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Biologically Available Phosphorus In Biocrust-Dominated Soils Of The Chihuahuan Desert

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BIOLOGICALLY AVAILABLE PHOSPHORUS IN BIOCRUST-DOMINATED SOILS OF THE CHIHUAHUAN DESERT

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DEDICATION

I would like to dedicate this to everyone who has supported me throughout this experience. To my advisors and professors, and to my amazing family and friends both near and far for their undeniable support and confidence in me.

BIOLOGICALLY AVAILABLE PHOSPHORUS IN BIOCRUST-DOMINATED SOILS OF THE CHIHUAHUAN DESERT

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ABSTRACT

In desert soils, phosphorus (P) is an important limiting nutrient and its cycling characteristics are less understood compared to nitrogen and carbon. Phosphorus cycling is controlled by both geochemical and biological factors. Traditionally, P availability has been characterized via sequential extraction analyses such as Hedley fractionation, but newly developed extraction methods allow for the examination of more biologically relevant P fractions, providing insight on mechanisms of P acquisition by plants and microbes. We examined these P cycling features in the context of biological soil crusts (biocrusts), which have been found to be important drivers of nutrient cycling and have the potential to release bound labile P for uptake by soil biota and plant roots. We adopted the biologically-based P (BBP) method that incorporates four extractions: calcium chloride ($CaCl_2$), citric acid, phosphatase enzymes, and hydrochloric acid (HCl) that mimic different P acquisition strategies. We coupled the extractions with a ^{33}P-labeled orthophosphate addition and incubation to assess the fate of freshly available phosphate (PO_4^{3-}). Low P concentrations in the $CaCl_2$ extraction suggests that drylands lack easily accessible P in soil solution, while higher amounts in the citric acid- and enzyme-extractable pools suggest dryland soil biota may acquire P through the release of organic acids and phosphatases. Radiolabel addition results showed that added PO_4^{3-} is, within 24 hours, quickly adsorbed onto mineral surfaces or incorporated into hydrolysable organic compounds, instead of remaining in the soil solution as very little recovery was observed in the $CaCl_2$ pool. Compared to areas of disturbance or with no intact biocrust, areas with biocrusts showed overall lower P concentrations across all four extractable pools. This suggests that biocrust organisms may prevent P adsorption onto mineral surfaces by incorporating P into their biomass. Overall, our results indicate that while P is not

immediately available in the soil solution, organisms have several viable strategies, including organic acid and enzyme production, to access P in dryland soils.

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INTRODUCTION

Phosphorus (P) is found in soil systems in a variety of organic or inorganic forms that are either soluble or insoluble. Unlike nitrogen (N), in the form of dinitrogen (N_2) and carbon (C) in the form of carbon dioxide (CO_2), P has no gaseous component that is of major importance in biogeochemical nutrient cycling. The replenishment of P into a system occurs via rock weathering of minerals such as apatite (Yang & Post 2011; Lajtha & Schlesinger 1988), or through dust mobilization and deposition (Porder & Ramachandran 2013). In dryland ecosystems, the degree of rock weathering, and crucially, the level of precipitation, are processes that control the dissolution and sorption of P in the soil (Belnap 2011). Chemical weathering of apatite ($Ca_{10}(PO_4)_6(OH,F,Cl)_2$) from rocks or leaching of dust-derived minerals releases P into the soil solution in available forms where it can be leached, taken up by plants or microbes, or adsorbed onto mineral surfaces (Lajtha & Schlesinger 1988). In comparison to more mesic soils, arid soils typically contain higher levels of calcium (Ca), aluminum (Al), and iron (Fe), which all bind with P making it unavailable to plants or for soil microbe mineralization (Belnap 2011). In many semi-arid lands, calcium carbonate ($CaCO_3$) is the prevailing soil component that interacts with P, providing a calcium source to make calcium phosphate, which accumulates as part of caliche horizons at depth, rendering P unavailable (Lajtha & Schlesinger 1988). Thus, the source of P to organisms in drylands depends on a mineral source from parent material or dust, and the chemical means to release that P into the soil solution.

Geochemical and biological controls are important for soil P availability, but function differently in arid systems than in the more often-studied mesic systems. In arid soils, the abundance of $CaCO_3$ stabilizes P and reduces available (labile) P due to the mineral associations

(Cross & Schlesinger 1995; Lajtha & Schlesinger 1988), while for example in forests, P is more likely found in soil organic matter (SOM), causing P availability to be linked to the amount of organic matter that accumulates over time. Thus, in mesic systems with higher levels of soil SOM and litter input, soil organic P is likely to be higher, which would result in an increase in phosphatase production as a strategy to meet P demand. Organic P can make up 80% of total P in mesic systems (Dalal 1977), while organic P has been found to make up less than 13% of total P in the Chihuahuan desert (Cross & Schlesinger 2001). In drylands, P is more likely to be found in mineral or mineral-associated forms than organic forms as drylands tend to have much smaller SOM pools (Sinsabaugh et al. 2008). Previous studies in the northern Chihuahuan Desert have found HCl extractable-P to be the largest P pool while directly available P in the soil solution makes up less than 1% of the total P and the overall P content to be low (Levy & Schlesinger 1999; Cross & Schlesinger 2001).

Many insights into dryland P cycling come from use of the Hedley fractionation method (Hedley & Stewart 1982; Tiessen & Moir 1993), which was created to characterize the physical and biochemical role of P (Cross & Schlesinger 2001). The Hedley fractionation quantifies different forms of soil P, provides information about the availability of P in soils (Johnson et al. 2003), and quantifies occluded P (DeLuca et al. 2015). The procedure separates P into inorganic and organic P pools that vary in their degree of availability for plants from labile to occluded. The sequential fractionation method first removes inorganic and organic P that is accessible in the soil solution with a resin membrane, followed by an extraction with sodium bicarbonate on the remaining material. The sum of these two fractions represent available (labile) P that may cycle over the short term (Fox et al. 2011; Bowman & Cole 1978; Cross & Schlesinger 1995; Johnson et al. 2003). The resin-extractable pool characterizes the available P that is directly accessible for

plant or microbial uptake. The remaining P fractions are accessed with NaOH, 1M HCl, concentrated HCl, H_2O_2, and concentrated H_2SO_4 to determine moderately labile (non-occluded) to highly recalcitrant forms of P (Tiessen & Moir 1993). Hedley data for the Chihuahuan desert suggests that P does not remain in the soil solution easily. Studies have consistently found resin-extractable P to be overall low, making up a small portion of total P in Chihuahuan desert systems (Cross & Schlesinger 1995, 2001; Levy & Schlesinger 1999; Lajtha & Schlesinger 1988). For arid environments, this suggests that when P is released by enzymatic breakdown of organically bound phosphorus, e.g. by the activity of phosphatases or liberated from mineral phases via leaching, it must be readily taken up by an organism, otherwise it may end up back in one of the occluded pools.

