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## Effects of drying and simulated flooding on soil phosphorus dynamics from two contrasting UK grassland soils

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/ejss.13196](https://doi.org/10.1111/ejss.13196)

## Abstract

Flooding is known to mobilise soil phosphorus (P). However, it is still not clear how climate change-driven extended periods of soil drying followed by flooding will affect soil-P dynamics. We tested the hypothesis under laboratory conditions that soil antecedent conditions (moist/dry) determine the amount of P mobilised upon flooding. A series of controlled laboratory experiments were carried out by flooding samples of two contrasting soils, which had each been either dried (40°C for 10-days) or kept at field moisture conditions (25% moisture content). Flooding was simulated by maintaining a 10-cm water-column depth in mesocosms. Periodically collected water samples were analysed for dissolved reactive P (DRP), total dissolved P (TDP) and dissolved unreactive P (DUP). The onset of flooding significantly ( $p < 0.001$ ) increased dissolved concentrations of all forms of P. The release of TDP coincided with a reduction in redox potential, suggesting reductive dissolution of P bearing Fe/Mn minerals as indicated by a significant positive correlation between TDP and dissolved Fe ( $r = 0.430$ ,  $p < 0.001$ ) and TDP and dissolved Mn ( $r = 0.622$ ,  $p < 0.001$ ). Flooding of the dried soils caused a significantly greater increase in the dissolved P concentrations of all forms of P relative to their moist-flooded counterparts. This could be due to a combination of factors which are associated with soil drying and flooding. The Crediton dry-flooded (CDF) soils released higher concentrations of DRP upon flooding (e.g., 0.14 mg P L<sup>-1</sup> on day 1 after flooding) perhaps due to its higher concentrations of water- and NaHCO<sub>3</sub>-extractable P than the Hallsworth dry-flooded (HDF) soil (0.03 mg P L<sup>-1</sup> on day 1 after flooding). However, most of the P in the water column of the dry-flooded soils was unreactive, with the HDF soil releasing higher concentrations of DUP, likely due to its higher organic matter and microbial biomass-P contents.

The results suggest that flooding of dried soils has greater potential to enhance mobilisation of soil-P than flooding of moist soils and thus has potential implications for soil fertility and surface water quality.

**Keywords:** Reductive dissolution, microbial biomass phosphorus, soil organic matter mineralisation, reactive and unreactive dissolved phosphorus

## 1.0 Introduction

The phenomenon of flooding induced mobilisation of nutrients (e.g., P, N, C) has been widely reported (e.g., Bai et al. 2017; Rapin et al. 2019; Kumaragamage et al. 2020; Shaheen et al. 2021). However, studies investigating the effect of flooding on P 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 for large parts of the year and therefore are likely to have microbial populations well adapted to the seasonal fluctuations in soil moisture content. Moreover, in soils experiencing repeated drying-wetting/flooding cycles aggregates may become slake resistant (Denef et al. 2001), thus lowering the risk of nutrient release due to breakdown of aggregates and exposure of new labile organic matter. There is a dearth of studies on P dynamics in soils which generally are not prone to seasonal flooding but could be flooded periodically due to climate change. Soils which are not normally flooded may have a microbial population which is not adapted to sudden changes in moisture. In such soils the microbial biomass may contain large quantities of P embedded in their cellular structure (Zhang et al. 2013), which may be released following flooding because of osmotic shock and cellular lysis if the microbial biomass is not adapted to tolerate sudden changes in soil moisture content. Climate change predictions (IPCC 2018) suggest that in many regions more intense episodic rains will cause soil saturation or flash floods (e.g., Otto et al. 2018). Climate-change is expected to increase run-off and soil erosion, with the increased risk of nutrient (e.g., P) losses in both particulate and dissolved forms from non-point sources (e.g., agricultural land) (Huttunen et al., 2015). Particulate forms in run-off dominate due to physical detachment of particles and colloids in response to abrasive forces applied by flowing surface water. The dynamics (retention and mobilisation) of dissolved forms of P across the soil-water interface in flooded soils are 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; Zhang et al., 2021). This is particularly important because mobilisation and transportation of P from seasonally flooded soils can contribute to eutrophication events (Dodds and Smith 2016).

Soil drying-rewetting is known to increase availability of P (Turner and Haygarth 2001; Turner and Haygarth 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 periods of soil drying

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followed by flooding can mobilise P. The significance of soils and sediments drying/aeration and subsequent re-flooding due to seasonal water level fluctuation in influencing P availability in lakes and wetlands has been widely reported previously (Dieter et al., 2015; Gilbert et al., 2014). Dieter et al. (2015) reported that sediment drying-reflooding increased the proportion of labile and soluble forms of P. However, in this study lake sediments were dried at ambient temperature (20°C) up to 3% residual gravimetric moisture. Similarly, in a recent study Baumann et al. (2020) investigated the effect of changes in soil redox potential due to water table fluctuation on P mobilisation in arable soils. Here, however, water level in the lysimeter was adjusted to mark wet (-25 cm water level depth) and dry (-80 cm water level depth) periods. To the best of our knowledge, no study has yet explored how climate-change predicted soil-drying followed by heavy rains with high risks of flash flooding, can potentially alter P dynamics in soils which generally are not subject to drying-flooding episodes. Climate change predictions suggest that many areas in North-West Europe will become significantly drier than they currently are (IPCC 2018). Extended periods of drought followed by 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 the influence of soil drying-rewetting on P 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-P mobilisation compared to flooding of soils which have not been dried. Moreover, it is still not clear how soils with different texture, organic matter content and concentrations of microbial biomass may respond to drying-flooding scenarios by mobilising P originating from microbial and non-microbial sources. The aim of this study was to test the hypothesis, under laboratory conditions, that (i) soil antecedent conditions determine the amount of P mobilised during flooding events, and (ii) higher quantities of P are mobilised from soils with high organic carbon and microbial biomass P concentrations following drying-flooding. The objectives of this study were to assess: (1) how antecedent soil conditions (moist or dried soil) alter soil P dynamics upon flooding, and (2) how soils with different organic matter, ammonium oxalate extractable Fe-/Al-oxide and microbial biomass P concentrations respond to soil drying-flooding.

