Hydrologic Controls on Phosphorus Speciation and Mobilization in a Subalpine Watershed (East River, Colorado)

Lucia Isobel Arthen-Long
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Hydrologic Controls on Phosphorus Speciation and Mobilization in a Subalpine Watershed (East River, Colorado)

A Thesis Presented

by

LUCIA ISOBEL ARTHEN-LONG

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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Plant and Soil Science
Hydrologic Controls on Phosphorus Speciation and Mobilization in a Subalpine Watershed (East River, Colorado)

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This Thesis is dedicated in loving memory of Lucia Walker Fairlie Pulgram and William Leopold Pulgram, whose hard work, support, and love made this research possible.

And to the soil itself, which answered the questions I asked and offered insight into what we might ask next.
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Field work for this project was conducted on the traditional land of the Ute Indian Tribe, who were forcibly removed by the United States Government in the late 1800s.
ABSTRACT

HYDROLOGIC CONTROLS ON PHOSPHORUS SPECIATION AND MOBILIZATION IN A SUBALPINE WATERSHED (EAST RIVER, COLORADO) SEPTEMBER, 2021

LUCIA ISOBEL ARTHEN-LONG B.A., MOUNT HOLYOKE COLLEGE M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Marco Keiluweit

The cycling of phosphorus (P) through floodplain environments is critical to ecosystem productivity and has significant implications on both water quality and soil fertility. P export from soils in response to saturation has been well documented, but the relative vulnerability of specific P pools to mobilization remains poorly constrained, as do the mechanisms mediating its release. The prediction of P availability in and export from mountainous floodplain soils is of great importance as global climate change is projected to significantly alter precipitation regimes in alpine systems. This study combined a thorough characterization of P distribution across a hillslope to floodplain transect with a high resolution analysis of P response to saturation, providing insight into both P cycling and flood-induced mobilization in the East River watershed in Gothic, Colorado. P-XANES, $^{31}$P NMR, and sequential extractions revealed that P concentrations were greatest in the floodplain and dominated by inorganic P ($P_i$) bound to primary minerals, while hillslope P was lower and characterized by more bioavailable and organic ($P_o$) pools. Time series analysis of P response to extended water saturation confirmed our hypothesis that P mobilization would
occur in two phases, first driven by lysis of microbial cells upon initial rewetting, and later driven by the reductive dissolution of Fe oxides with extended saturation. These findings indicated that (i) the interaction of weathering and hydrology dictate P speciation across the transect; (ii) hillslope P may be concentrated in microbial pools while floodplain P is primarily bound by minerals; (iii) bioavailable P pools may be dominated by $P_{\text{o}}$; and (iv) $P_{\text{o}}$ is more vulnerable to mobilization during initial rewetting of dried soils while $P_{\text{i}}$ responds more to changes in redox potential during extended saturation. Results from this study underscore the significance of hydrology in determining P speciation and mobilization, indicating that long-term changes in precipitation may influence P bioavailability and export from alpine watersheds. Understanding the mechanisms by which P is mobilized is therefore of critical importance to better predict soil P response to saturation in a changing climate.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2. APPROACH</td>
<td>7</td>
</tr>
<tr>
<td>3. RESULTS</td>
<td>15</td>
</tr>
<tr>
<td>4. DISCUSSION</td>
<td>23</td>
</tr>
<tr>
<td>5. CONCLUSIONS</td>
<td>44</td>
</tr>
<tr>
<td>6. APPENDIX: SUPPLEMENTARY INFORMATION</td>
<td>46</td>
</tr>
<tr>
<td>REFERENCES CITED</td>
<td>49</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Absolute quantification of P concentration across transect and with depth.</td>
<td>16</td>
</tr>
<tr>
<td>2. Correlation coefficients of Ca and Fe/Al/P$_0$ pools measured by P-XANES with H$_2$SO$_4$- and DI/NaHCO$_3$-extractable P quantified by sequential extraction.</td>
<td>17</td>
</tr>
<tr>
<td>3. Changes in a) dissolved total, organic, and inorganic P, b) pH, dissolved Fe, and DO, and c) DOC and accumulated CO$_2$ emissions over 21 days of saturation.</td>
<td>20</td>
</tr>
<tr>
<td>4. Conceptual model of P distribution across hillslope to floodplain transect.</td>
<td>26</td>
</tr>
<tr>
<td>SI 1. Relative contributions of P pools across transect and with depth.</td>
<td>46</td>
</tr>
<tr>
<td>SI 2. $^{31}$P NMR spectra of hillslope, toeslope, and floodplain sites.</td>
<td>47</td>
</tr>
<tr>
<td>SI 3. Example linear combination fits of P-XANES spectra.</td>
<td>48</td>
</tr>
<tr>
<td>SI 4. Results of preliminary experiment testing incubation of floodplain surface (20-30 cm bgs) and hillslope deep soils (70-80 cm bgs).</td>
<td>48</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION

The cycling of phosphorus (P) through floodplain environments is critical to ecosystem productivity and has significant implications on both water quality and soil fertility (Boström et al. 1988; Elser et al. 2007; Vitousek 1984). However, much remains unknown about how P cycling will respond in a changing climate. P is subject to mobilization and export from soils in response to inundation (Baldwin and Mitchell 2000; E. K. Bünemann et al. 2013; de Vicente 2021; Gu et al. 2019; Hömberg and Matzner 2018; Parsons et al. 2017; Schönbrunner, Preiner, and Hein 2012; Benjamin L. Turner and Haygarth 2001), representing a gap in the P cycle that may be exacerbated by increased precipitation, particularly in environments characterized by episodic flooding. The loss of P from floodplains not only depletes essential nutrients from soils often prized for their agricultural value, but also poses a significant threat to downstream water quality through the addition of excess nutrients (Diaz and Rosenberg 2008; National Research Council 1995). This is of particular concern in mountainous environments, which supply the majority of river discharge (Viviroli, Weingartner, and Messerli 2003). The increasingly variable timing and magnitude of flooding events makes floodplains particularly susceptible to the impacts of climate change, producing uncertain consequences for the cycling and export of this critical nutrient. Predicting P availability in and export from floodplain soils in a changing climate requires detailed consideration of the biogeochemistry regulating its mobility.
P accumulation in upland soils can be attributed primarily to the weathering of apatite minerals and subsequent biological uptake (Benjamin L. Turner et al. 2013; Walker and Syers 1976). Here, inorganic phosphorus (P$_i$) released from apatite is first mobilized by weathering processes and then transformed into organic phosphorus by microbes and plants. Over time, progressive weathering and subsequent biological uptake removes P$_i$ from the underlying bedrock and subsoil, concentrating it in surface horizons where it primarily exists in organic forms (Benjamin L. Turner et al. 2013; Walker and Syers 1976). P stocks in floodplains, while subjected to similar processes, are additionally influenced by seasonal hydrological dynamics that add nutrient rich sediments through depositional processes. In addition to these deposition events and subsequent burial, floodplain soils tend to be waterlogged, creating anoxic environments that slow the rate of decomposition (Boye et al. 2017). The depositional nature of floodplain environments combined with frequent saturation results in reduced in-situ weathering and horizonation in comparison with their less dynamic hillslope and upland neighbors (Goyne, Brantley, and Chorover 2006; Welch, Taunton, and Banfield 2002). Thus, the discrepancy in flooding regime between hillslope and floodplain systems likely establishes a gradient in P characteristics within a watershed.