While Hedley fractionation results suggest that microbial and plant uptake play a role in P availability in the Chihuahuan desert, they provide incomplete information about the mechanisms used by plants and microorganisms to acquire P, such as root interception, acid dissolution, or enzyme hydrolysis (Deluca et al. 2015). To get a better idea of the differences between different biological P acquisition strategies that organisms may employ, a method has been introduced by Deluca et al. (2015) for assessing biologically-based P pools (BBP). This procedure uses parallel instead of in sequential extractions and the four steps mimic strategies used by plants or microbes to access P. The first pool is a dilute (0.1 mmol $CaCl_2$) salt-extractable pool, which simulates what is available in soil pore water through diffusion or mass flow, and directly available for plant and microbial uptake. This pool is similar to the resin-extractable pool used during Hedley fractionation. Next, there are two acid-extractable pools: citric acid and HCl (Table 2.1). The citric acid-extractable pool assesses P that is sorbed to clay particles or P that is weakly bound to other parts of the soil matrix. This is similar to the bound P assessed in the Hedley fractionation

bicarbonate and hydroxide fractions; however, citric acid is thought to be a better analogue for the organic acids released by plant roots and microbes (Darch et al. 2016). The HCl extractant assesses P that is strongly bound to mineral surfaces, which may be less accessible to plants and microbes (Deluca et al. 2015). Finally, the phosphatase pool assesses the availability of labile organic P through enzyme hydrolysis (Deluca et al. 2015). Building on the Hedley-type approaches, the BBP method includes a weak acid similar to organic exudates such as citric acid, oxalic acid, acetic acid (Belnap 2011) and also assesses the phosphatase-available pool consisting of organically bound P, providing a means to more directly illuminate the bioavailable forms of P.

While there is little information on the size of weak acid and enzyme-extractable P pools in dryland soils, knowledge about the fate of freshly available PO_4^{3-} is even more scarce. In mesic systems, the incorporation of P into different soil P fractions has been investigated through the use of radioisotopes (Fardeau 1996). Because P has only one stable isotope (^{31}P) that makes up almost all the phosphorus on earth, the two of the seven radioisotopes of P with the longest half-life, ^{32}P (14 days) and ^{33}P (25 days), have been used in numerous agricultural and forest soil studies, along with aquatic and sediment studies, to understand microbial biomass, plant P uptake, transformations of P, and other aspects of P cycling (Di et al. 1997; Frossard el at. 2011). Some of the earliest studies using radio-labeled P were to differentiate the labile forms of phosphorus (McAuliffe et al. 1948, Larsen 1952). Frossard et al. (2011) demonstrated the applicability of radiolabeled P in soils with low P availability and high P-sorbing capacity to trace P transfer between inorganic P in the soil solution and organically bound P, along with the mineralization of organic P and P uptake via soil microbes, but such studies have not been applied in drylands. Furthermore, no investigations we are aware of have coupled a radioisotope approach with an examination of different biologically available fractions in the Chihuahuan desert.

In this study, we investigate biologically based sources of P and the fate of freshly available PO_4^{3-} using dryland soils with and without biological soil crusts (biocrusts). We chose to focus on biocrusts because they have been shown to be particularly important for nutrient cycling in arid systems and are a crucial component of most dryland soils (Belnap 2011). Organisms found in surface biocrusts such as of nitrogen-fixing cyanobacteria, algae, fungi, lichens, and mosses (Belnap & Lange 2001) may contribute to the release of unavailable P from surface rock or dust deposits through the release of organic compounds via microbial weathering (bioweathering). Most P and other nutrients enter systems via dust or litter deposition and weathering, thus high nutrient concentration occurs on the surface (Jobbagy & Jackson 2001; Thomas & Dougill 2007). In desert systems, P bioavailability has been found to be more tightly connected to the amount of accessible P that enters the system through surface adsorption (i.e. dust deposition) than to the release of P from carbonate-bound forms (Murman & Peach 1969). Organic acid release by surface microbes in these systems may increase the amount of available P in the soil solution even in absence of labile soil organic matter (Cross & Schlesinger 2001). It has been shown that the secretion of low molecular weight organic acids (e.g., citric acid or oxalic acid) by soil biota can prevent P from being occluded and that this mechanism releases larger amounts of organic P into solution than what is hydrolyzed by phosphatases (Fox et al. 2011; Reed et al. 2011). Cyanobacteria, algae, and fungi have been found to a) excrete phosphatases that access P through enzyme hydrolysis; b) secrete organic acids that may release P from mineral surfaces; or c) excrete hydrogen ions (H^+) during respiration that induce carbonate dissolution, thereby releasing carbonate-bound P, all of which increase P availability for plants and other soil biota (Belnap 2011; Jones & Oburger 2011). For example, dryland cyanobacteria have shown to aid in the liberation and acquisition of P by mobilizing inorganic P hydroxyapatite (Whitton 2000). Once P is in biotic

pools, it can be recycled via decomposition of organically bound P driven by phosphatase enzyme production. Indeed, phosphatase activity has been shown to increase under developed biocrusts compared to areas with bare soil (Bolton et al. 1993), despite phosphatase release being highly controlled by soil organic matter (Sinsabaugh et al. 2008). In addition, biocrusts may aid in the capture of P-containing dust particles and prevent P loss or redistribution through the formation of an extracellular sheath through the excretion of exopolymers that create a sticky surface (Belnap 2011). Through these processes, concentrated microbial communities may substantially influence P cycling in drylands, creating conditions that are significantly different from areas with disturbed crusts or bare soils.

The aim of this study is to investigate the influence biocrust-dominated soils have on P dynamics in dryland soils that have characteristically low levels of organic matter and low P availability. Using the BBP method coupled with a ^{33}P orthophosphate label addition, we address the following questions: (1) How do biologically available P pools in drylands compare to those in mesic systems? (2) How does added phosphorus become incorporated into the different P pools? and (3) How do intact surface crusts affect P cycling in comparison to areas of disturbance?