## **2.0 Materials and Methods**

### **2.1 Site description, sample collection and preparation**

Bulk samples of the Hallsworth and Crediton series top (0-10 cm) soils were collected in March 2016 from sheep and beef cattle grazed permanent grassland fields located at North Wyke (Devon, UK). The study site is described in Harrod and Hogan (2008). The first soil was a typical non-calcareous soil of the Hallsworth series (FAO stagni-vertic cambisol) (hereafter referred to as Hallsworth soil) and the second soil was a typical brown earth (FAO dystic cambisol) (hereafter referred to as Crediton soil). Field moist soils were passed through a 2 mm sieve, removing all non-soil material (grass, roots, earthworms and stones). For the purpose of analysis, sub-samples of the soils were oven-dried at 40°C till no further moisture loss occurred.

## **2.2 Flooding experiment design**

Moist samples of the Hallsworth and Crediton series soils were oven-dried for 10-days at 40°C. The soils were weighed before and after drying to measure the moisture loss, and subsequently 84 replicates comprising 150 g dry-weight equivalent (DWE) of soil (3 replicates for each sampling day x 7 sampling days x 4 treatments (Hallsworth moist-flooded (HMF), Hallsworth dry-flooded (HDF), Crediton moist-flooded (CMF) and Crediton dry-flooded (CDF)), were placed in 500-ml polypropylene, translucent, wide neck bottles (hereafter referred to as mesocosms). The control counterparts of these soils (a further 84 moist soil samples) were similarly prepared and stored moist (25% moisture content) at 3°C and maintained at room temperature (21°C) for one day before subjecting to flooding. Soil was gently packed (1.1 g/cm<sup>3</sup>, similar to these top-soils' typical bulk density) in the mesocosms and flooded with 400 ml of deionised water. Water columns were maintained to a depth of 10 cm throughout the flooding duration. Surface water samples (supernatant) were collected (~100 ml/replicate) using syringes on day 1, 3, 7, 10, 15, 22 and 31 after flooding by sacrificing 3 replicates of each treatment on each sampling day. To compensate for any water loss by evaporation during the flooding period (31-days), the level in the mesocosm was maintained daily by replenishing with deionised water.

## **2.3 Laboratory analysis**

Redox potential and pH were measured at the soil-water interface periodically throughout the flooding duration, using a redox/ORP electrode (Thermo scientific Orion metallic combination electrode) and a pre-calibrated pH meter (Oakton™ pH 700 Benchtop Meter). The water samples were filtered (Whatman 0.45 µm cellulose nitrate membrane) immediately after

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collection and analysed for DRP using the Murphy and Riley (1962) method. For the determination of TDP, filtered water samples were digested using the persulfate digestion method (O'Dell 1993) using a mixture of H<sub>2</sub>SO<sub>4</sub> (11 N) and ammonium persulfate, followed by analysis using the Murphy and Riley (1962) method. Dissolved unreactive phosphorus (DUP) was calculated as the difference between TDP and DRP. For the determination of total dissolved concentrations of Fe, Mn and Al, filtered (Whatman 0.45 µm cellulose nitrate membrane) water samples were acidified (Aristar HNO<sub>3</sub>; 2-3 drops per 50 ml sample) and analysed by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700 series) following standard protocols.

Soils were characterised (Table 1) using standard procedures and quality assurance protocols. 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 was determined gravimetrically by oven-drying soil samples at 105°C for 16 hours. Soil texture was determined by the hydrometer method following pre-treatment with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), as outlined in Rowell (1994). Soil organic carbon was determined by the Walkley and Black dichromate oxidation method as described in Carter and Gregorich (2007). Soil total phosphorus (TP) was determined by digesting finely ground soil in perchloric acid (HClO<sub>4</sub>). The digests were filtered (Whatman 541), diluted to 250 ml with de-ionised water and P in the digestates was determined by ICP-AES (Carter and Gregorich 2007). For the determination 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<sub>2</sub>SO<sub>4</sub>) before analysing them on ICP-AES (Carter and Gregorich 2007). Total organic P was then measured as the difference between ignited and unignited samples.

Water extractable P was determined as described by Carter and Gregorich (2007). Briefly, 20 ml of DI water was added to 2 g DWE soil, and the mixture was then shaken for 1 hour, followed by centrifugation at 3000 g, filtration (Whatman No. 42) and determination of P colorimetrically (Murphy and Riley 1962). Bicarbonate extractable P (Olsen-P) was measured based on the method described by Olsen et al. (1954) as outlined in Carter and Gregorich (2007). Briefly, 5 g DWE (dry weight equivalent) soil was extracted in 100 ml 0.5 M NaHCO<sub>3</sub> 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 (0.25M H<sub>2</sub>SO<sub>4</sub>), and P concentrations determined colorimetrically (Murphy and Riley, 1962). Microbial biomass P was measured in both moist and dry soil replicates using the 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 2007) and the metals (Fe, Mn, and Al) were analysed by ICP-AES (Jobin Yvon Horiba – ULTIMA 2C / 2CE).

## 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 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<sub>10</sub> transformation as they were not found to follow a normal distribution. The significance of difference between individual means was determined by one-way ANOVA (significance at  $p < 0.05$ ). Tukey's post-hoc test was employed for multiple comparisons. Three-way ANOVA was also performed to find out the effect of interaction between independent variables (flooding duration, soil antecedent condition and soil type) and dependent measured parameters in water samples (Table 2). The data are reported as means ( $n = 3$ )  $\pm$  standard deviation. Pearson's correlation analysis was performed to explore relationships among the parameters (Table 3). Analysis of variance and correlation analysis were performed using SPSS (IBM SPSS statistics 26).

