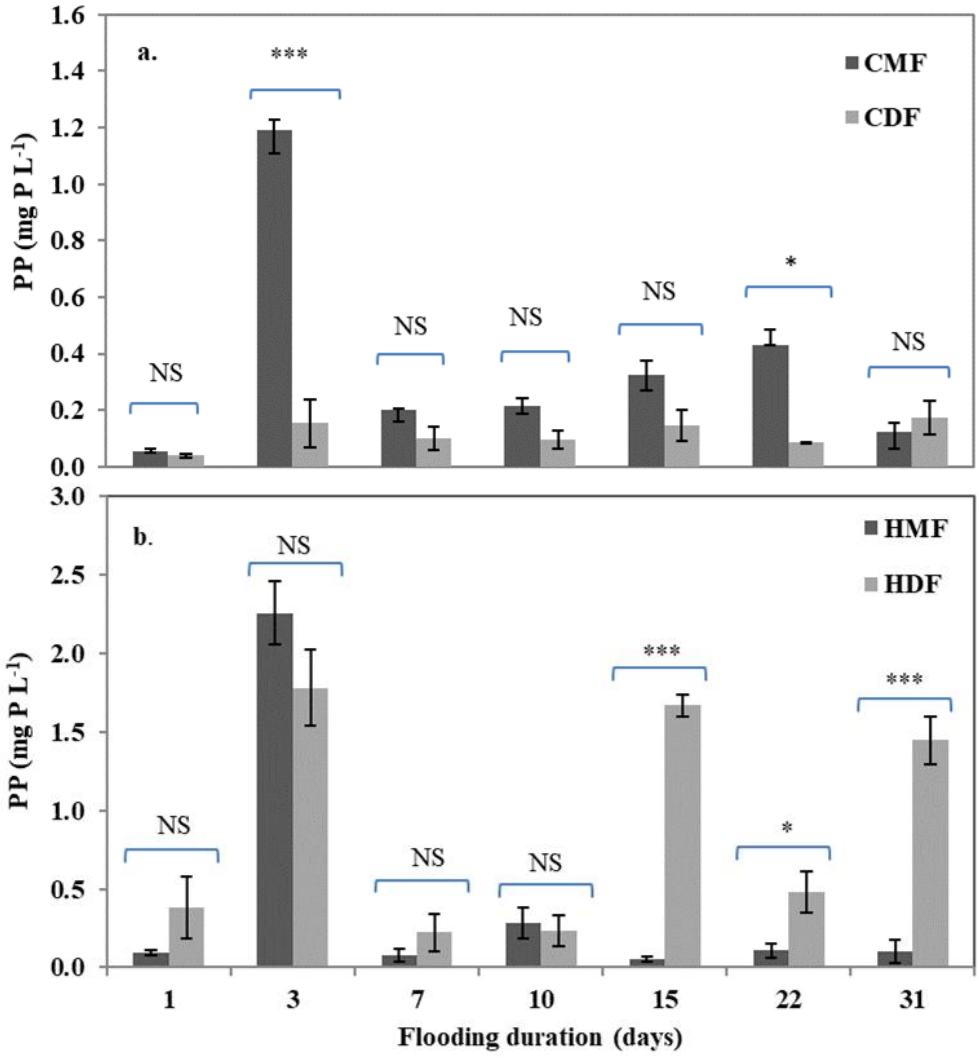

Figure 2a-b: Changes in concentrations of PP (mg P L^{-1}) during 31-days of flooding period (**a**. PP released from Crediton moist-flooded (CMF) and Crediton dry-flooded (CDF) soils and **b**. PP released from Hallsworth-II moist-flooded (HMF) and Hallsworth-II dry-flooded (HDF) soils. Error bars represent standard deviation, n = 3. The means difference is significant at $p < 0.05$ as determined using LSD post-hoc test (***** Significant at $p < 0.05$ probability level, *******Significant at $p < 0.001$ probability level). Where 'NS' means not significant.