Although soil P is often overwhelmingly organic, an emphasis on plant-available P$_i$ has left P$_o$ dynamics understudied, particularly in floodplain soils (Chardon et al. 1997). Several methodological limitations make it difficult to address this knowledge gap (George et al. 2018; C. Gu et al. 2020; Kruse et al.
2015). First, most established methods of measuring P offer snapshot, rather than dynamic insights, impeding our ability to monitor P as it cycles. Second, P<sub>o</sub> tends to be associated with minerals, introducing additional barriers to its measurement. Finally, no single methodology can fully speciate P, requiring the integration of various methods to understand its distribution across soil pools. Sequential extraction, which is widely used to characterize P, is useful to determine operational pools and measure bioavailability, but cannot adequately quantify or isolate organic pools or distinguish P species (Barrow et al. 2021; Cade-Menun and Liu 2014; Condron and Newman 2011; Gu et al. 2020). True speciation can be obtained through 31P nuclear magnetic resonance (NMR), and P x-ray absorption near edge structure (P-XANES) spectroscopy, but the former can only measure P extracted prior to analysis (Cade-Menun and Liu 2014), and the latter is limited in its accurate identification of P<sub>o</sub> species (Werner and Prietzel 2015). In order to overcome these limitations, recent studies have combined these three methods to produce robust characterizations including the speciation and relative bioavailability of soil P. However, although these methods have been successfully combined in numerous studies (Bauke et al. 2017; Cade-Menun and Liu 2014; C. Gu et al. 2020; Prietzel, Klysubun, and Werner 2016; B. L. Turner 2008; Werner and Prietzel 2015), very few have applied them to floodplain soils.

While the release of P in response to soil re-wetting has been well documented (Bünemann et al. 2013, Gu et al. 2019, Parsons et al. 2017), the processes mediating its mobilization are poorly defined. Four primary
mechanisms of release have been previously proposed and investigated, including microbial lysis, physical disruption, reductive dissolution of iron (Fe) oxides and changes in pH. The microbial lysis hypothesis posits that the inundation of dry soils will trigger osmotic shock in microbes, causing cells to burst and release a pulse of P\(_o\) into the porewater (Baldwin and Mitchell 2000; S. Gu et al. 2018; Vendelboe et al. 2011). This initial release of P has also been attributed to the physical disruption of soil particles at the time of rewetting which could prompt the release of colloidal P (Bünemann 2013). Redox driven P release is proposed to occur as inundated soils become anoxic. In such conditions, Fe oxides which are known to associate with P are reduced, releasing previously sorbed P into the soil solution (Gomez et al. 1999; Gross, Pett-Ridge, and Silver 2018; Parsons et al. 2017; Rahutomo, Kovar, and Thompson 2018; Upreti, Maiti, and Rivera-Monroy 2019). Finally, Gu et al. (2019) found that redox reactions may not only release P directly, but also indirectly by influencing the pH of the soil solution. The authors propose that as pH increases, soil particles will become electronegative, repelling organic matter and associated P (Penn and Camberato 2019). Although each of these mechanisms has been found to contribute to P mobilization after rewetting, it remains unknown how these processes behave in sequence, what the relative contribution of each is to total P (P\(_t\)) released, and which pools of P are particularly susceptible to each mechanism.

Here, we aim to determine how episodic flooding affects mobilization and transformation of P in soils in a subalpine watershed, with a particular emphasis
on the fate of understudied $P_o$. To this end, we have conducted a two-part study integrating both field and lab-based approaches. An in-depth characterization of $P$ speciation across soil profiles was performed on a hillslope-to-floodplain transect in order to define how the relative abundance of $P_i$ and $P_o$ forms change in different physical pools across the transect. Then, soils from the same transect were subjected to a three-week laboratory experiment mimicking seasonal flooding dynamics of the site to observe the $P$ response over short and long time scales. With these approaches we hope to accomplish the following research objectives:

**Objective 1: Characterize the types and sizes of $P$ pools across a hillslope-floodplain transect**

By determining the spatial distribution and physical state of $P$ across the transect we will define how the relative abundance of $P_o$ and $P_i$ change in different physical pools across the transect. Additionally, we will be able to quantify the pools and forms of often obscured mineral-associated $P_o$. We hypothesize that total and $P_o$ will increase with proximity to the river and decrease with soil depth, driven by distinctions in redox dynamics and extent of weathering.
Objective 2: Identify P release mechanisms and quantify their relative contributions

By subjecting soils to prolonged wetting we can determine how different mechanisms act in sequence during flooded periods, which mechanisms are most effective in mobilizing P, and which P pools are most susceptible to different mobilization mechanisms. We hypothesize that P release will occur in two phases, the first driven by $P_0$ release via microbial lysis promptly after rewetting and the second by P release via reductive Fe dissolution after anoxic conditions are reached.
CHAPTER 2

APPROACH

2.1. Research site and soil sampling.

Our site is located on the East River in Gothic, CO, which serves as a test bed for the Department of Energy funded Watershed Function Scientific Focus Area and associated research initiatives (Hubbard et al. 2018). Situated in a continental, subarctic climate, the East River watershed encompasses montane, subalpine, and alpine life zones (Hubbard et al. 2018) and receives an average of 1200 mm of precipitation annually, primarily in the form of snow (PRISM Climate Group n.d.). The biogeochemistry of the East River floodplain is largely controlled by seasonal hydrological dynamics (Hubbard et al. 2018). These cycles are characterized by an influx of snowmelt in the late spring followed by a period of inundation lasting an average of three weeks before gradually drying over the summer months (Wan et al. 2019). The hydrology of these lowlands is in contrast with that of the hillslopes cradling the floodplain zone, which remain saturated by snowmelt for close to two weeks (Wan et al. 2019) but fail to hold stagnant water for extended periods of time. In order to understand the movement of P through the East River watershed, our study site encompasses this topographic and hydrologic gradient in a transect including hillslope, toeslope, and floodplain sampling sites.
Samples were collected from the lower montane pump house site from established research sites. Hillslope and toeslope soils were sampled at PLM (Pumphouse Lower Montane) 1 and 3 (Wan et al. 2019), while floodplain soils were collected from meander C (Fox et al. 2020), approximately 25 m from the East River. Soils were collected in June of 2019 by auger at hillslope, toeslope, and floodplain sites. Three replicate cores were sampled and homogenized at each depth and site. Sampling depth was constrained in the hillslope and floodplain by weathering bedrock and gravel deposits, respectively. For practical reasons, soils in the floodplain were sampled at slightly different intervals from the toeslope and hillslope. Soil depths were chosen for experimental soils based on results of sequential extraction analysis in order to capture apparent shifts in P distribution across operational pools. All samples remained refrigerated through collection and transport to the lab where they were air dried, hand ground, and sieved to 560 µm.

2.2. Site characterization

To understand how P is distributed among soil pools across our transect and with depth, a series of analyses were performed to characterize and speciate P. Relative bioavailability was determined by sequential extraction, while speciation of Po and Pi were determined spectroscopically by $^{31}$P NMR and P-XANES. As discussed above, although none of these methods would sufficiently
speciate P alone, they combine to produce a robust characterization of P
distribution across our study site.

2.2.1. Sequential Extraction

In order to quantify P pools based on bioavailability, a Hedley fractionation
adapted from Tiessen and Moir (1993) was performed. Soils were sequentially
extracted in DI-H2O, 0.5 M NaHCO3, 0.1 M NaOH, and 1 M H2SO4. Often
termed “labile P,” DI-H2O and NaHCO3-P are intended to respectively represent
freely exchangeable and plant available pools (Moir and Tiessen 2007; Olsen
1954). NaOH is used to extract primarily Al- and Fe-bound P (Gu and Margenot
2021; Moir and Tiessen 2007). While DI-, NaHCO3-, and NaOH- extractable
pools are likely to contain both P₀ and Pᵢ, P extracted by H₂SO₄ is expected to
reflect Ca-associated Pᵢ, which can be interpreted to represent P bound in
primary apatite minerals (Hedley, Stewart, and Chauhan 1982; Moir and Tiessen
2007). Soils were shaken for 18 hours during each extraction and supernatants
filtered to 0.22 µm following centrifugation. Pᵢ concentrations in extracts were
determined by DRC-ICP-MS. Residual P was quantified as the difference
between Pᵢ determined by microwave acid digestion (US EPA 2019) and the sum
total of extracted P.

“Bioavailability,” as measured by sequential extraction is a qualitative term
used to define the relative accessibility of P pools to plants and microbes. It is
used in this study as a gradient rather than a category (for example, Fe-
associated P₀ is not freely accessible to microbial mineralization, but it is typically
more accessible than P bound to primary apatite minerals), and with the caveat that soil solution chemistry has great influence on bioavailability of a given pool. Additionally, it must be noted that the “residual” P pool remains poorly defined and may in fact be accessible to plant uptake (Condron and Newman 2011).