**Table 1**  
Initial soil properties of the Hallsworth and Crediton soils

Soil Properties	Hallsworth	Crediton
pH	5.13	5.41
% Organic carbon	3.65	1.08
Water extractable DRP, mg P kg <sup>-1</sup>	13.45	21.63
Sodium bicarbonate extractable P, mg P kg <sup>-1</sup>	26	42
Total soil P, mg P kg <sup>-1</sup>	1283	883
Total organic P, mg P kg <sup>-1</sup>	725	396
Sand, %	35	77
Silt, %	46	12
Clay, %	19	11
Moist soil microbial biomass P, mg P kg <sup>-1</sup>	68	14
Dry soil (40°C, 10 days) microbial biomass P, mg P kg <sup>-1</sup>	11.43	7.45
Fe-ox <sup>a</sup> , %	1.06	0.47
Mn-ox <sup>a</sup> , %	0.04	0.09
Al-ox <sup>a</sup> , %	0.02	0.01

<sup>a</sup>Acid ammonium oxalate extractable metal (Fe/Mn/Al) oxides. DRP, dissolve reactive phosphorus

## 3.0 Results and Discussion

### 3.1 Soil Characterisation



Initial physical and chemical properties of the two grassland soils used in the flooding experiment are presented in Table 1. Both soils were acidic ( $\text{pH} < 6$ ). The organic carbon and clay contents of the Hallsworth soil were higher than those of the Crediton soil, reflecting the better ability of Hallsworth soil to retain nutrients (e.g., P) by increasing sorption capacity (Bruland and Rishardson 2006; Bruland and Dement 2009). However, the  $\text{NaHCO}_3$  extractable P and water extractable P concentrations in the Crediton soil were higher than those in the Hallsworth soil (Table 1), indicating a higher bio-available P status of the Crediton soil. The microbial biomass P of the moist Hallsworth soil was about 5 times higher than that of the moist Crediton soil which perhaps is at least partly due to its higher organic carbon content (Table 1). A marked reduction in microbial biomass P occurred in both soils upon drying ( $40^\circ\text{C}$  for 10-days) from  $68 \pm 1 \text{ mg P kg}^{-1}$  to  $11.43 \pm 1.73 \text{ mg P kg}^{-1}$  (83% decrease) and from  $14 \pm 4 \text{ mg P kg}^{-1}$  to  $7.45 \pm 1.58$  (47% decrease) in the Hallsworth and Crediton soils, respectively (Table 1). This drying induced reduction in microbial biomass as indicated by the decrease in microbial biomass P could contribute to increased P in the water column when these soils are flooded. The detrimental effect of soil drying on microbial biomass has been reported in many drying-rewetting experiments (e.g., Khan et al. 2019; Pezzolla et al. 2019). Microbial cells may accumulate large amounts of P into their cellular structures (Zhang et al. 2013) and the species which are not adapted to tolerate these stresses release P into the soil solution due to osmotic shock and cell lysis upon rewetting.

### 3.2 Flooding induced changes in redox potential and pH

Soil flooding duration caused a significant reduction in redox potential ( $p < 0.001$ ; Table 2), ranging from 390 mV to -57 mV and 246 mV to -230 mV for the CMF and CDF treatments, and from 281 mV to -114 mV and -9 mV to -282 mV for HMF and HDF, respectively (Figure 1a). Both HDF and 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 mineralisation of organic matter and dead microbial biomass, because redox potential of soils well supplied with organic matter drops rapidly due to increased availability of oxidizable organic carbon for microbial decomposition and concomitant  $\text{O}_2$  depletion (Kogel-knabner et al. 2010; Jayalath et al. 2016). The decrease in redox potential was larger in the HDF soil than in the CDF soil (Figure 1a), reflecting the higher organic matter and microbial biomass concentrations in this soil.

The onset of flooding caused an increase in soil pH in all treatments (Figure 1b). 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 to 7.1 respectively. The pH increase upon flooding was larger in the dry-flooded soils relative to the moist-flooded counterparts. The increase in pH was larger in HDF soil than in CDF soil (Figure 1b). 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 most likely caused by increased availability of labile organic matter when the dried soil is flooded. This is due to the detrimental effects of soil drying on organic matter and microbial biomass. The availability of oxidizable organic carbon can stimulate an increase in pH due to increased proton consumption under reducing conditions, because many anaerobes are heterotrophic (Jayalath et al. 2016).

### **3.2 Effect of soil antecedent conditions on solubilisation of phosphorus**

Antecedent soil conditions (moist or dry) prior to flooding had a highly significant effect ( $p < 0.001$ ) on mobilisation of all forms of P (DRP, DUP and TDP) in the water column (Table 2). The DRP concentrations in the floodwater of the HDF soils were significantly ( $p < 0.001$ ) higher (ranging from  $0.031 \text{ mg P L}^{-1}$  on day 1 after flooding to  $0.223 \text{ mg P L}^{-1}$  on day 15 after flooding) than in their moist-flooded counterparts (ranging from  $0.014 \text{ mg P L}^{-1}$  on day 1 after flooding to  $0.028 \text{ mg P L}^{-1}$  on day 15 after flooding). The DRP concentrations in the floodwater of the CDF soil also increased relative to the CMF soil until day 10 after flooding, beyond which DRP concentration showed a declining trend (Figure 2a-b). The DUP concentrations in the CDF floodwater were significantly ( $p < 0.05$ ) higher than the CMF counterparts except on day 15 and 22 after flooding where the differences in the mean concentrations were not significant ( $p > 0.05$ ) (Figure 3a). Similarly, DUP concentrations in the HDF floodwater were significantly ( $p < 0.001$ ) higher than in the HMF floodwater except for day 31 after flooding when the difference in the mean concentrations was not significant ( $p > 0.05$ ) (Figure 3b). The TDP concentrations in the CDF floodwater were significantly ( $p < 0.05$ ) higher than those in the CMF floodwater until day 7 after flooding beyond which TDP concentrations showed a declining trend (Figure 4a-b). The TDP concentrations in the HDF floodwater were significantly ( $p < 0.001$ ) higher than in the HMF counterpart except on day 31 after flooding when the difference was not significant (Figures 5a-b). Higher concentrations of all forms of P in floodwater over dried soils could be due to greater destruction of organic matter and microbial biomass during drying induced dehydration (Turner and Haygarth 2003; Sun et al.

2017; Brodlin et al. 2019). Drying induced loss of sorption capacity in previously dried soils could also be a possible cause of elevated dissolved P concentrations in the water column, since reduction in P sorption capacity as a result of increased crystallisation of minerals (mineral-aging) prevents sorption of P originating from microbial cell lysis 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 change may also have played a role (see section 3.3).

These results suggest that if soils are dried, they are likely to release larger amounts of P with the onset of flooding, thus supporting the first hypothesis that soil antecedent conditions (moist/dry) determine the amount of P mobilised upon the onset of flooding. The DRP concentrations measured in the floodwater, particularly when the soils were dried prior to flooding are higher than the revised standard (40 µg P/L) required to achieve good ecological status in lowland low alkalinity rivers (UK TAG, 2013). In the field the nutrients released may be transported to surface waterbodies. However, if the soil is moist the slower changes in redox upon flooding may mean the mobilisation of P is reduced. It is also possible that under natural field conditions the moist flooded soil may not cause substantial transportation of P as the water drains-off and mobilised P is re-precipitated/sorbed in the crystal lattice of metal oxides, reducing its transportation. However, if dry soils are flooded, the faster onset of reducing conditions is likely to result in greater mobilisation of P.