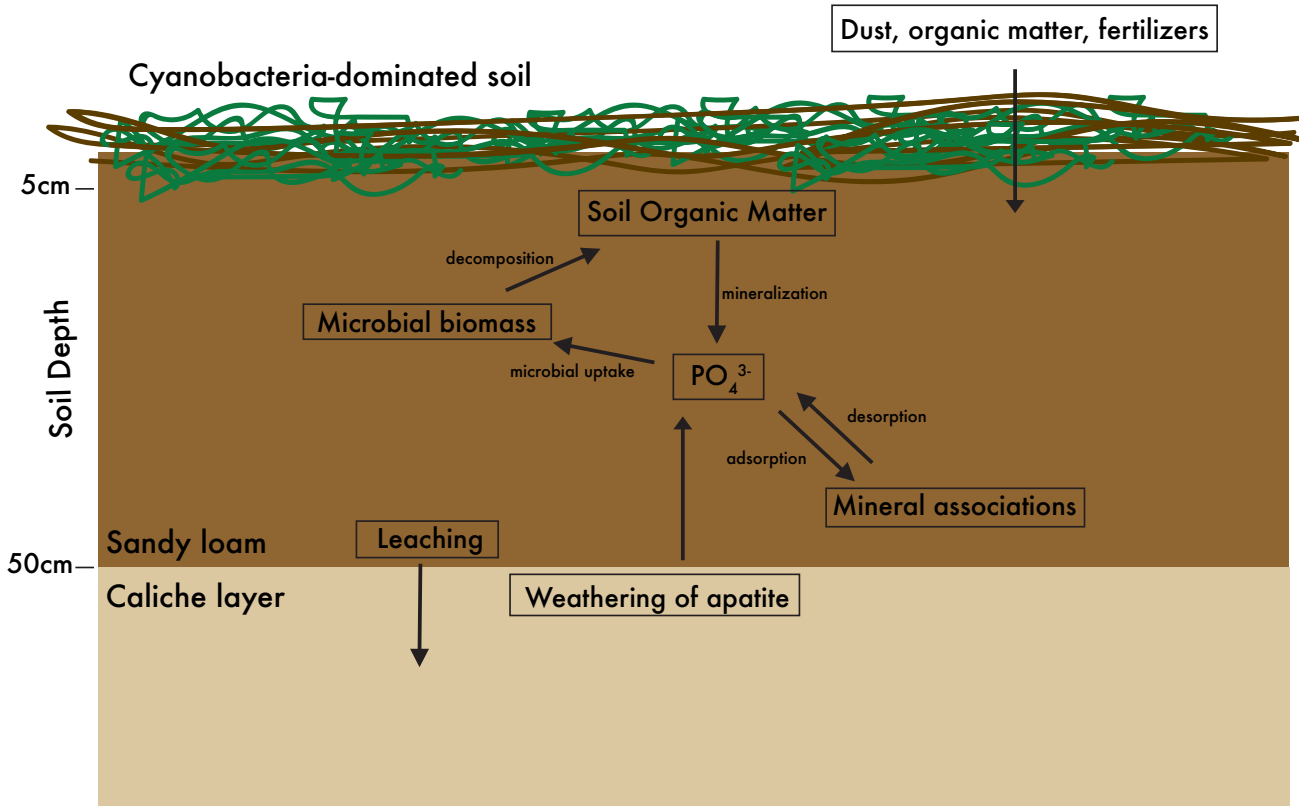

Figure 1.1 Hypothesized phosphorus cycle in a cyanobacteria-dominated soil in the Chihuahuan desert.

METHODS

2.1 Site Information and sample collection

Samples were collected from areas with intact biocrusts (crusts) and areas of disturbance (no intact crusts) from a cattle exclosure site within the Jornada Experimental Range outside Las Cruces, New Mexico (32.5876689, -106.740027). The areas of disturbance chosen for sampling previously had intact crusts but have been disturbed due to recent and constant human trampling that began approximately 18 months prior to sample collection. The intact and disturbed crust samples were collected from 12 distinct areas (six replicates for each crust type) within the exclosure at least six feet apart in all directions. The site is dominated by black grama (*Bouteloua eriopoda*) (Figure 2.1), and light cyanobacterial biocrusts mostly consisting of *Microcoleus vaginitus* (Garcia-Pichel et al. 2013).

Figure 2.1 Field site location is located within the Jornada Long Term Experimental Research Site in Las Cruces, New Mexico.

Figure 2.2. Black grama is the dominant vegetation at this site (left). Light cyanobacteria-dominated crusts on the soil surface (right).

2.2 Radiolabel Addition and Incubation

Soil cores were collected at 10 cm depth from areas with and without intact surface crusts (figure 2.2). Samples were sieved at 2 mm and 20 g fresh soil was subset into 50 mL tubes for radio-label addition and incubation, while 5 g of sample was dried for three days at 60 °C for gravimetric water content. Fresh soils were labeled with 1 mL of 0.0384kBq g^{-1} orthophosphate (Perkin Elmer Health Sciences, Sheldon, CT) and incubated at 3 °C for 24 hours.

Figure 2.3. Areas of intact surface crusts (left) versus areas of disturbance (right).

2.3 Extractions and P determination

Following the 24-hour incubation, 0.5 g of sample was subset into 15 mL tubes for the extraction procedure (4 vials per sample). Extractions were conducted in parallel by shaking sample with 10 mL of respective extractant (Table 2.1) for 3 hours (200 rev min^{-1}), followed by a centrifugation for 30 min (3020 rev min^{-1}; Deluca et al., 2015).

Following the four extractions, a 10 mL aliquot of supernatant was placed into a clean 15 mL tube for phosphate determination. If necessary, samples may be placed in the fridge (2 days maximum) or frozen for storage. Samples were diluted appropriately and analyzed colorimetrically (630 nm) using a modified malachite-green method (D'Angelo et al., 2001) on a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, Vermont, USA).

Table 2.1 Extractants used to access different forms of P that provide evidence for different microbial or plant acquisition strategies (Deluca et al. 2015).

Extractant Type	Form of P accessed	Biotic system mimicked by extraction method
0.01 M $CaCl_2$	Weakly adsorbed inorganic P	P accessed by root interception & diffusion
0.01 M citric acid	Active inorganic P sorbed to clay particles or weakly bound in inorganic precipitates	Organic acid release by plants and microorganisms
0.2 enzyme unit (wheat germ phosphatase)	Organic P readily attached by acid phosphatase enzymes	Enzyme release by plants and microorganisms to access labile organic P
1 M HCl	Soluble, active and moderately stable inorganic P adsorbed to mineral surfaces or present in inorganic precipitates.	Proton release by plants and microorganisms to access adsorbed and precipitated P.