2.2.2. $^{31}$P Nuclear Magnetic Resonance

While sequential extraction is useful to identify operational pools, it cannot adequately isolate specific forms of P (Barrow et al. 2021; Condron and Newman 2011; C. Gu et al. 2020). Quantitative solution state $^{31}$P NMR was used to identify and quantify P-containing compounds in bulk soils. Prior to analysis, soils were extracted at a 1:20 solid to solution ratio in a 0.25 M NaOH - 50 mM Na$_2$EDTA solution, frozen at -80°C and subsequently lyophilized according to Turner (2008). Following extraction, samples were reconstituted in 900 µL of 1 M NaOH, 0.05 M EDTA and a 70:30 H$_2$O/D$_2$O solution, vortex mixed and centrifuged for 10 minutes at 20 °C and 15,000 RPM.

All NMR analyses were performed at the Environmental Molecular Sciences Laboratory (EMSL) on a Bruker 750 MHz NMR spectrometer with Avance III console and room temperature 5 mm BBO probe. Parameters were optimized by mixing 350 µL of supernatant with 50 µL of 0.5 M Na$_2$PO$_4$, providing a strong signal to measure. The 90° pulse width was measured by nutation curve with a 60 s delay between scans, and all samples came out between 17-17.5 µs. T1 measurements were performed with a single scan inversion recovery (Bruker pulse sequence T1ir) experiment with 16 points ranging from 0.001 s to 60 s,
with a 60 s delay. T1 values came out between 1-5 s. For sample analysis, 550 μL of supernatant was deposited into a 5 mm NMR tube. NMR spectra of the samples were acquired after locking, tuning, and shimming using a pseudo-2D NMR sequence (zg2d) with 256 transients acquired per increment for a total of 2048 transients. The spectral width was 101 ppm collected into a time domain of 37k points over 0.6 s. D1 was set to 5 times the measured T1, and the receiver gain was fixed at maximum (2050). Experiment time varied from 4-17 hours. The use of these parameters allows the direct comparison of signal between spectra. Spectra were processed at EMSL using TopSpin 4.0.6 (Bruker BioSpin) and MestreNova 10.0 (MestreLab), where they were Fourier transformed after zero-filling to 64k points and application of a 3 Hz line broadening function and referenced for orthophosphate at 6 ppm.

2.2.3. Phosphorus X-ray Absorption Near Edge Structure analyses

P-XANES was performed on soils to primarily speciate Pi. Soils were ground to a fine powder, and then thinly spread over a P-free double-sided tape attached to a sample holder. Spectra were obtained at Canadian Light Source on a Soft X-ray Microcharacterization Beamline calibrated to 2152 eV (E0) using AlPO₄. Two scans were collected for each sample at an energy range set from 2119.5 to 2210 eV and a dwell time between 1-16 seconds. Spectra were averaged, normalized and fit using the Athena program of the DEMETER software package (Ravel and Newville 2005). Linear combination fitting was performed in Athena using 12 common soil compounds including: FePO₄ x 2H₂O, RNA, DNA,
Mg_3(PO_4)_2, K_2HPO_4, AlPO_4, Mg_2P_2O_7, NH_4MgPO_4 x H_2O, MgHPO_4 x 3H_2O, Ca_{10}(OH)_2(PO_4)_6 and phytic acid. Goodness of fit was determined by R-Factor (≥ 0.01) and visible deviation from sample spectra. In order to prevent overfitting, results including more than five standards or a contribution of less than 5% from any standard was discarded.

2.3. Flood Induced Mobilization Experiment.

To assess which P mobilization mechanisms are operative during flooding events, four soils collected between 20-30 cm and 70-80 cm from hillslope and floodplain sites were subjected to three weeks of water saturation, simulating typical site flooding duration. Over the course of the incubation, soil suspensions and headspace were sampled for measurement of P, pH, dissolved oxygen (DO), dissolved Fe, dissolved organic carbon (DOC), and CO_2 to infer contributions of lysis, dissolution of Fe oxides, and pH-driven mobilization mechanisms. Three replicates of each sample were combined with DI-H_2O (46.93 g of soil to 190 ml DI) in serum vials sealed air-tight with septa. Headspace and soil suspensions were collected five minutes after rewetting and again at hours 1 and 6 to capture rapidly occurring biogeochemical processes, and then six times more over the remaining 20 days of the experiment. Between sampling events jars were continuously shaken in the dark for 21 days. All soil suspensions were collected using 16 gauge needles, centrifuged for five minutes at 4000 G and then filtered to 0.22 µm through cellulose acetate syringe filters.
2.3.1. Quantification of total, inorganic, and organic phosphorus in porewater

$P_t$, $P_i$, and $P_o$ concentrations were measured to illuminate the timeline of P release during flooding and distinguish between mobilization mechanisms. Following filtration, porewater P samples were promptly frozen at -80 °C and subsequently thawed at 4°C immediately before analysis. $P_i$ was determined by colorimetric analysis at 882 nm using a Molybdenum Blue method adapted from Murphy and Riley (1962). It must be noted that analysis of P by the molybdenum method measures only orthophosphate and is unable to detect molybdate-unreactive pyrophosphate and polyphosphate. While this may result in the slight underestimation of $P_i$ (Cade-Menun and Liu 2014), NMR spectra indicated that $P_i$ was dominated by orthophosphate at all sites, suggesting that this had little effect on our results (Fig 1). $P_t$ was measured using ICP-MS. In order to account for background interferences sometimes associated with P measurement by ICP-MS, selected samples were spiked with internal standard calibrations, yielding consistent results. Organic phosphorus was estimated as the difference between $P_t$ and $P_i$.

2.3.2. Indications of redox status

In order to determine the contribution of reductive dissolution of Fe oxides in mobilizing P during saturation, several indicators of redox potential were monitored during incubation. DO was measured in 10 ml of sampled soil suspension with a Mettler Toledo potentiometric probe and was used to confirm
the progressive depletion of available O$_2$ required for the establishment of reducing conditions. pH was measured using the same probe, and was used to inform the interpretation of biogeochemical processes including soil organic matter (SOM) decomposition, redox activity, and changes in mineral solubility. Dissolved Fe was measured to monitor the reductive dissolution of Fe oxides as anaerobic conditions were established. In order to prevent precipitation, porewater samples were acidified by 1:1 dilution with 1 M HCl following filtration, and concentrations determined by ICP-MS.

2.3.3. Dissolved organic carbon and CO$_2$

DOC concentrations and CO$_2$ fluxes were used as indicators of microbial respiration during incubation. Suspensions sampled for DOC measurement were stored anaerobically and quantified using a TOC analyzer (Shimadzu TOC-L CPH) calibrated with potassium hydrogen phthalate. Headspace samples were drawn by syringe at each sampling point and CO$_2$ concentrations measured by direct injection on an infrared gas analyzer (LI-COR LI-8100A).
CHAPTER 3
RESULTS

3.2. Phosphorus speciation across the transect

3.2.1 Sequential Extraction

Sequential extraction revealed distinctions in $P_i$ concentrations and pool distribution across the transect and with depth. $P_i$ was lowest in hillslope soils and highest in the floodplain, with concentrations of 1542, 1813, and 2307 $\mu$mol g$^{-1}$ in surficial hillslope, toeslope, and floodplain soils, respectively (Fig. 1a). $P_i$ decreased with depth by $\sim$25% at all sites. Approximately 50% of hillslope $P$ existed in NaOH-, DI-, and NaHCO$_3$-extractable pools. These pools accounted for less than 20% of floodplain $P_i$, which was dominated instead by H$_2$SO$_4$-extractable $P$ and contained relatively larger residual pools. In addition to having greater concentrations of $P$ associated with bioavailable pools - here assumed to be DI-, NaHCO$_3$-, and NaOH-extractable $P$ - in comparison with the floodplain, hillslope soils also retained >70% of these pools with depth, while toeslope and floodplain soils retained <20% of NaOH-extractable pools and <40% of NaHCO$_3$- and DI-extractable pools in equivalent soils (note that floodplain sample depths vary from those of hillslope and toeslope sites).
Figure 1. Absolute quantification of P concentration across transect and with depth. A) P sequentially extracted by DI/NaHCO$_3$ (freely exchangeable and ‘plant-available’ P), NaOH (Fe-/Al-associated P), and H$_2$SO$_4$ (P bound by Ca/apatite minerals), as well as un-extracted residual component. B) Speciation of NaOH-EDTA-extractable P by $^{31}$P NMR (green and grey representing organic and inorganic P compounds, respectively), and C) Speciation by LCF of P-XANES spectra, normalized to total P measured by sequential extraction.