In the case of the HDF treatment, DRP was not detected in the flood water samples collected on the third day after flooding (Figure 2b). Phosphorus sorption by freshly exposed surfaces of soil minerals could have played a role in DRP removal from the water column on the third day of flooding. Secondly, microbial community shift with the prevalence of more stable and resilient microbial species (Randle-Boggis et al. 2018) and assimilation of P released in the water column from other less adapted members of the microbial community during cell lysis and organic matter mineralisation could also be a reason why DRP was not detected on the third day after flooding.

### **3.3 Effect of redox on phosphorus solubilisation**

Flooding duration had a highly significant effect ( $p < 0.001$ ) on the mobilisation of all forms of P (DRP, DUP and TDP) (Table 2). The increase in TDP showed the same trend as the increase in total dissolved Fe and Mn (Figures 4a-b and 5a-b). The increase in P concentration

is likely reflective of reductive dissolution of metal (Fe/Mn) oxides as is evident by the highly significant ( $p < 0.001$ ) positive correlation between total dissolved P and metals (Fe/Mn) (Table 3). However, in the case of the CDF treatment, Mn demonstrated an irregular trend which was not similar to the TDP pattern (Figure 4b). Metal oxy/hydroxides are well recognised as P sorbents (Amarawansa et al., 2015; Sun et al., 2017; Kumaragamage et al., 2020; Shaheen et al., 2021). Iron ( $\text{Fe}^{+3}$ ) is redox sensitive and reduces to  $\text{Fe}^{+2}$  with the development of reducing conditions, subsequently releasing previously sorbed P 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-organic matter- $\text{PO}_4$  (Al-OM- $\text{PO}_4$ ) complexes (Darke and Walbridge et al. 2000). The significant positive correlation ( $p < 0.05$ ) (Table 3) between total dissolved concentrations of Al and P indicates that non-reductive dissolution of P bearing Al minerals (Al-OM- $\text{PO}_4$ ) may have also been partly responsible for P release to the water column. However, the relationship between TDP and Al was less robust (Table 3) compared to that with Fe and Mn (Table 3), which perhaps is reflective of their predominant role in P solubilisation due to reductive dissolution.

The increase in TDP concentration in floodwater was also concurrent with the increase in pH in all treatments as indicated by the highly significant positive correlation between TDP and pH ( $p < 0.01$ ) (Table 3). This could be because the rise in pH under anaerobic conditions reduces the P binding capacity of Fe and Al minerals primarily due to ion-exchange reactions in which hydroxide ( $\text{OH}^-$ ) replaces orthophosphate ( $\text{PO}_4^{3-}$ ) (Sun et al., 2017).

Another factor contributing to P release following flooding could be the release of microbial biomass P either through microbial decay under anaerobic condition e.g., fungi are more sensitive to flooding induced anaerobic conditions relative to the bacterial biomass (Drenovsky et al. 2004; Mentzer et al. 2006; Voroney 2007), or as a protective mechanism whilst shifting between oxic-anoxic environments (Wright et al. 2001; Hupfer and Lewandowski 2008). Under oxic conditions and adequate nutrient supply, microorganisms assimilate dissolved P. However, as conditions turn anaerobic, this previously stored P is released back into the soil solution (Wright et al. 2001; Khoshmanesh et al. 2002).

The CDF treatment floodwater had higher concentrations of TDP relative to the HDF treatment floodwater during the initial stages of flooding (up to day 7 after flooding). However, from day 7 onwards the HDF treatment, whose soil had higher oxalate-extractable Fe/Al and

organic matter contents had higher concentrations of TDP in the floodwater (Figures 5b). Phosphorus sorption capacities have been known to be significantly positively correlated with concentrations of oxalate-extractable Fe and Al (amorphous) oxides (Darke and Walbridge, 2000). Metal oxy/hydroxides are important in fixing P in soils. However, in flooded soils, with the development of reducing conditions, one of the strongest phosphate binders, Fe<sup>+3</sup> oxy/hydroxide, becomes unstable and results in reductive dissolution of Fe<sup>+3</sup> and release of previously sorbed P (Rapin et al. 2019). Nevertheless, the oxidation state of Al 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 to releasing P upon flooding as stated above through a number of mechanisms - blocking or occluding P binding sites on mineral surfaces by organic matter (Dieter et al. 2015), competing with phosphate anions 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 P by these mechanisms cannot be ruled out.

**Table 2**

Summary statistics of three-way ANOVA

Assessment of the effects of independent factors (flooding-duration, soil antecedent condition and soil type) on the dependent measured parameters (pH, redox, DRP, DUP and TDP) in water column