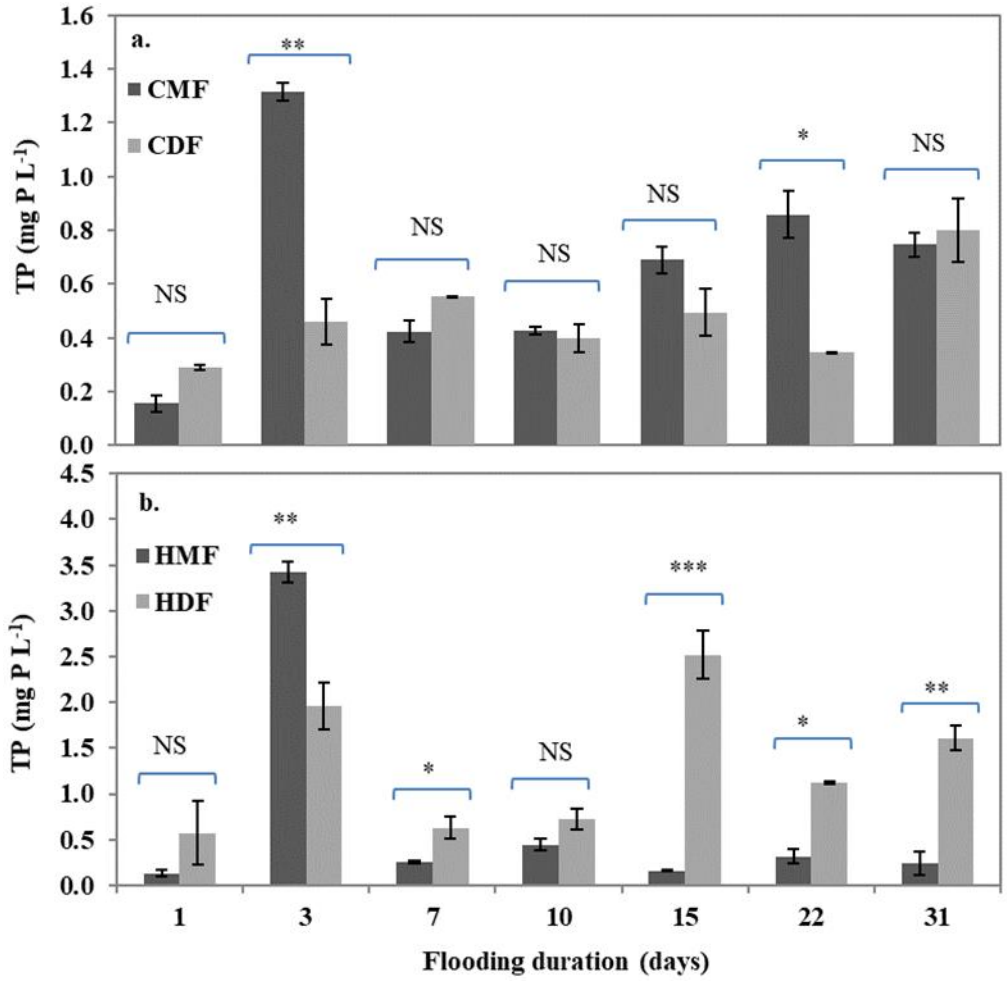

Figure 3a-b: Changes in concentrations of TP (mg P L^{-1}) during 31-days of flooding period (**a**. TP released from Crediton moist-flooded (CMF) and Crediton dry-flooded (CDF) soils and **b**. TP released from Hallsworth-II moist flooded (HMF)and Hallsworth-II dry-flooded (HDF) soils. Error bars represent standard deviation, n = 3. The means difference is significant at $p < 0.05$ as determined using LSD post-hoc test (***** Significant at $p < 0.05$ probability level, ****** Significant at $p < 0.01$ probability level, *******Significant at $p < 0.001$ probability level). Where 'NS' means not significant.