3.2.2. $^{31}$P NMR

$^{31}$P NMR spectra demonstrated changes in $P_i$:P$_0$ ratio across the transect and with depth in NaOH-EDTA the extractable pool. In contrast to $P_i$ trends, the highest concentrations of NaOH-EDTA-extractable P were found in the hillslope and the lowest in the floodplain (Fig. 1b). NaOH-EDTA extractable P decreased with depth at all sites, mirroring the trajectory of the more “bioavailable” extractable pools (i.e., the sum of DI, NaHCO$_3$, and NaOH extractable P) ($R^2 = 0.73$, 0.99, and 0.96 in the hillslope, toeslope, and floodplain respectively). $P_i$ was
dominated by orthophosphate, which accounted for 64-97% of inorganic pools while $P_o$ was primarily monoester, accounting for 84-100% of organic pools. While there was little difference in the $P_i$: $P_o$ ratio among surficial soils, the proportion of $P_i$ species gradually increased with depth in the floodplain, with $P_i$ accounting for nearly 50% of P in the deepest floodplain samples. These results indicate that hillslope soils remain more consistent in $P_i$: $P_o$ ratios in comparison with floodplain soils with depth, and that $P_o$ declines disproportionately to $P_i$ in the floodplain.

![Confirmation of Sequential Extraction by P-XANES Analysis](image)

**Figure 2.** Correlation coefficients of Ca and Fe/Al/$P_o$ pools measured by P-XANES with $H_2SO_4$- and DI/NaHCO$_3$-extractable P quantified by sequential extraction.

3.2.3 P-XANES

P-XANES analysis identified stark contrasts in pool distribution between the hillslope and floodplain (Fig. 1c). Linear combination fitting of P-XANES spectra identified a total of seven reference standards present across all sites. RNA and
phytic acid were pooled to represent $P_{o}$, $\text{FePO}_4 \times 2\text{H}_2\text{O}$ and $\text{AlPO}_4$ were pooled to represent “Fe/Al-associated P”, $\text{Ca}_{10}\left(\text{PO}_4\right)_6(\text{OH})_2$ was used to represent P bound in primary apatite minerals, and finally, $\text{Mg}_3(\text{PO}_4)_2$ and $\text{K}_2\text{HPO}_4$ were combined to represent P precipitated in salts. Spectral fits demonstrated a shift in dominance from Fe/Al-associated P in the hillslope to Ca-associated P in the floodplain, with Fe/Al-associated P accounting for approximately 50% of deep hillslope P and only 14% at comparable depth in the floodplain. $P_{o}$ pools decreased with depth at all sites, resulting P distributions dominated by Fe/Al- and Ca-bound P in deep soils.

In sum, our extraction and spectroscopy results demonstrated that while $P_t$ increased from the hillslope towards the floodplain, P speciation shifted from easily extractable and $P_{o}$ pools in the hillslope to P bound to primary minerals and other $P_t$ pools in the floodplain. P concentrations decreased with depth in all sites, but while the hillslope saw little differentiation in pool distributions with depth, floodplain soils demonstrated more dramatic shifts.

3.3. Time-series analysis of P response to extended saturation

3.3.1 Evolution of dissolved total, inorganic, and organic phosphorus during incubation

Upon rewetting, greatest $P_t$ concentrations were measured in surficial floodplain soils (Fig. 3.a). However, within the first six hours of saturation floodplain surface $P_t$ declined sharply while $P_t$ in the hillslope surface increased
by 143% from initial concentrations, surpassing those of the floodplain. Initial pulses of $P_t$ were less dramatic in floodplain and deep hillslope sites (increases of 5%, 32% and 29% in the floodplain surface, floodplain subsurface, and hillslope subsurface, respectively). $P_t$ concentrations declined gradually over the remaining 20 days of inundation within hillslope and floodplain soils by ~60% and ~90%, respectively.

$P_o$ accounted for ~70-99% of porewater $P_t$ in both surficial soils during the first 3 days of saturation. Porewater $P_t$ remained overwhelmingly organic in the hillslope surface through the remainder of the experiment, but $P_o$ plummeted in the floodplain surface, leaving only trace amounts (~0.01%) by the final week of saturation. Consistent with trends observed in $^{31}$P NMR spectra (Fig. 1.b), initial contributions of $P_t$ varied more significantly with depth in the floodplain (4% in surface, 47% in subsurface) than in the hillslope (25% in surface, 14% in subsurface). An initial pulse of porewater $P_t$ approximately coincided with maximum $P_t$ concentrations, peaking at 6 hours post rewetting. This peak was immediately followed by a sharp decline in porewater $P_t$, most notably in hillslope soils where nearly 100% was lost between hours 6-24. A second release of porewater $P_t$ was observed 2-3 days after rewetting in all soils. Surficial soils differed in P response to continued saturation, with floodplain surface $P_t$ reaching maximum concentrations at 14 days (~4x higher than initial concentrations), while $P_t$ in hillslope surface increased to day 6 before plateauing, ultimately recovering only half of initial concentrations. Although $P_t$ declined in all sites,
these trends illustrate a distinction between the hillslope and floodplain in timing, magnitude, and speciation of porewater P in response to saturation.

3.3.2 Changes in porewater chemistry during incubation

Though vials were constantly shaken over the course of the 21-day incubation, sedimentation was observed in all replicates, establishing a gradient in DO concentration within experimental units and excluding settled materials from porewater sampling. This compromised our use of dissolved Fe and DO to estimate redox potential and limited the interpretation of solid-state analyses to sampling points at the start and end of the incubation, when soil suspensions were thoroughly mixed. These end point samples helped clarify the impact of sedimentation on porewater measurements and provided insight into changes in mineral phases and P sorption dynamics. Figures illustrating end point data can be found in the supplementary information associated with this thesis (SI Fig. 4).
Initial DO concentrations were consistent across all soils upon rewetting and decreased to similar extents in all sites (Fig 3.b). DO declined steadily during the first 5 days of saturation but subsequently plateaued, likely due to sedimentation effects described above. Hillslope soils were consistently more acidic than those of the floodplain, remaining one unit lower throughout the course of incubation (Fig. 3.b). The pH of all soils rapidly decreased by approximately 1 unit during the first 6 hours of saturation before gradually increasing towards neutral and ultimately plateauing by day 5. Dissolved Fe was highest in deep hillslope samples and lowest in the deep floodplain, reflecting trends observed by P-XANES analysis in soils of comparable depths. Little variation was observed in porewater concentrations over time, however supplemental samples discussed above demonstrated that dissolved and HCl-extractable Fe increased by orders of magnitude over three weeks of incubation.

3.3.3 DOC and CO₂

The initial DOC concentration of the floodplain surface soil (~40 µmol g⁻¹) was approximately 2x higher than all other sites (Fig. 3.c). Both surficial soils experienced a spike in DOC during the first hour of saturation, but while the hillslope surface increased by 112%, a notably higher peak was observed in the floodplain, which increased by 830%. A second, weaker pulse of DOC was apparent in both surficial soils 2 days post-rewetting. Accumulated CO₂ emissions in surface soils were more than double those measured in deep soils, and hillslope soils amassed higher concentrations of CO₂ than floodplain
samples at corresponding depths (Fig. 3.c). The evolution of CO$_2$ concentrations over time demonstrated higher fluxes (and therefore activity) during the several days of incubation (with the exception of the deep floodplain), indicating a decline in microbial activity after six days of saturation (Fig. 3.c). Results of an earlier test experiment corroborate these results, demonstrating high rates of CO$_2$ production during the first 24 hours which subsequently decline with extended saturation (SI Fig. 4).
CHAP БER 4
DISCUSSION