2.3 ^{33}P Label Recovery

A 3 mL aliquot of supernatant from each sample with respective extractant was placed in a scintillation vial with 15 mL of scintillation cocktail (Ultima-Gold Scintillation Cocktail, Perkin Elmer) and counted for radioactivity using a 2900 TriCarb Liquid Scintillation Counter at the Biomolecule Analysis Core Facility (BACF) in the UTEP Biological Sciences Department. Samples were counted for two minutes; the counting was repeated three times. The average counts per minute (CPM) and disintegrations per minute (DPM) were determined by the scintillation counter and converted to kBq and used to determine the sample and introduced radioactivity. The label recovery of each sample was determined using the following equation:

$$\mathbf{^{33}P\ Recovery\ (\%) = (r/R) \times 100}$$

where r and R represent the amount of radioactivity or sample activity (kBq kg^{-1}) recovered in each individual and the total applied (introduced) radioactivity in each sample prior to incubation, respectively (Bünemann et al. 2004).

2.4 Statistics

To examine the relative sizes of phosphate pools from the BBP fractions and label recovery within each sample, mean pool sized from the 12 samples were divided by one another (e.g. citric acid / $CaCl_2$) to obtain a proportion. Uncertainty for those proportions was estimated by generating 95% bootstrap confidence intervals (Carpenter and Bithell, 2000). Bootstrap confidence intervals were calculated using the bias corrected and accelerated (BCa) method (Efron, 1987). All statistical analyses were conducted using the R software program (version 1.13.5, R Development Core Team, 2018).

RESULTS

3.1 Biologically available phosphorus pools across crust type

The phosphatase-enzyme-extractable pool was lower on average (intact: 2.07 mg kg^{-1}; disturbed: 7.12 mg kg^{-1}) compared to the citric acid-extractable (intact: 60.2 mg kg^{-1}; disturbed: 44.1 mg kg^{-1}) and HCl-extractable (intact: 58.4 mg kg^{-1}; disturbed: 72.6 mg kg^{-1}) pools in intact and disturbed areas. The $CaCl_2$ pool contained very little phosphate in both crust types (intact: 0.03 mg kg^{-1}; disturbed: 0.17 mg kg^{-1}) (Figure 3.1). Phosphorus concentration in the citric acid, enzyme, and HCl pools were higher in areas of disturbance compared to areas with intact crusts (Figure 3.1).

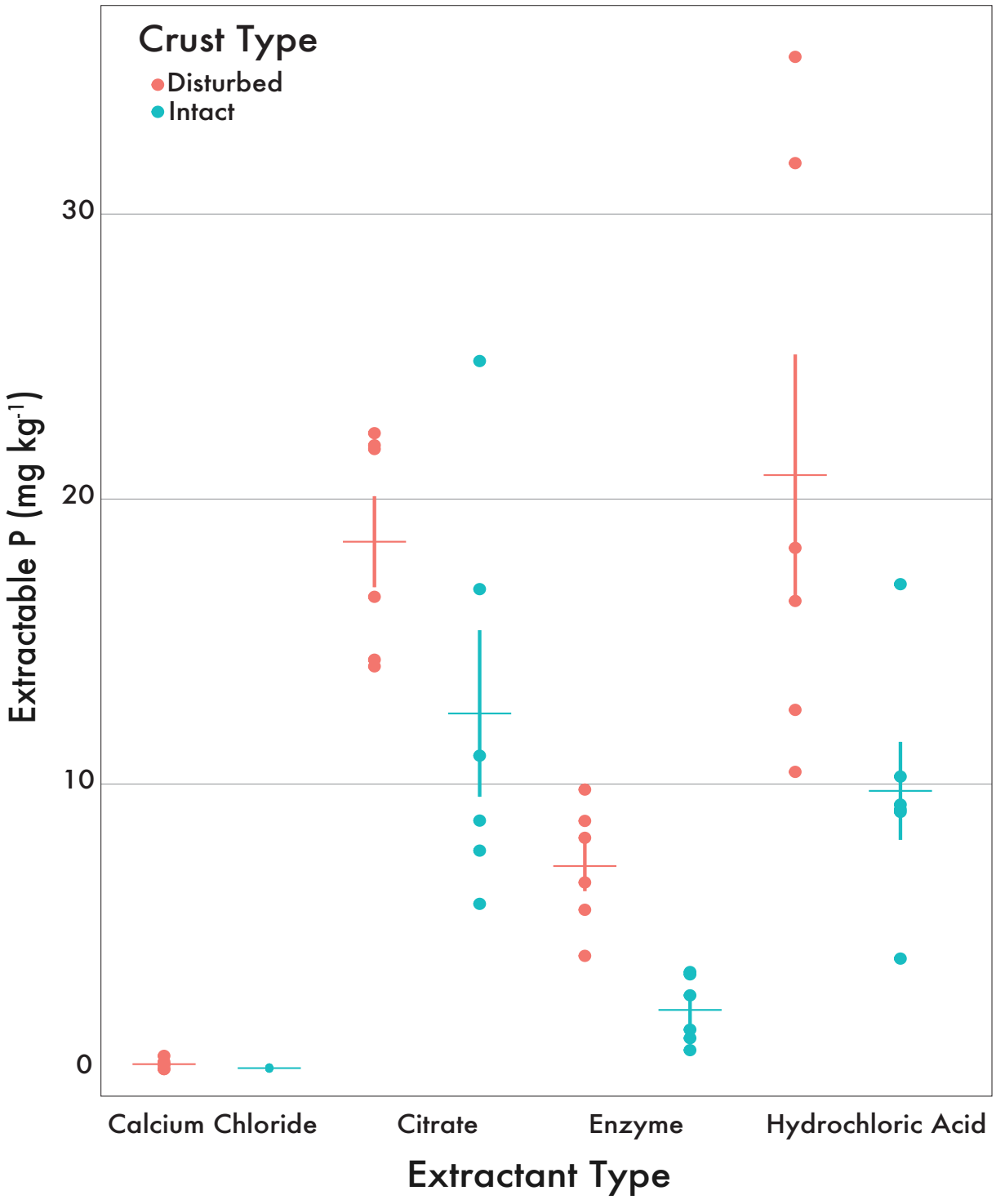

Figure 3.1 Extractable P across all four extractant types and crust types (blue = intact; pink = disturbed). The citric acid and hydrochloric acid showed the highest extractable P compared to the calcium chloride and phosphatase extractants in both crust types. The horizontal lines indicate the mean and the vertical lines indicate the standard error.