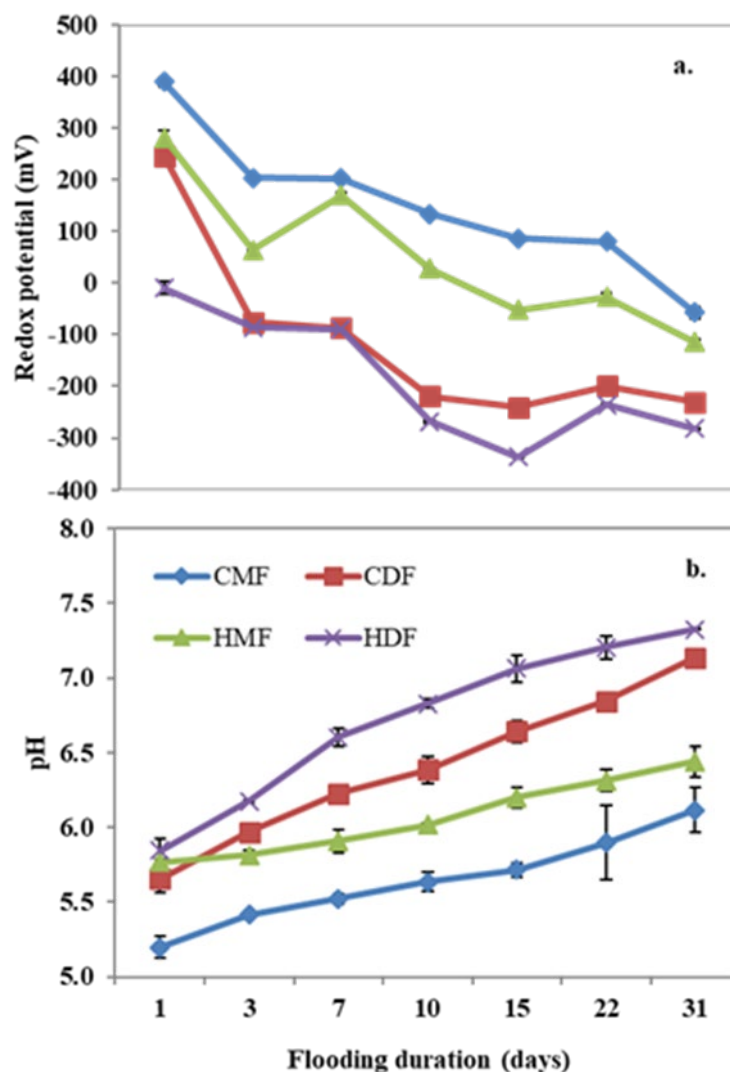
<b>Tests of between subjects effects</b>	<b>pH</b>	<b>Redox</b>	<b>DUP</b>	<b>DRP</b>	<b>TDP</b>
<b>Source</b>	<b>F</b>	<b>F</b>	<b>F</b>	<b>F</b>	<b>F</b>
Flooding duration	492 <sup>***</sup>	2466 <sup>***</sup>	173 <sup>***</sup>	142 <sup>***</sup>	100 <sup>***</sup>
Soil antecedent condition	2573 <sup>***</sup>	14572 <sup>***</sup>	1011 <sup>***</sup>	213 <sup>***</sup>	449 <sup>***</sup>
Soil type	763 <sup>***</sup>	1743 <sup>***</sup>	0.004 NS	2020 <sup>***</sup>	95 <sup>***</sup>
Flooding duration x Soil antecedent condition	52 <sup>***</sup>	102 <sup>***</sup>	16 <sup>***</sup>	56 <sup>***</sup>	24 <sup>***</sup>
Flooding duration x Soil type	6 <sup>***</sup>	78 <sup>***</sup>	148 <sup>***</sup>	48 <sup>***</sup>	76 <sup>***</sup>
Soil antecedent condition x Soil type	16 <sup>***</sup>	27 <sup>***</sup>	120 <sup>***</sup>	722 <sup>***</sup>	165 <sup>***</sup>
Flooding duration x Soil antecedent condition * Soil type	5 <sup>**</sup>	52 <sup>***</sup>	62 <sup>***</sup>	276 <sup>***</sup>	50 <sup>***</sup>

Data on pH and redox did not require transformation prior to the analysis as the data were normally distributed. However, the values for DRP, DUP, and TDP were normalised using a  $\log_{10}$  transformation prior to analysis

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

\*\*\* Significant at  $p < 0.001$

NS, Not significant ( $p > 0.05$ )



**Figure 1a-b:** Flooding induced variations in soil redox potential and pH (a. variation in redox potential of Credition moist-flooded (CMF), Credition dry-flooded (CDF), Hallsworth moist-flooded (HMF) and Hallsworth dry-flooded (HDF) treatments, b. Variation in pH of CMF, CDF, HMF and HDF treatments). Error bars represent  $\pm$  standard deviation,  $n = 3$ . In some cases, error bars are too small to be visible.

### 3.4 Flooding induced variations in phosphorus forms

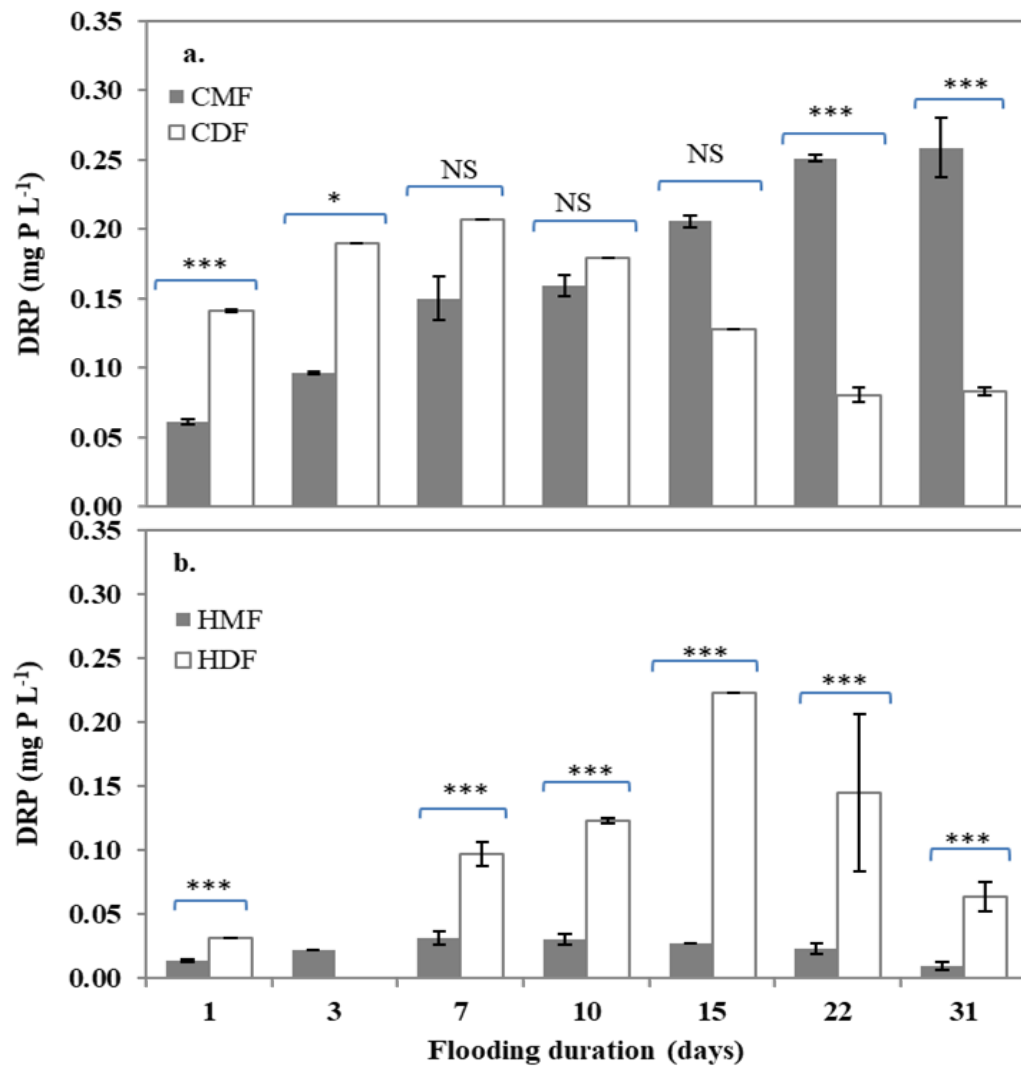
The various forms of P in the flood water of all treatments were compared. The results showed that the CMF and CDF soils generally released higher concentrations of DRP into the water column than the HMF and HDF soils which was perhaps due to the higher concentrations of water and  $\text{NaHCO}_3$  extractable P fractions in the Credition soil, indicative of easily bio-available (soluble) P upon rewetting (Table 1). Another factor which may have caused relatively less mobilisation of DRP from the HMF and HDF soils could be the higher organic matter and clay contents of this soil (Table 1), which may have re-sorbed some of the DRP