Hydrological dynamics are known to mediate P release from soils, but understanding of the mechanisms by which this occurs has been obscured due to methodological limitations. This study combined a thorough characterization of P distribution across a hillslope to floodplain transect with high resolution monitoring of the P response to saturation, providing insight into both P cycling and flood-induced mobilization at our study site. Sequential extraction and spectroscopic analyses found that $P_{\text{t}}$ was greatest in the floodplain and dominated by $P_{\text{i}}$ bound to primary minerals, while hillslope $P_{\text{t}}$ was lower and characterized by more accessible and organic pools. Prolonged soil saturation, as expected during snowmelt, revealed two phases of P mobilization into the porewater, a first release of $P_{\text{o}}$ occurring within the first six hours and the second dominated by $P_{\text{i}}$ between 2-5 days after rewetting. Both phases of release varied in timing and magnitude among sites, indicating differences in the dominance of P pools and mobilization mechanisms across the transect. In sum, these results suggest that (i) the interaction of weathering and hydrology dictate P speciation across the transect; (ii) hillslope P may be concentrated in microbial pools while floodplain P is primarily bound by primary minerals; (iii) bioavailable P pools were dominated by $P_{\text{o}}$; and (iv) $P_{\text{o}}$ is more vulnerable to mobilization during initial rewetting of dried soils while $P_{\text{i}}$ responds more to changes in redox potential during extended saturation.
4.1 Hydrologic controls on P distribution across transect

4.1.1. Influence of water table depth on soil development

Weathering of apatite minerals is the primary mechanism by which P enters soil, and is controlled by a variety of environmental and biogeochemical factors (Heindel et al. 2018; Benjamin L. Turner et al. 2013; Walker and Syers 1976). Floodplain environments may stunt weathering of apatite through the neutralization of soil pH via proton-consuming redox processes (Ponnamperuma 1972) and by limiting the production of organic acids due to reduced microbial activity (Goyne, Brantley, and Chorover 2006; Welch, Taunton, and Banfield 2002). A growing body of research at our study site confirms that hydrology is a primary control on weathering across the transect, delineating weathering fronts at the deepest extent of the water table below ground surface at each site (~4 m, ~3.5 m, and ~1 m bgs in the hillslope, toeslope, and floodplain respectively) (Tokunaga et al. 2019; Wan et al. 2019; 2021). Though this research was focused primarily on the weathering of carbonates and pyrite, apatite weathering is controlled by similar factors influencing that of carbonate including moisture availability and pH, and is likely to follow general trends (Goyne, Brantley, and Chorover 2006; Heindel et al. 2018; Welch, Taunton, and Banfield 2002). In addition to contrasting water table depth, distinctions in hydrological regimes may differentiate weathering rates between the hillslope and floodplain. While hillslope soils are saturated directly after snowmelt each spring, floodplain soils are inundated for longer periods due to hillslope and river discharge, further limiting
chemical weathering in the floodplain (Tokunaga et al. 2019; Wan et al. 2019; 2021; Winnick et al. 2017). Sequential extraction, $^{31}$P NMR and P-XANES analyses all revealed that toeslope surficial and intermediate depths mimicked hillslope dynamics in distribution across operational and mineral-associated pools, changes with depth, and $P_i:P_o$ ratios, while deep toeslope soils more resembled floodplain distributions at comparable depths (Fig. 1). Given landscape position and the abrupt transition to organic-rich materials between 60-70 cm, deep toeslope soils may be comprised of former floodplain soils that have since been buried by eroded hillslope materials (Scharrón, Castellanos, and Restrepo 2012).

It must be noted that while hillslope soils developed in-situ, floodplain sediments are comprised of river deposits, reflecting distinctions in soil genesis between the two sites. This may result in slight differences in mineralogy, as the river catchment traverses a transition from igneous to sedimentary parent materials (Carroll et al. 2018). Floodplain sediments have also likely been subjected to intense physical weathering during residence times in the meandering river channel and during repeated flooding events after deposition in floodplain (Föllmi et al. 2009; Sutfin, Wohl, and Dwire 2016). However, the slightly alkaline water of the East River is unlikely to encourage the chemical dissolution of apatite minerals, which are nearly insoluble at neutral pH (Bryant et al. 2020; Föllmi et al. 2009; Heindel et al. 2018). We therefore propose that differences in P speciation across the transect are determined primarily by
hydrologic controls on weathering rates and associated in situ biogeochemical factors to be discussed more extensively below (Fig. 4).

**Figure 4.** Conceptual model of P distribution across hillslope to floodplain transect: total P increases with proximity to East River while organic P, relative bioavailability, and degree of soil weathering decrease.

### 4.1.2. Influence of soil development on P distribution across transect

The majority of hillslope P was stored in DI-H$_2$O-, NaHCO$_3$-, and NaOH-extractable pools (Fig. 1), which are generally associated with greater bioavailability relative to stronger acid extractants. These results are consistent with the Walker and Syers (1976) model of P evolution during pedogenesis, which predicts the storage of P in bioavailable pools and in association with Fe and Al oxides in well-weathered soils. Though sequential extraction measures only operational pools and cannot be used for purposes of speciation (Barrow et al. 2021; Condron and Newman 2011; C. Gu et al. 2020), $^{31}$P NMR and P-XANES extend our ability to interpret operationally defined fractionation results
(Cade-Menun and Liu 2014; Condron and Newman 2011; C. Gu et al. 2020; C. Gu and Margenot 2021; Prietzel, Klysubun, and Werner 2016; Werner and Prietzel 2015). As shown in Figure 2, we found that the sum of DI-H₂O-, NaHCO₃-, and NaOH-extractable P was strongly correlated with the sum of Al-, Fe-, and organically-bound P measured by XANES spectroscopy ($R^2 = 0.73$) and with the overwhelmingly organic NaOH-EDTA extractable pool measured by NMR ($R^2 = 0.95$). Given these correlations we infer that these “bioavailable” pools and Al-/Fe-bound P may be dominated by $P_o$.

Across all analyses, $P_i$ showed a tendency to increase with depth and proximity to the floodplain. $P_i$ accounted for approximately 10% of NaOH-EDTA-extractable P in all surface soils, but increased with depth in toeslope and floodplain soil, respectively contributing ~20% and ~50% of NaOH-EDTA-extractable P in deep soils (Fig 1). While $P_i$ was present in bioavailable pools of all sites, the majority of $P_i$ was found in H₂SO₄-extractable P, an extractant intended to quantify Ca-bound $P_i$ (Condron and Newman 2011; Moir and Tiessen 2007). Investigations comparing sequential extraction results with P-XANES have identified various inconsistencies, including a potential for the overestimation of Ca-P (Barrow et al. 2021; C. Gu et al. 2020). However, the results of this study indicate a strong correlation between H₂SO₄-extractable P and Ca-P (Fig. 2). Ca-P, typically used as a measurement of primary apatite P, increased with depth at all sites and was the dominant pool in the deepest toeslope soil and throughout the floodplain profile (Fig. 1.c). The abundance of
apatite P in the floodplain relative to hillslope soils contributes further empirical evidence validating the limited extent of weathering in the floodplain.

Despite the characteristically organic nature of floodplain soils, P-XANES and NMR spectra indicated P₀ accounted for a greater proportion of Pᵣ in the hillslope (Fig. 1). These results reflect a well-established shift in dominance from weathering-driven P cycling in less developed soils, to more biologically-mediated cycling in weathered soils (Davies et al. 2016; Benjamin L. Turner et al. 2013; Walker and Syers 1976). The abundance of P₀ in the hillslope indicates that progressive weathering has released substantial apatite P into the soil matrix, where it now cycles predominantly between biological pools (vegetation and microbial biomass), the soil solution, and associations with clays and secondary minerals (Benjamin L. Turner et al. 2013; Walker and Syers 1976). In the floodplain, though some degree of weathering has surely occurred within the first meter, P bound in apatite has seemingly remained largely inaccessible to microbial or plant uptake, limiting the accumulation of P₀. The release of P from primary minerals and subsequent transformation into P₀ may also facilitate its export from a system via leaching, microbial lysis, dissolution of Fe oxides and other mechanisms (Bol et al. 2016; Brödlin et al. 2019; Maranguit, Guillaume, and Kuzyakov 2017; Walker and Syers 1976). Our results indicate that Pᵣ measured by microwave digestion was 50-75% higher in floodplain soils relative to comparable depths in the hillslope (Fig. 1), demonstrating the transformation of apatite-bound P stocks into more mobile P₀ and bioavailable pools in hillslope soils. We therefore conclude that advanced weathering in hillslope soils has
facilitated the incorporation of P into organic pools while P in the floodplain is retained predominantly in primary minerals.