Table 3.1 shows the results of the paired bootstrap statistical analysis for the phosphate concentration by individual sample, crust types and extractant. The citric acid pool was 78% [48, 139] as large as the HCl pool in the intact areas and 113% [79, 144] in the disturbed areas, putting them at a similar magnitude. The citric acid and HCl extractants had the smallest effect size across all comparisons. When compared with the enzyme extractant, the citric acid and HCl extracts revealed comparable effect sizes between the two crust types (intact: 6.02, 4.71; disturbed: 2.60, 2.93 respectively). Extractant comparisons with $CaCl_2$ were several orders of magnitudes smaller and had a larger degree of variability from comparisons made between the citric acid, enzyme, and HCl extractants (Table 3.1). The $CaCl_2$ extractant was distinct from the other three extractants and had the least effect on P concentration across individual samples.

Table 3.1 Results of the paired bootstrap analysis across individual samples and crust type for phosphate concentration following four extractants. The asterisk (*) represents the similarity between the citric acid and HCl extractants. The carrot (^) represents the similar effects the citric acid and HCl extractants had in comparison with the enzyme extractant.

Crust Type	Comparison	Proportional difference	Lower 95% CI	upper
Intact	Citric acid / $CaCl_2$	374	211	966
	Enzyme / $CaCl_2$	62.2	32.4	245
	HCl / $CaCl_2$	293	124	667
	Citric acid / Enzyme	6.02^	4.17	7.63
	HCl / Citric acid	0.782*	0.483	1.39
	HCl / Enzyme	4.71^	3.21	7.69
Disturbed	Citric acid / $CaCl_2$	107	65.6	324
	Enzyme / $CaCl_2$	41.3	21.8	136
	HCl / $CaCl_2$	121	51.1	422
	Citric acid / Enzyme	2.60^	2.17	3.13
	HCl / Citric acid	1.13*	0.794	1.44
	HCl / Enzyme	2.93^	2.31	3.77

3.2 ^{33}P Label Recovery

On average, little ^{33}P label was recovered in the $CaCl_2$ extractable pool (5-7%), while significant amounts of label recovery were observed in the citric acid, HCl, and enzyme pools across both crust types (Figure 3.2; Table 3.2). The citric acid and HCl pools, in both intact and disturbed areas, showed more P accumulation and label recovery compared to the other two extractants with about 45-70% recovery of the added phosphorus. The citric acid pool was the only pool to show increased recovery (15%) in areas with intact crusts compared to areas of disturbance. The enzyme pool showed similar amounts of recovery in both crust types (intact: 27.6%; disturbed: 36.1%).

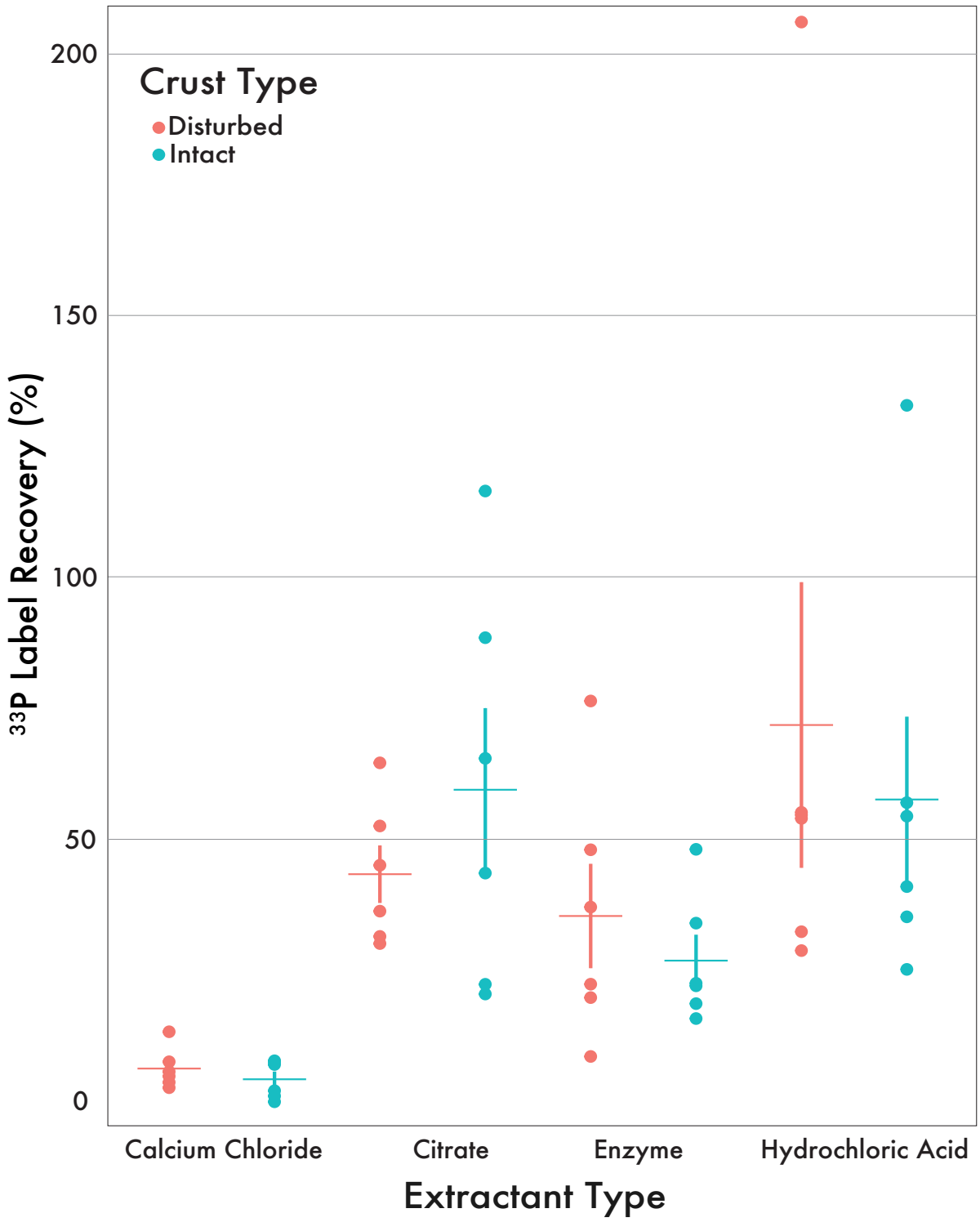

Figure 3.2 ^{33}P recovery across all four extractant types and crust type (blue = intact; pink = disturbed). The citric acid, phosphatase, and HCl pools showed the highest label recovery in comparison to the calcium chloride extractant. The horizontal lines indicate the mean and the vertical lines indicate the standard error.