released from microbial cell lysis and reductive dissolution of P-bearing minerals. Also soils 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.

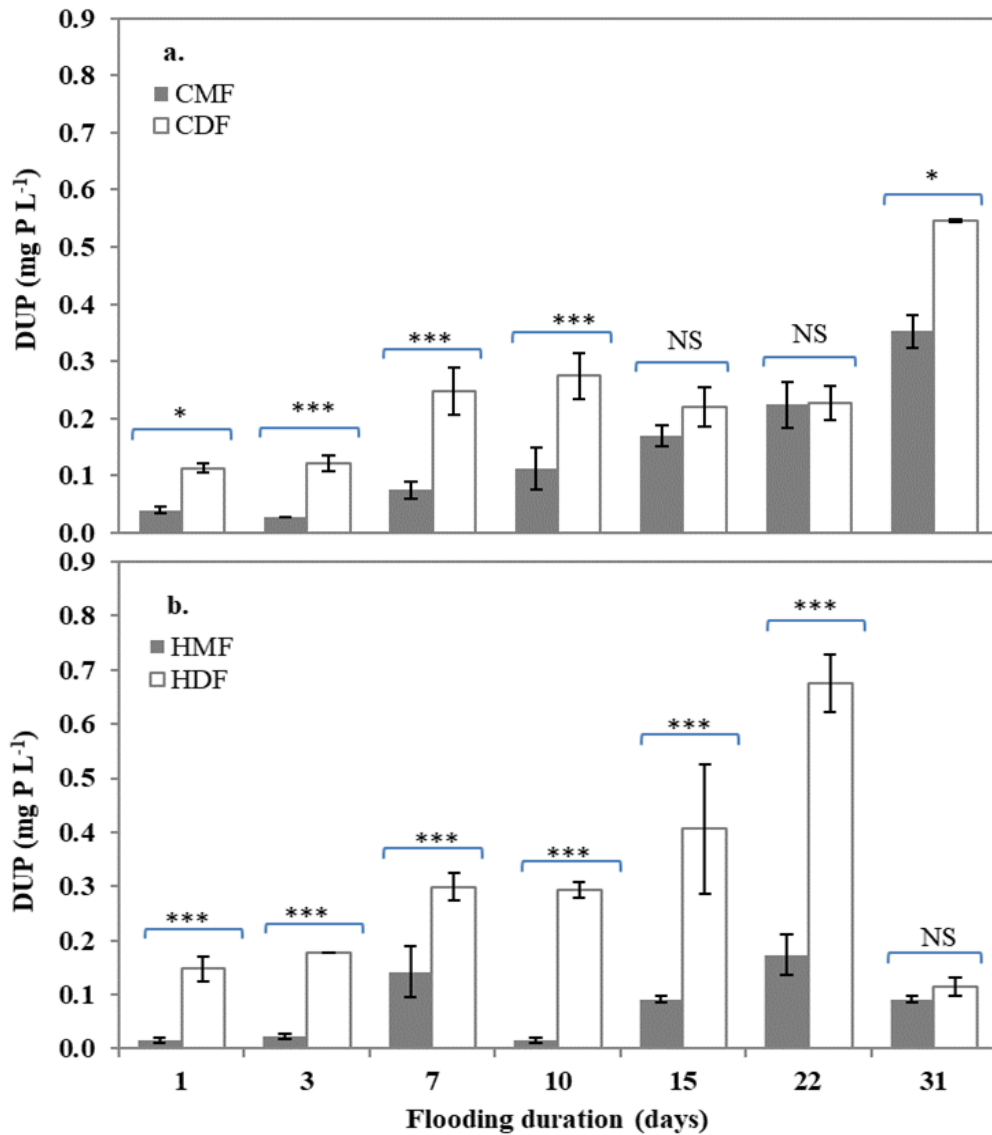
Most of the P in the floodwater of the dry-flooded soils was unreactive with the HDF soil releasing larger quantities of DUP relative to the CDF soil (Figure 3a-b), most probably due to greater detrimental impact of soil drying on organic matter and microbial biomass P in the Hallsworth soil relative to that of the Crediton soil (Table 1), because unreactive P mostly comprises organic forms of P, usually derived from microbial lysis (Blackwell et al. 2013; Gu et al. 2018). These results support our second hypothesis that soils with greater % organic carbon and microbial biomass P (in this study the Hallsworth soil) tend to release greater concentrations of P following drying-flooding processes (Table 1). It is further supported by the reduction in microbial biomass P due to soil drying (40°C; 10 days) which was larger in the case of Hallsworth dry soil (-83%) relative to that of the Crediton dry soil (-47%) (Table 1).

Nevertheless, in both CDF and HDF treatments, DRP and DUP floodwater concentrations declined after attaining their maximum concentrations (Figures 2 and 3). However, in CDF treatment DUP floodwater concentration gradually declined up to day 22 after flooding followed by an increase (Figure 3a). Studies demonstrating initial increases in P concentrations in the water column upon flooding have also reported decreases in P concentrations at later stages (Chacon et al. 2005; Amarawansa et al. 2015; Tian et al. 2017), due to re-sorption by soil minerals, microbial uptake and/or re-precipitation of Fe-/Mn-oxide as they diffuse into the water column across the anoxic-oxic zone, resulting in sorption of P (Peltovuori and Soinne 2005).