In summary, the distribution of P across the hillslope to floodplain transect illustrates systems in two pedogenic stages following the model of Walker and Syers (1976) (Fig. 4). Weathering in the hillslope has resulted in sizeable pools of $P_{o}$ and bioavailable P. The floodplain, due to extended periods of saturation has been weathered to a lesser degree, preserving P in primary apatite minerals. These differences in P storage likely have significant implications on microbial and plant communities as well as the efficacy of P mobilization mechanisms and export.

4.2. P mobilization mechanisms and potential across the hillslope-floodplain transect

The release of P upon the rewetting of dry soils is mediated by a number of mobilization mechanisms (Achat et al. 2012; Arenberg, Liang, and Arai 2020; Baldwin and Mitchell 2000; Bünemann et al. 2013; Brödlin et al. 2019; S. Gu et al. 2018; Gu et al. 2019; Hömberg and Matzner 2018; Mitchell, A. and Baldwin, n.d.). However, our understanding of the extent to which these mechanisms impact specific P pools and how they act in sequence remains limited. Insufficient speciation of porewater P (particularly $P_{o}$) constrains our ability to identify mobilization mechanisms, and typical sampling cadences fail to capture rapidly cycling P, overlooking important patterns that may contribute to mobilization and export of P. As discussed above, the characterization of P distribution across the hillslope to floodplain transect demonstrates the influence
of hydrology and weathering in determining P speciation across the transect.

Results of our flooding simulation incubation indicate how differences in P speciation in the hillslope and floodplain influence the extent and timing of P release in response to these hydrological changes. We hypothesized that P mobilization would occur in two phases, with a first pulse of $P_0$ released immediately upon rewetting, driven by the lysis of microbial cells, and a second release after the establishment of reducing conditions, mediated by the dissolution of Fe oxides. Consistent with our predictions, two phases of P release were observed in all sites, the first within 6 hours of initial saturation and the second between 3-5 days after rewetting, following the decline of porewater $DO$ (Fig. 3.a). Many trends observed over the course of the incubation were in line with speciation determined by sequential extraction and spectroscopic analyses, but others diverged, raising questions about the ability of such techniques to predict P response to rewetting on short timescales.

4.2.1. Rapid P response to saturation

High-resolution sampling during the first hours of rewetting demonstrated the rapid release of P in all sites. Over the first 6 hours of saturation we observed a sharp increase ($+141\%$) in porewater $P_i$ in the surface hillslope soil, while in the floodplain surface, initially high concentrations of $P_i$ dropped by 67% after two hours of saturation (Fig. 3.a). Porewater P in the floodplain surface during this period was composed almost entirely (96%) of $P_0$ and coincided with a dramatic release of DOC (Fig. 3). While subsurface soils in the hillslope and floodplain
exhibited pulses of dissolved P upon rewetting, the response of these soils was significantly dampened, likely due to the decreased availability of P at depth (Fig 1.a).

The amount and speciation of P released into the porewater during this period was largely consistent with site characteristics determined by sequential extraction and spectroscopic techniques discussed above. Hillslope soils were slightly acidic, reflecting coarser, better-draining textures and advanced weathering in comparison to the nearly neutral floodplain soils (Fig 3.b). Porewater P$_t$ concentrations between 6-24 hours in both surface soils align with total DI-extractable P pools, which were extracted for 18 hours during sequential fractionation (Fig 1.a). Further conforming to trends observed in the DI-extractable pool, porewater P$_t$ was substantially higher in surface soils, confirming a decline in bioavailable P with depth. The relative contribution of P$_o$ likewise decreased with depth, as was generally observed by our spectroscopic analyses and others (Li et al. 2013; Prietzel, Klysubun, and Werner 2016; Werner et al. 2017). Upon rewetting, porewater P was overwhelmingly organic in all sites (>75%) apart from the deep floodplain (Fig 3.a), reaffirming our conclusion that rapidly mobilized and thus bioavailable P across the study transect is dominated by P$_o$.

Though porewater dynamics were generally aligned with the results of characterization analyses, P concentrations in hillslope and floodplain surface soils during the first 24 hours of saturation revealed dynamics that were undetected by bulk soil analyses targeting different P pools. Most notably, though
sequential extraction, NMR, and P-XANES agreed that $P_o$ and bioavailable P in hillslope surface soils exceeded that of the floodplain surface, high resolution sampling found that initial $P_I$ in the floodplain surface was approximately 2x that of hillslope $P_I$ (Fig. 3.a). Further diverging from characterization trends, $P_o$ in the floodplain surface accounted for approximately 97% of $P_I$, while hillslope surface $P_o$ made up only 75% of $P_I$ immediately upon rewetting. This relationship was rapidly inverted, as $P_I$ decreased in the floodplain surface and increased in the hillslope surface over the first 6 hours. It can be presumed that these rapid changes in porewater P were unobserved by sequential extraction because by the time concentrations were measured after 18 hours of extraction, hydrolysis of $P_o$ would have already occurred (Bünemann 2015; George et al. 2018). This is further confirmed by the alignment of $P_I$ concentrations measured in incubation porewater between 6-24 hours and DI-extractable $P_I$ measured at 18 hours. That rapid P cycling was not accounted for in sequential extraction results calls into question the use of extraction-based techniques to accurately predict P bioavailability in our system and demonstrates the importance of in-situ measurements of P quantify and speciation in the lab or field environment.

The difference in timing and magnitude of rapid P mobilization between surface soils is not only in contrast with trends observed by characterization analyses, but interestingly indicates a distinction in mobilization mechanisms and sources of P activated by initial rewetting in the two sites. There are a number of factors that may differentiate $P_o$ response between the hillslope and floodplain, including distinctions in quantity and quality of plant inputs, the degree of
aggregation and hydro/ bioturbation (Bünemann et al. 2013), differences in the extent of SOM decomposition and its distribution between plant and microbial sources, and finally, legacy effects of drying and wetting cycles (Baldwin and Mitchell 2000; de Vicente 2021). First, floodplain vegetation is characterized as riparian shrubland, while the hillslope is comprised of lupine meadow, sagebrush, and forbs (Falco et al. 2019). The quantity and bioavailability of P in plant litter is known to vary depending on plant species (Wallace, Ganf, and Brookes 2008; Zou et al. 2021), which is likely to create distinctions in SOM decomposition between the two sites. Decomposition may additionally be influenced by the degree of aggregation in hillslope and floodplain soils, as preservation of SOM may be prolonged by physical occlusion within soil aggregates (Schmidt et al. 2011; Sollins, Homann, and Caldwell 1996). In a study P release from disaggregation, the size and stability of soil aggregates were found to influence the quantity of P released in response to saturation, as was the speed at which soils were saturated (E. K. Bünemann et al. 2013).

Differences in P0 response to saturation may also indicate a distinction in the relative contributions of plant-derived and microbial P in the two sites. Our NMR spectra were able to distinguish monoester-, diester-, and phosphonate- P0, but did not have sufficient resolution to distinguish between pyhtic acid, RNA, and other more specific P0 species, limiting our ability to directly distinguish P0 from plant and microbial sources. However, insight can be drawn from concurrent changes in porewater chemistry during this period of rapid P transformation. The sharp decrease in pH observed in all soils would be expected to accompany the
rapid release of SOM into the soil solution, indicating that all sites received significant organic inputs (Fig. 3.b). DOC concentrations spiked dramatically in the floodplain surface during the first 6 hours, ultimately reflecting a typical stoichiometry of plant litter (C:P = 3291:1) while DOC in the hillslope during the same period produced C:P ratios more characteristic of microbial biomass (C:P = 410:1) (Cleveland and Liptzin 2007; McGroddy, Daufresne, and Hedin 2004). Considered in combination with more extensive weathering observed in hillslope soils discussed above, this is consistent with the model proposed by Turner et al. (2013), which predicts that soil P will become increasingly dominated by microbial, rather than plant litter-derived, P with progressive weathering.