Table 3.2 shows the results of the paired bootstrap statistical analysis for the ^{33}P label recovery by individual sample, soil type and extractant. The analysis revealed a similar trend as observed in the phosphorus concentration, that the citric acid pool was 96% [49, 157] as large (i.e. similar in size) as the HCl pool. These extractants were the most similar across individual samples and crust types compared to the other extractants. When compared with the enzyme extractant the citric acid and HCl extractants had similar effect sizes with little variability (intact: 2.18, 2.11; disturbed; 1.22, 2.01 respectively). Unlike the P concentration, the extractant comparisons with $CaCl_2$ revealed overall lower effect sizes when compared with the citric acid, enzyme, and HCl extractants, along with less variability (Table 3.2). The $CaCl_2$ extractant was several orders of magnitudes smaller compared to the other three extractants and had the least effect on ^{33}P label recovery across individual samples.

Table 3.2 Results of the paired bootstrap analysis across individual samples for ^{33}P label recovery among four extractants and crust types. The asterisk (*) represents the similarity between the citric acid and HCl extractants. The carrot (^) represents the similar effects the citric acid and HCl extractant had in comparison with the enzyme extractant.

Crust Type	Comparison	Proportional difference	Lower 95% CI	upper
Intact	Citric acid / $CaCl_2$	12.1	7.54	31
	Enzyme / $CaCl_2$	5.56	3.29	11.7
	HCl / $CaCl_2$	11.7	6.69	19.7
	Citric acid / Enzyme	2.18^	1.48	2.61
	HCl / Citric acid	0.969*	0.491	1.57
	HCl / Enzyme	2.11^	1.07	3.12
Disturbed	Citric acid / $CaCl_2$	6.29	4.43	11.5
	Enzyme / $CaCl_2$	5.15	2.68	9.84
	HCl / $CaCl_2$	10.4	5.13	29.8
	Citric acid / Enzyme	1.22^	0.76	1.73
	HCl / Citric acid	1.65*	1.06	2.81
	HCl / Enzyme	2.01^	1.05	3.63

Table 3.3 Results from phosphorus concentration (extractable-P) and label addition (^{33}P recovery) for each soil sample across extractants and crust type. Averages by extractant and crust type are in bold.

Sample ID	Extractant	Crust	Extractable-P (mg kg-1)	^{33}P Recovery (%)
1	$CaCl_2$	Intact	0.000	0.7
2			0.018	1.8
3			0.019	8.3
4			0.039	8.5
5			0.071	7.8
6			0.053	2.8
Average			**0.03**	**5.0**
7		Disturbed	0.000	4.4
8			0.000	14.0
9			0.253	5.5
10			0.148	3.4
11			0.171	6.4
12			0.463	8.3
Average			**0.17**	**7.0**
1	Citric acid	Intact	16.840	21.2
2			8.718	23.1
3			5.797	66.2
4			7.665	44.3
5			10.997	89.3
6			24.843	117.3
Average			**12.48**	**60.2**
7		Disturbed	16.570	30.9
8			21.756	53.3
9			21.887	45.8
10			14.130	65.4
11			14.364	32.2
12			22.312	37.1
Average			**18.50**	**44.1**
1	Enzyme	Intact	3.404	16.6
2			2.587	23.3
3			0.664	19.4
4			1.085	22.8
5			1.384	34.8
6			3.323	48.9
Average			**2.07**	**27.6**
7		Disturbed	8.708	9.3
8			8.114	37.8
9			9.807	77.2
10			5.589	48.8
11			3.972	20.6
12			6.548	23.1
Average			**7.12**	**36.1**

1	HCl	Intact	9.109	25.9
2			17.014	57.8
3			9.279	41.8
4			9.025	55.2
5			3.876	133.6
6			10.263	36.0
Average			**9.76**	**58.4**
7		Disturbed	18.286	29.5
8			35.516	54.8
9			31.787	55.4
10			16.425	206.8
11			10.428	55.9
12			12.603	33.1
Average			**20.8**	**72.6**

DISCUSSION

4.1 Biologically available P in drylands

Consistent with the lower SOM levels in dryland soils, our results show overall low average concentrations of extractable-P accessed with the enzyme extractant (mean 4.6 mg kg^{-1}) and the $CaCl_2$ extractant (mean 0.10 mg kg^{-1}), compared to concentrations from the acid-extractable extractants (mean 15.4 mg kg^{-1}). In contrast, a BBP study conducted by Deluca et al. (2015) in a series of much more mesic vegetation types across Northern Wales observed higher P concentrations in the soil solution ($CaCl_2$-extractable) and enzyme-extractable P pools, between 2 to 30 mg kg^{-1} and ~500 mg kg^{-1} respectively. This contrast suggests that our dryland system maintains relatively low levels of available P in solution that is directly accessible for microbial or plant root uptake via diffusion or transpiration-driven mass flow. We conclude that very little P is directly available in the soil solution, and that most phosphorus is surface-bound or incorporated into organic matter.

Our BBP results show that the highest concentrations of P were in the two acid-extractable pools. We found similar P concentrations following the citric acid and HCl extractions (mean 15.49 mg kg -1 and mean 15.28 mg kg -1, respectively). This similarity suggests that both acid extractants are effective at accessing adsorbed or mineral forms of P (Deluca et al. 2015; Hoang & Marschner 2017). Deluca et al. (2015) also observed a strong correlation in the acid-extractable pools ($r = 0.856$), and it is likely that the HCl-extractable pool will include P extractable via a weak acid (Hoang & Marschner 2017). We observe that release of citric acid or similar low molecular weight organic acids such as acetate or oxalate may be an adequate and energetically less costly strategy to acquire P compared to the release of a stronger acid.