**Figure 2a-b:** Changes in floodwater DRP concentrations ( $\text{mg P L}^{-1}$ ) during 31-days flooding period (**a.** DRP in the floodwater of Crediton moist-flooded (CMF) and dry-flooded (CDF) treatments and **b.** DRP in the floodwater of Hallsworth moist-flooded (HMF) and Hallsworth dry-flooded (HDF) treatments. Error bars represent  $\pm$  standard deviation,  $n = 3$ . The means difference is significant at  $p < 0.05$  as determined using Tukey's post-hoc test (\* Significant at 0.05 probability level, \*\* Significant at 0.01 probability level, \*\*\*Significant at 0.001 probability level). Where 'NS' means not significant. DRP was not detected in the floodwater of HDF treatment on the third day of flooding.



**Figure 3a-b:** Changes in floodwater DUP (dissolved unreactive phosphorus) concentrations ( $\text{mg P L}^{-1}$ ) during 31-days flooding period (**a.** DUP in floodwater of Crediton moist-flooded (CMF) and Crediton dry-flooded (CDF) treatments, **b.** DUP in floodwater of Hallsworth moist-flooded (HMF) and Hallsworth dry-flooded (HDF) treatments. Error bars represent  $\pm$  standard deviation,  $n = 3$ . Where, 'NS' means not significant. The means difference is significant at  $p < 0.05$  as determined using Tukey's 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 3**

Pearson's correlation coefficients comparing the relationship among the studied parameters in floodwater of the Hallsworth and CREDITON moist and dry-flooded treatments.

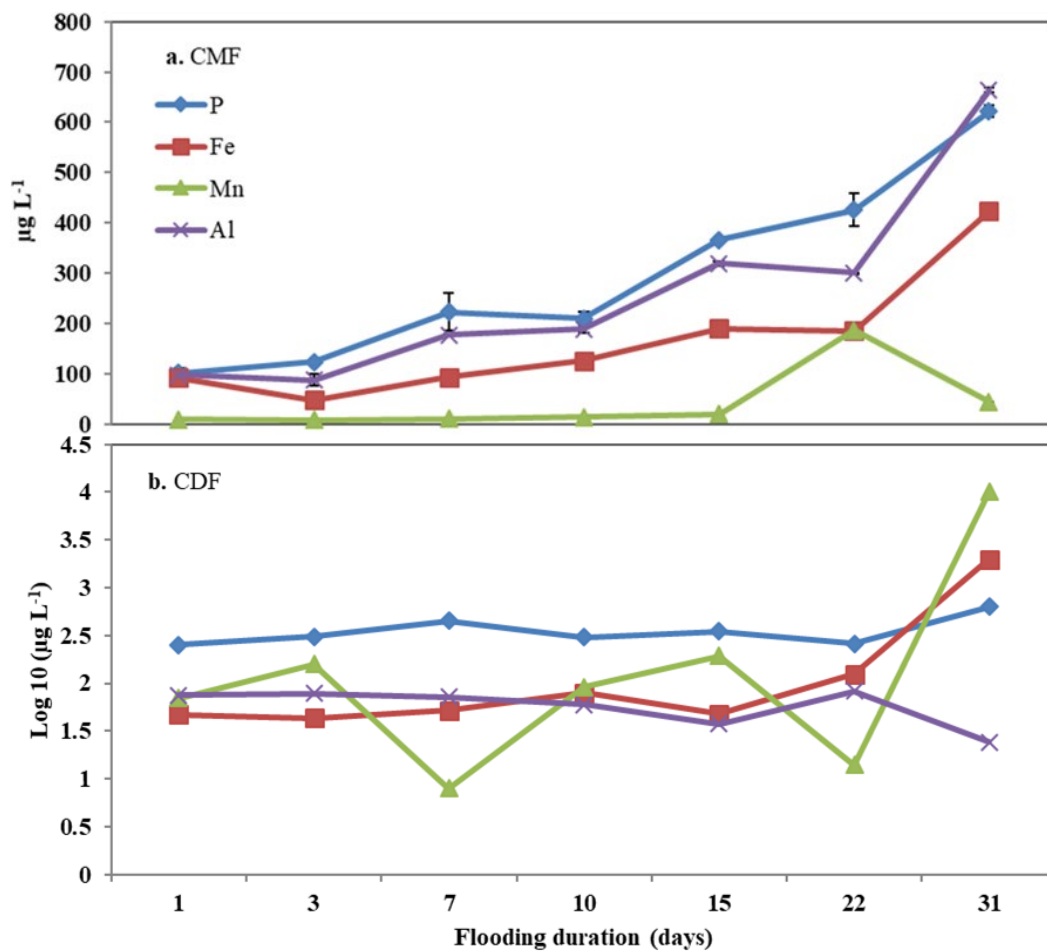
	<b>pH</b>	<b>Redox</b>	<b>DUP</b>	<b>TDP</b>	<b>Fe</b>	<b>Mn</b>	<b>Al</b>
Redox	-0.586***						
DUP	0.369**	-0.660***					
TDP	0.330**	-0.542***	0.886***				
Fe	0.541***	-0.565***	0.491***	0.430***			
Mn	0.511***	-0.684***	0.678***	0.622***	0.755***		
Al	NS	NS	NS	0.218*	NS	NS	
DRP	NS	NS	0.527***	0.728***	NS	0.306**	0.348**