Differences in P response to rewetting may also reflect distinctions in drying-induced changes in P storage and soil organic matter composition between hillslope and floodplain sites. Numerous studies have confirmed that a strong pulse of dissolved P is released upon the rewetting of desiccated soils (Achat et al. 2012; Baldwin and Mitchell 2000; Bartlett and James 1980; Birch 1960; Blackwell et al. 2009; Brödlin et al. 2019; Bünnemann et al. 2013; de Vicente 2021; S. Gu et al. 2018; Mitchell and Baldwin; Schönbrunner, Preiner, and Hein 2012; Turner and Haygarth 2001; Qiu and McComb 1995). Various drying-induced changes are expected to influence the form and quantity of P released, including fluctuations in mineral crystallinity and rates of microbial respiration, which directly influence a soil’s sorption capacity and nutrient and C availability (Baldwin and Mitchell 2000; de Vicente 2021). The process of soil drying can be considered in two phases: the first, which we will term ‘aeration’ brings a
waterlogged soil from anoxic to oxic conditions. This stage will see a boom in microbial activity, leading to the rapid decomposition of organic materials, as well as the oxidation of reduced Fe species, increasing the amount of amorphous Fe hydroxides (and therefore attractive binding sites) in the soil (Baldwin and Mitchell 2000; Bartlett and James 1980; de Vicente 2021). The second phase can be considered ‘desiccation,’ in which the remaining moisture of a soil is removed. During desiccation, microbial activity decreases linearly with moisture content, slowing rates of SOM decomposition, and forcing microbial cells to either rapidly accumulate solutes to withstand changes in moisture, or lyse (W. Attygalla et al. 2016; Baldwin and Mitchell 2000). Floodplain soils spend much of the growing season in waterlogged, reducing conditions, and thus experience both aeration and desiccation during the drying process, facilitating rapid SOM decomposition followed by a sharp decline in microbial processing. This may account for the substantial pulse of TOC measured in floodplain porewater during the first hours of saturation. Surficial hillslope soils retain moisture but remain generally unsaturated and aerobic, and thus, apart from aggregate-protected anoxic microsites, would not have experienced the increased microbial activity associated with the aeration phase.

The rapid decline in $P_o$ that followed rewetting in surficial hillslope and floodplain soils within the first 24 hours has been similarly observed in previous study and can likely be attributed to a combination of microbial uptake and sorption to mineral surfaces (Bünemann 2015; D’Angelo 2005; Gross, Pett-Ridge, and Silver 2018; Qiu and McComb 1995; Yevdokimov, Larionova, and
More rapid accumulation of CO₂ production was observed during the first days of incubation (Fig. 3.c), and elevated rates of CO₂ production during the first 24 hours observed during a test experiment (SI Fig. 4) indicate high rates of microbial respiration coinciding with decreasing porewater $P_0$, suggesting rapid microbial uptake of bioavailable $P_0$. Though mineral crystallinity and sorption capacity were not directly measured, visible mottling in both deep soils and the floodplain surface suggests precipitation of Fe oxides, which tend to have a high affinity for $P_0$ (Baldwin and Mitchell 2000; Bartlett and James 1980; Celi and Barberis 2007).

To our knowledge, no other study has monitored the P response of re-wetted soils at a finer resolution than once per day, offering no direct comparison to these results. However, differences in porewater stoichiometry, rates of SOM decomposition indicated by pH and CO₂ fluxes, and timing of P release in the context of site hydrological dynamics support the conclusions that (i) porewater P in the floodplain surface may be dominated by plant-derived SOM while P released in the hillslope surface is likely of primarily microbial origin; and (ii) floodplain $P_0$ may be more vulnerable to drying effects while hillslope $P_0$ responds more dramatically to flooding. Taken in sum, monitoring of P response during the first 24 hours after saturation illustrated highly dynamic changes in porewater chemistry, supporting our hypothesis that rewetting would induce the mobilization of $P_0$ to the soil solution, but additionally revealed that P released in the hillslope and floodplain may vary in origin and mobilization mechanisms.
4.2.2. *P response to extended saturation*

Continued saturation of experimental soils demonstrated further distinctions in mobilization mechanisms between sites. We hypothesized that the establishment of anaerobic conditions would trigger a second phase of *P* mobilization due to the reductive dissolution of Fe oxides (Gomez et al. 1999; Gross, Pett-Ridge, and Silver 2018; Parsons et al. 2017; Rahutomo, Kovar, and Thompson 2018; Upreti, Maiti, and Rivera-Monroy 2019). Though direct monitoring of redox potential in our incubations was limited by sedimentation, we observed a steady increase in dissolved *P* in all sites between 2-5 days after rewetting (Fig. 3.a). Elevating concentrations of *P* coincided with a gradual increase in pH that approached but never reached neutral (Fig 3.b), a trend typical of anaerobic soils undergoing reduction processes that consume H+ ions from the soil solution (Ponnamperuma 1972). DOC and porewater *P* indicated a strong pulse of microbial activity during in the first 24 hours which was followed by a steady decline in porewater DO, indicating that anoxic conditions were reached in all incubations. Increased dissolved Fe was not clearly observed in our incubations. However Parsons et al. (2017) found in a similar batch experiment that 99.4% of reduced Fe was not released to porewater, but instead quickly redistributed within the solid phase during anoxic periods. Our additional end point measurements showed dramatic increases in porewater and HCl-extractable Fe after re-suspension of settled sediments, demonstrating active Fe reduction (SI Fig. 5). Taken in sum, this gives credence to our hypothesis that a
second pulse of P would be released driven by the reductive dissolution of Fe-oxides.

Mobilization of $P_i$ during extended incubation deviated from porewater $P_i$ and $P_o$ trends, which continually decreased after the first day of saturation, and was in contrast with early mobilization dynamics which were most prominent in $P_o$. That P released in response to reductive dissolution was exclusively $P_i$ suggests the preferential desorption of $P_i$ from Fe association and/or the preferential redistribution of $P_o$ within solid phases. Studies comparing P sorption to Al and Fe oxides have demonstrated that phytic acid is consistently sorbed at higher rates than orthophosphate, suggesting a particular affinity for this abundant $P_o$ compound (Anderson, Williams, and Moir 1974; Celi and Barberis 2007). Furthermore, it has been observed that $P_i$ tends to be liberated predominantly from anoxic regions in soils (Baldwin and Mitchell 2000). This stark distinction in P speciation demonstrates that dissolved $P_o$ is more vulnerable to mechanisms acting on rapid timescales, while dissolved $P_i$ responds more strongly to extended saturation.

P mobilization dynamics with extended saturation additionally highlights the distinct response of the hillslope surface, which was the only site to maintain significant levels of dissolved $P_o$ during the second half of incubation, and had the least pronounced porewater response to reducing conditions compared to the other three sites (Fig. 3.a). Whereas $P_i$ in the hillslope surface plateaued at around day 5, ultimately recovering approximately half of initial values, concentrations continued to rise in the other sites. At 14 days after rewetting,
dissolved $P_i$ concentrations more than doubled in both deep soils and increased by 5x in the floodplain surface. That $P_o$ remained dominant in the hillslope surface while $P_i$ in all other sites was exclusively $P_i$ implies that microbial and/or sorptive mechanisms removing $P_o$ from hillslope surface were less of a factor, particularly over the final week of saturation where changes in dissolved P were not observed. Differences in microbial community composition and sorption equilibria may provide some explanation for this divergence from the other three sites.

The relative dominance of mobilized $P_o$ in the hillslope surface may be explained by the lack of microbial adaptation to stronger redox cycling. It has been observed that soil environments associated with fluctuating redox conditions select for microbial communities that are adapted to withstand variable conditions (Baldwin and Mitchell 2000; DeAngelis et al. 2010; Pett-Ridge, Silver, and Firestone 2006; Schimel, Balser, and Wallenstein 2007). In an investigation of community composition across our transect, Lavy et al. (2019) identified significant differences in microbial community structure based on proximity to the water table, where redox fluctuations commonly occur. This finding suggests that hillslope surface soils may be characterized by a predominantly aerobic microbial community less adapted to sustained periods of anoxia. With the depletion of $O_2$ from the soil solution during incubation, it is expected that obligate aerobes would be unable to facilitate the reduction of alternate oxidizing agents. A microbial community less suited for anaerobic respiration may then limit the release of $P_i$. 