Phosphatase production, in contrast to acids, may be a less suitable P acquisition strategy in drylands. Deluca et al. (2015) observed P concentrations that were about two times higher in the phosphatase-extractable pool compared to the citric acid-extractable pool (mean ~200 mg kg -1), while we observed the opposite relationship. We found overall low phosphate levels following the enzyme extraction. The citric acid-extractable pool was roughly three times higher than the phosphatase pool. However, the low P concentrations amounts still suggest the formation of phosphatase-available organic P (i.e., organic P); thus, we cannot rule out the role of enzyme-based P acquisition in our system because it has been shown that phosphatase activity will increase in response to P-deficient soils (Richardson & Simpson 2011). The phosphatase pool may suggest that P is being taken up by plants or microbes following enzyme hydrolysis but may not be the primary strategy used in drylands. Thus, our results indicate that dryland soil biota are more likely to release a weak or low molecular weight acid, like citric acid, while in a mesic system, phosphatase production may be the preferred strategy.

4.2 Short-term fate of PO_4^{3-} added to biocrust-dominated soils

The radioisotope recovery results reveal that P added to the soil solution is quickly adsorbed to mineral surfaces or subject to microbial uptake as opposed to remaining free in the soil solution. The increased radiolabel presence in the acid-extractable pools provides insight into the likelihood that the P introduced into this system is quickly sorbed, which agrees with other studies that examined recovery after 24 hours (Schneider et al. 2017). Thus, the release of organic acids, like citric acid, or a stronger acid may increase the solubility of inorganic P that is added to the system via dust deposition.

The incorporation of radiolabeled P into the enzyme-extractable pool was unexpected. The labile organic P pool in the Chihuahuan desert has been found to be low in comparison to the labile

inorganic pool (Lajtha & Schlesinger 1988). As mentioned, drylands tend to have lower levels of SOM in comparison with other systems, and phosphatase activity is highly correlated with SOM (Sinsabaugh et al. 2008) and higher levels of microbial biomass. However, we observed 25 - 40% of the ^{33}P added was found in the enzyme-extractable pool. This result suggests that the PO_4^{3-} was converted to a hydrolysable organic form during the 24-hour incubation. Microorganisms have been shown to rapidly synthesize organic P following an inorganic P addition (Tate 1984; Stewart & Tiessen 1987). Also, because arid soils are subject to P limitations due the high $CaCO_3$ content and lack of precipitation, organisms may be adapted to quickly take up and incorporate the P into biotic pools. In a similar radiolabel study in calcareous soils, it was observed that soils with low microbial biomass and low available P had a higher label recovery following a 24-hour incubation in the microbial pool (Schneider et al. 2017). We did not directly assess the microbial P pool, though the amount of recovery observed in the enzyme-extractable pool after the radiolabel addition may indicate increased microbial activity in dryland soils.

The short-term fate of added PO_4 provides insight on the path of P coming into the system via dust deposition, as dust is one of the main sources of nutrient input in drylands, and biocrusts that occupy surface soils are hypothesized to aid in the capture of P-containing dust particles (Belnap 2011). Dryland organisms may be assimilating the added inorganic P into their biomass. Crust organisms, including cyanobacteria, have the ability to mobilize inorganic phosphate (Whitton 2000) from mineral surfaces, and aquatic cyanobacteria have been found to remove orthophosphate from water sources, even at low phosphate levels (Gaffney et al. 2001). A previous study using an inorganic P addition and the BBP method to identify available P pools in a South Australian arid soil found low P concentration in the $CaCl_2$- and enzyme-extractable pools in soils grown with plants suggesting that the P was taken up by the plants and removed from the soil

(Hoang & Marschner 2017). This is comparable to what we observed in the soils with intact crusts. Active microbial communities like biocrusts may illicit a similar response in taking up added P. Hoang and Marschner (2017) also observed an increase in the $CaCl_2$-extractable and enzyme-extractable pool in bare soils compared to soils growing with plants suggesting that P released from the acid extractants may remain available in solution in the absence of plants (Hoang & Marschner 2017). However, the amount of variability between areas with and without intact surface crusts in this study does not provide strong evidence to differentiate between organismal strategies. The relative recovery of across all samples regardless of crust type represents the possibility of a variety of soil microorganisms contributing to the release and uptake of P following organic acid or enzyme release.

4.3 Bioavailable phosphorus in intact crusts versus areas of disturbance

Across all four biologically available phosphorus pools ($CaCl_2$, citric acid, enzyme and HCl- extractable P), P concentrations were higher in areas of disturbance and lower in areas with intact crusts. A possibility is that in areas with intact surface crusts, cyanobacteria and other crust organism may be rapidly taking up the P by incorporating P into their biomass or aiding in the retention of P in biotic pools, preventing a buildup on mineral surfaces in comparison to with areas of disturbance. The disturbed areas are loose soils with no intact surface crusts and the observed high concentrations in these areas may be due to the lack of microbial activity. Similarly, Hoang and Marschner (2017) found extractable-P across all pools to decrease in the presence of plants compared to bare loose soils. We cannot rule out that the disturbed areas are devoid of free-living crust organisms or other soil biota that may be contributing to microbial uptake or retention as these areas had intact crusts prior to disturbance. Though, on average, we found that areas with intact crusts have 30-80% less phosphorus compared to areas of disturbance.

Biocrusts in drylands have been shown to produce compounds such as oxalic acid, citric acid, and malic acid to release P (Belnap 2011), and cyanobacteria and microbes in this system are likely to produce a weak organic acid to access available inorganic forms of P. Biocrusts have been shown to induce bioweathering (Baumann et al. 2017) through the release of weak acidic compounds. The low concentrations in the citric acid-extractable pool in areas with intact crusts may indicate a microbial strategy to induce bioweathering through the release of a weak organic acid. As a byproduct, P is released from the mineral soil and possibly lost via hydrologic transport to roots or leaching. Bioweathering initiated by crust organisms and other soil biota may lead to an increase in P bioavailability in the system. Our data are consistent with a system in which microbes in the Chihuahuan desert are contributing to the acquisition and release of phosphorus through the production of organic acids and increasing the bioavailable P pool.