\*\*\* Significant at  $p < 0.001$

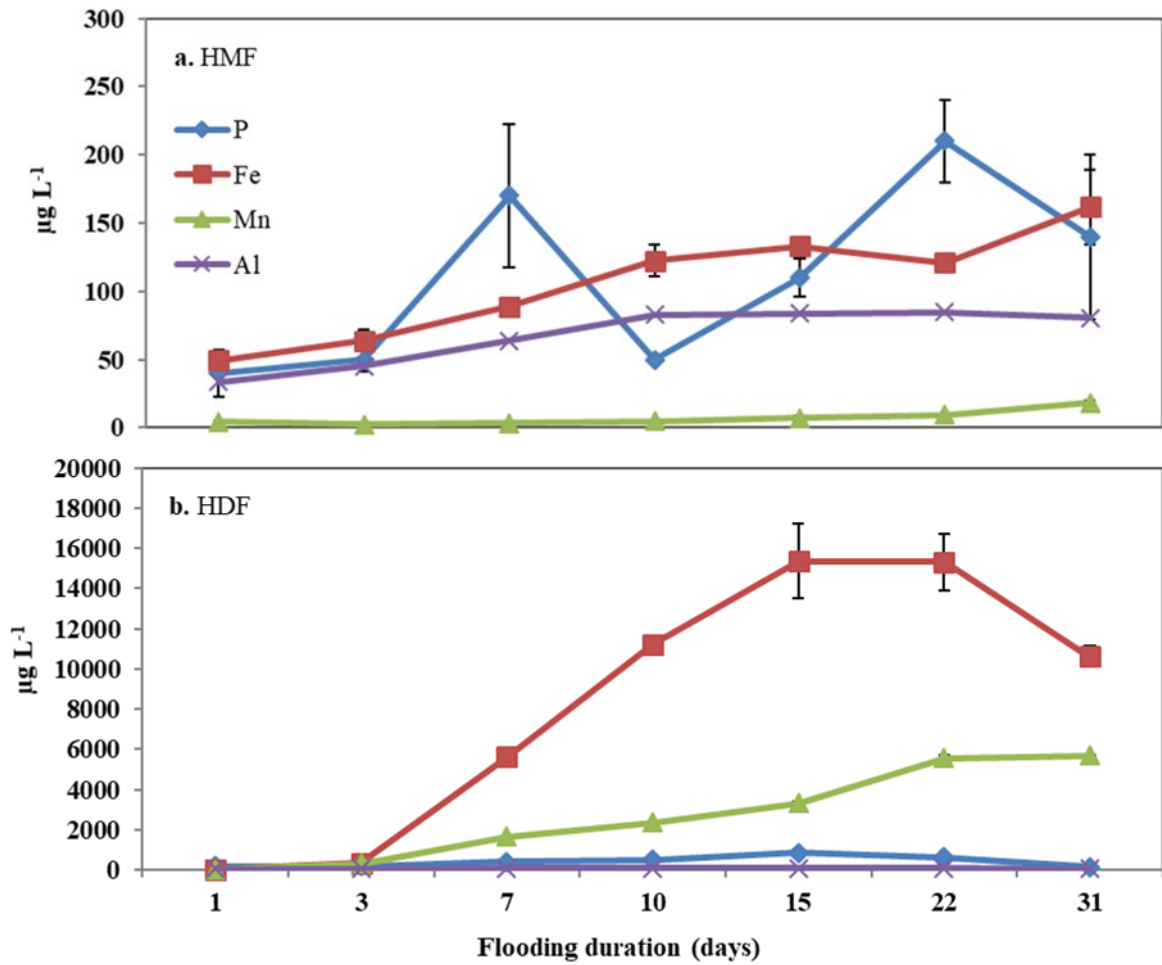
\*\*Significant at  $p < 0.01$

\*Significant at  $p < 0.05$

NS, Not significant ( $p > 0.05$ )



**Figure 4a-b:** Changes in floodwater total dissolved concentrations ( $\mu\text{g L}^{-1}$ ) of P, Fe, Mn, and Al during 31-days flooding period, **a.** Crediton moist-flooded (CMF) and **b.** Crediton dry-flooded (CDF) treatments. Error bars represent  $\pm$  standard deviation,  $n = 3$ . In some cases, error bars are too small to be seen. In case of chart 'b' data were log transformed ( $\text{Log}_{10}$ ) as some of the concentrations were too small while others too high.



**Figure 5a-b:** Changes in floodwater total dissolved concentrations of P, Fe, Mn, and Al during 31-days flooding period, **a.** Hallsworth moist flooded (HMF) and Hallsworth dry-flooded (HDF) treatments. Error bars represent  $\pm$  standard deviation,  $n = 3$ . In some cases, error bars are too small to be seen.

### 3.5 Conclusion

The onset of flooding increased dissolved concentrations of all forms of P in floodwater. The increase in floodwater P concentration 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 the observed elevated dissolved concentrations of P. This was further supported by a strong significant positive correlation between TDP and dissolved Fe ( $r = 0.430$ ,  $p < 0.001$ ) or TDP and dissolved Mn ( $r = 0.622$ ,  $p < 0.001$ ). Upon flooding the soils which were previously dried released significantly greater ( $p < 0.05$ ) concentrations of P into the 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 DUP concentrations measured in the floodwater of the HDF treatment were generally higher than the CDF treatment throughout the flooding duration, possibly due to the higher organic matter (OM) and microbial biomass P concentrations in this soil. However, the DRP concentrations in the floodwater of the CDF treatment remained generally higher than the DRP concentrations in the floodwater of the HDF treatment possibly due to higher water- and bicarbonate-extractable P concentrations in this soil, indicative of easily soluble P upon rewetting/flooding. Once in solution, P can either be resorbed by non-redox sensitive minerals/elements (e.g., Al, Ca, Mg) or transported to surface water through runoff. The results suggest that soil drying followed by flooding has the potential to promote increased mobilisation of soil-P compared to flooding of moist soils and thus has implications for soil fertility, crop-plant productivity, and surface water quality. The loss of P from flooded agricultural soils is mainly attributed to redox/pH driven reductive dissolution of P from Fe/Mn oxides. The soils used in this study were acidic ( $\text{pH} < 6$ ). Acidic soils because of their tendency for pH to increase under flooding are known to pose greater threats to aquatic ecosystems by promoting eutrophication (Zhang et al., 2021) compared to alkaline soils under reducing conditions by mobilising phosphorus associated with Fe/Mn minerals. Therefore, the results obtained from this study will help understand the processes and mechanisms (controlling P mobilisation from Fe/Mn system under reducing condition) required for effective agricultural practices, management of P loss and abating water pollution e.g., maintaining soils in a moist state by irrigation where possible, particularly in vulnerable soils. However, further studies are needed to investigate microbial community 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 and microbial biomass concentrations. The results in this

study are based on mesocosm/laboratory experiments. Therefore, nutrients mobilised in a field site upon moist-/dry-flooding, may be retained elsewhere in the landscape and hence to assess the potential of P release field-scale studies are needed.

### Acknowledgments

We thank Kingston University London for funding this research in the form of a PhD Studentship award to SK. Rothamsted Research is supported by strategic funding from UKRI-BBSRC via its Institute Strategic Programmes including projects BBS/E/C/000I0321, BBS/E/C/000I0320 and BBS/E/C/000I0330.

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