39
from the dissolution of Fe oxides in the hillslope surface and impede further mineralization of porewater $P_0$.

Differences in sorption capacity may also contribute to distinctions in P mobilization during extended incubation. As discussed above, hillslope and floodplain sites vary significantly in soil development, impacting the relative abundance of primary and secondary minerals and consequently the distribution of P across mineral pools. P is known to associate strongly with Ca, Fe, and Al in soils, and sorption dynamics (used here to encompass both adsorption and precipitation) are strongly influenced by Eh, pH, and the relative concentrations of P and minerals in a solution (Celi and Barberis 2007; D'Angelo 2005; Gomez et al. 1999; Gross, Pett-Ridge, and Silver 2018; Grybos et al. 2009; Kopáček et al. 2005; Loganathan et al. 2014; Lopez-Hernandez and Burnham 1974; Penn and Camberato 2019). Though characterization of changes in mineral-associated P at each sampling point was beyond the scope of this study, measurement of dissolved and HCl-extractable Ca, Fe, and Al can help us speculate on interactions among P and metals in the porewater. Dissolved Ca increased in all sites over the three week incubation, establishing increasingly favorable conditions for P-immobilizing Ca precipitation to occur (Penn and Camberato 2019). Porewater Al was relatively unchanging in all sites except the hillslope surface, where concentrations began to decline around day 5, when Fe reduction is presumed to have begun. P is understood to sorb strongly to Al oxides between pH 4.5-6.5 (Penn and Camberato 2019). Al oxides also have the potential to re-capture P released from the reductive dissolution of Fe oxides,
which may result in decreased liberation of $P_i$ to hillslope surface porewater (Kopáček et al. 2005). End point measurement of dissolved metals and P show no substantial variation between settled and re-suspended incubations except in the case of Fe, which increased by approximately 50x in surface soils and by 4x in deep soils (SI Fig. 5). In contrast, HCl-extractable measurements revealed substantially higher concentrations of HCl-extractable Fe, Al, Ca, and P upon re-suspension of settled sediments, indicating a general shift towards solid phases over the course of incubation. Notably, disparities in Ca, Fe, and P between settled and suspended samples were 3-9x greater in the hillslope surface in comparison to the floodplain, indicating a stark difference in extent of sorption between the sites (SI Fig. 5). That dissolved and HCl-extractable Fe increased to such great extent in the hillslope surface suggests that Fe reduction occurred, despite having a microbial community presumably more skewed towards aerobes. This, along with the exceptional increases observed in the solid phase, indicates that increased sorption to solids may account for differences in P response to extended saturation in the hillslope surface.

4.3 Implications of P speciation and flooding-induced mobilization on bioavailability and export

Characterization of P distribution and high-resolution monitoring of P response to saturation highlight the significance of hydrological dynamics in controlling P speciation and mobilization across the hillslope to floodplain.
transect. The influence of groundwater depth on apatite weathering suggests that long-term changes in precipitation patterns would have significant implications on P bioavailability and abundance of $P_o$. This effect could have particularly pronounced effects in floodplains where weathering (and thus soil development) is retarded close to the surface due to high water tables, preserving P in unweathered primary minerals (Goyne, Brantley, and Chorover 2006; Ponnamperuma 1972; Welch, Taunton, and Banfield 2002). Increased cycling of P into bioavailable pools may result in changes in nutrient availability to microbial and plant communities, and increase potential for leaching during flooding events (Bol et al. 2016; Brödlin et al. 2019; Maranguit, Guillaume, and Kuzyakov 2017; Walker and Syers 1976). The potential for P release during such events is illustrated by significant mobilization of $P_o$ observed during the first 24 hours of saturation. The magnitude of this release is expected to be modulated by the extent and duration of soil drying, rate of rewetting, and frequency of moisture oscillations (Schönbrunner, Preiner, and Hein 2012), which influence the composition of SOM (Borken and Matzner 2009) and vulnerability of microbial populations to moisture stress (Baldwin and Mitchell 2000; Schimel, Balser, and Wallenstein 2007). Release of P due to the reductive dissolution of Fe oxides during extended saturation may have diminished leaching potential in comparison with early mobilization at our site given that (i) $P_t$ release observed during this phase was significantly lower; (ii) dramatic increases in HCl-extractable P and Fe indicate that the majority of P mobilized during reductive dissolution was redistributed within the solid phase rather than exported to the
porewater; and (iii) reducing conditions are associated with relatively stagnant flow of water, increasing the likelihood that mobilized P will remain within the soil profile. However, variations in hydrologic regimes and sorption capacity among soils may influence the retention of P mobilized via these mechanisms.
CHAPTER 5
CONCLUSIONS

Investigations targeted at understanding the cycling of $P_0$, and development of analytical methodologies improving its speciation have increased significantly over the past 20 years, reflecting increased prioritization of this research (George et al. 2018). While the use of spectroscopic techniques allows for detailed characterization of P distribution in soils, high resolution sampling revealed that rapid cycling dynamics observed during the first hours of incubation were undetected by characterization analyses. These novel results underscore the importance of methods that allow for dynamic, in situ measurement of P. Though P mobilization to porewater can be estimated through the measurement of dissolved P, the integration of solid state and spectroscopic measurements would provide powerful insight into the interplay of sorption and mobilization. This would be further improved with dynamic quantification of microbial P and differentiation between colloidal vs. truly dissolved P (Bol et al. 2016). Better understanding of the many biogeochemical factors influencing P sorption is critical to improve prediction of P dynamics in response to saturation, particularly the influence of Al in retaining P mobilized by the reduction of Fe oxides.

Increased investigation of P response to saturation is particularly necessary in floodplains, which are environments especially prone to wetting and drying cycles. Floodplains contribute significant ecosystem services and are critically important to global economies and food security (Dugan 2010; Turpie et
al. 1999; Warner, Schattman, and Hatch 2017). Our ability to predict and manage P cycling may have substantial implications on agricultural sustainability, particularly as sources of phosphate ore used for fertilization are dwindling (Daneshgar et al. 2018). Understanding P cycling and export potential from floodplains also has significant implications on the quality of downstream surface water, as nutrient enrichment is known to facilitate eutrophication of water bodies (Diaz and Rosenberg 2008; National Research Council 1995). P export from alpine floodplains may have particular influence on nutrient contamination as mountainous rivers supply the majority of river discharge (Viviroli, Weingartner, and Messerli 2003). Climate projections indicate that alpine systems will be subjected to dramatic changes in timing and volume of precipitation (Gobiet et al. 2014; Horton et al. 2006; Lapp et al. 2005). Given the considerable influence of hydrology on P speciation and mobilization demonstrated in this study, climatic changes may have significant implications on the export of P from mountainous floodplains. Improved understanding of P response to saturation in these dynamic environments is therefore crucial to better predict and manage soil P cycling and export in a changing climate.
SI Fig. 1. Relative contributions of P pools across transect and with depth. A) P sequentially extracted by DI/NaHCO\(_3\) (freely exchangeable and ‘plant-available’ P), NaOH (Fe-/Al-associated P), and H\(_2\)SO\(_4\) (P bound by Ca/ apatite minerals), as well as un-extracted residual component. B) Speciation of NaOH-EDTA-extractable P by \(^{31}\)P NMR (green and grey representing organic and inorganic P compounds, respectively), and C) Speciation by LCF of P-XANES spectra.
SI Fig. 2. $^{31}$P NMR spectra of hillslope, toeslope, and floodplain sites delineated by organic (green) and inorganic (grey) signal regions.
SI Fig. 3. Example linear combination fits of P-XANES spectra.

SI Fig. 4. Results of preliminary experiment testing incubation of floodplain surface (20-30 cm bgs) and hillslope deep soils (70-80 cm bgs). Vials in this experiment were sealed for 3 hours before CO\textsubscript{2} measurement in headspace but otherwise remained covered only by parafilm, enabling measurement of CO\textsubscript{2} flux. Settled sediments during test were re-suspended by stirring during measurement of DO, correcting the O\textsubscript{2} gradient established through sedimentation.
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