Like we observed in the acid extractions, the lower concentration in the intact surface crusts following the enzyme extraction may represent an acquisition strategy used by biocrust organisms and soil biota to access P. Biocrust organisms, specifically cyanobacteria, have the potential to increase phosphatase activity in soils by the release of extracellular phosphatases (Belnap 2011), and our data suggests that the release of phosphatases may be an additional mechanistic approach to acquire P. For example, aquatic cyanobacteria have been found to increase phosphatase activity as a response to P-limiting conditions (Whitton et al. 2005). Also, the nitrogen-fixing characteristic of cyanobacteria may increase phosphatase activity because as N uptake increases, phosphatase amount and activity may also increase resulting in an increase in available P (Belnap 2011). In a study focused on biocrust organisms and associated soils, Baumann et al. (2017) found that biocrust organisms are considerable factors influencing concentrations of accessible P in temperate soils, and crust associated organisms may excrete phosphatases to release P from Fe or Al. This is

a likely mechanism used by dryland cyanobacteria and crust organisms in the Chihuahuan desert due to the Fe and Al mineralogy, and high carbonate composition. Thus, increased phosphatase production and excretion is a likely strategy used by biocrust organisms and other dryland soil biota to access inorganic P.

Community structure and activity may influence P dynamics and bioavailable P concentrations (Alamgir & Marschner 2013; Delgado-Baquerizo et al. 2015), and we observe differences in extractable P between areas with and without intact surface crusts. Soil microbes associated with biocrusts may have similar or differing P acquisition strategies based on the community structure and soil characteristics, and with the BBP method we found that the preferred strategies may be through the release of organic acids and excretion of phosphatase, with the organic acids potentially being the most effective strategy. Overall, our study suggests that microbes within areas with intact surface crusts play a strong role in P bioavailability in dryland soils.

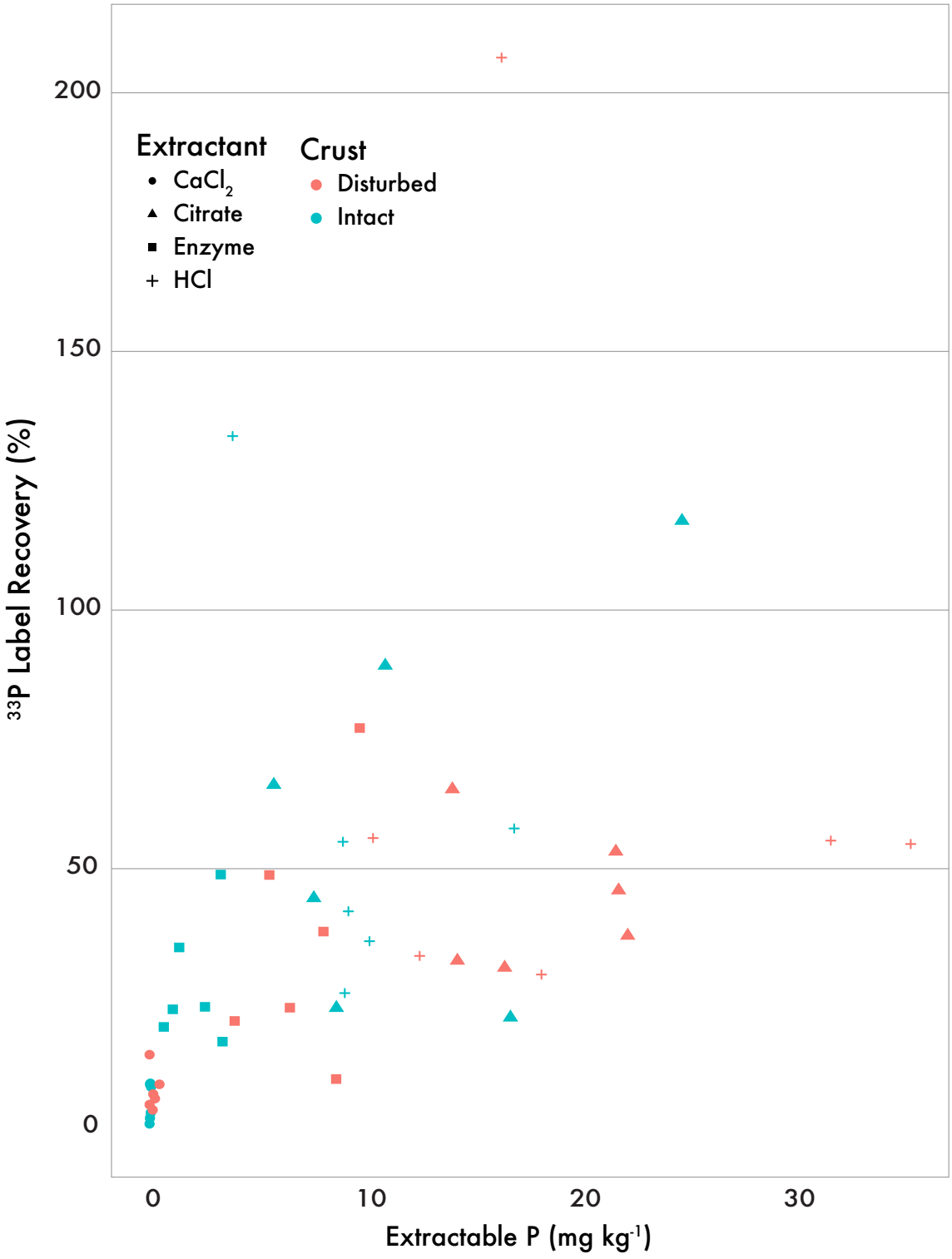

Figure 4.3 Relationship between extractable phosphorus concentrations and ^{33}P label recovery extractant and crust type.

CONCLUSION

Our BBP results revealed phosphorus stocks that were mostly bound to mineral surfaces, showing the importance of geochemical controls on P cycling in drylands. However, our data also indicated that biological processes may be the key to the release of this bound P, especially in areas with intact biocrusts. Our results show that microbes may be accessing P through the release of organic acids or phosphatase enzymes. In particular, the size of the citric acid-extractable pool suggests that organic acid exudation is a viable strategy to acquire P and is nearly as effective as the release of a stronger acid. Phosphatase activity may also be a viable strategy to acquire P as we observed substantial P concentrations in the enzyme-extractable pool. The rapid incorporation of radiolabel into the enzyme-extractable pool further suggests that the organic P pool is still an important part of the soil P stocks despite low organic matter levels. The higher radiolabel recovery observed in the acid and enzyme pools also suggests that newly added P can be quickly adsorbed or subject to microbial uptake and does not remain in the soil solution for root uptake. The P concentrations and increased recovery in the citric acid- and enzyme-extractable pools suggest that there are two mechanistic approaches used by biocrust organisms and other soil microbes to prevent the sequestration of P into the mineral soil, which may increase the uptake potential and P available for plants and other soil biota in drylands.

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