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1 # XANES demonstrates the release of calcium

2 # phosphates from alkaline Vertisols to moderately

3 # acidified solution

4 *Karl O. Andersson,* [*,†] *Matthew K. Tighe,* [†] *Christopher N. Guppy,* [†] *Paul J. Milham,* [†,‡] *Timothy*

5 *I. McLaren* [†,§] *, Cassandra R. Schefe,* [⊓] *and Enzo Lombi* [⊥]

6 [†] Agronomy and Soil Science, School of Environmental and Rural Science, University of New

7 England, Armidale, 2350, Australia.

8 [‡] Hawkesbury Institute for the Environment and the School of Science and Health, University of

9 Western Sydney, LB 1797, Penrith, NSW 2751, Australia.

10 [§] Group of Plant Nutrition, Institute of Agricultural Sciences, Swiss Federal Institute of

11 Technology (ETH) Zurich, Eschikon 33, CH-8315 Lindau, Switzerland.

12 [⊓] Schefe Consulting, 59 Sheridan Court, Rutherglen, Victoria, 3685, Australia.

13 [⊥]Future Industries Institute, University of South Australia, Building X, Mawson Lakes Campus

14 Mawson Lakes, SA, 5095, Australia.

15 ABSTRACT

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16   Calcium phosphate (CaP) minerals may comprise the main phosphorus (P) reserve in alkaline

17   soils, with solubility dependent on pH and the concentration of Ca and/or P in solution.

18   Combining several techniques in a novel way, we studied these phenomena by progressively

19   depleting P from suspensions of two soils (low P) using an anion exchange membrane (AEM),

20   and from a third soil (high P) with AEM together with a cation exchange membrane. Depletions

21   commenced on untreated soil, then continued as pH was manipulated and maintained at three

22   constant pH levels: the initial pH (pH_i), pH 6.5, and pH 5.5. Bulk P K-edge X-ray absorption

23   near-edge structure (XANES) spectroscopy revealed the main forms of inorganic P in each soil

24   were apatite, a second more soluble CaP mineral and smectite-sorbed P. With moderate

25   depletion of P at pH_i or pH 6.5, CaP minerals became more prominent in the spectra compared to

26   sorbed species. The more soluble CaP minerals were depleted at pH 6.5 and all CaP minerals

27   were exhausted at pH 5.5, showing that the CaP species present in these alkaline soils are soluble

28   with decreases of pH in the range achievable by rhizosphere acidification.

29   INTRODUCTION

30   The concentration of phosphorus (P) in the soil solution of alkaline soils is ultimately

31   controlled by the dynamics of calcium phosphate (CaP) minerals.[1,2] However, slow equilibration

32   rates and ongoing soil processes (sorption, biotic activity) also influence P availability.[3,4] The

33   most common CaP minerals found in soil (and molar Ca:P ratios), in order of decreasing

34   solubility under alkaline conditions, are dicalcium phosphate dihydrate (brushite, 1:1), dicalcium

35   phosphate (monetite, 1:1), octacalcium phosphate (OCP, 1.33:1), β-tricalcium phosphate (β-TCP,

36   1:5), hydroxyapatite (HAp, 1.67:1) and fluorapatite (FP, 1.67:1).[5] In addition, P sorption occurs

37   on the surfaces of iron (Fe) and aluminium (Al) (oxy/hydr)oxides, calcite, organic matter and

2

38   some clay minerals.[6-10] As pH decreases from pH 8, the solubility of OCP, β-TCP and apatites

39   increase more quickly than monetite and brushite.[5] In alkaline soils, P sorbs rapidly to calcite,

40   and a small surface area of sorbed P can serve as the nucleus for CaP precipitation, although

41   modified by the presence of organic ions like citrate.[7,11,12] Conversely, precipitation of Fe

42   phosphate minerals on the surface of Fe (oxy/hydr)oxides can occur when Fe minerals dissolve

43   at low pH.[13] Sorbed P forms an important pool of P that buffers solution P concentration, and a

44   recent review of P sorption studies over the last 70 years found that clay minerals may be as or

45   more important than Fe and Al (oxy/hydr)oxides for binding P.[10] It is possible that both sorption

46   and precipitation/dissolution are important processes operating at the mineral-water interface.[14]

47   Labile P that replenishes P in solution of alkaline soils is commonly measured using a sodium

48   bicarbonate extractant.[15,16] The bicarbonate soil P test is generally considered to extract P sorbed

49   to the surfaces of Fe and Al (oxy/hydr)oxides and calcite, and some labile CaP minerals.[17]

50   However, it is well established that plants access acid-soluble pools of P from alkaline and

51   neutral soils that are not extracted with bicarbonate.[18-27] Recognising this, commercial soil

52   testing of alkaline Vertisols[28] in some regions,[29-31] includes two targeted tests for soil P. For

53   example, in the Northern Grains Region of eastern Australia, soils are routinely extracted with

54   0.5 M bicarbonate,[16] (Colwell-P), and 0.005 M H_2SO_4,[32] (BSES-P). The additional quantity of P

55   generally measured in the acid extract is termed reserve-P, and is considered to consist of CaP

56   minerals as well as some sorbed forms.[33-36] The mechanism by which plants access this reserve-

57   P is likely driven by two factors: 1) the replenishment of labile-P via slow dissolution of CaP

58   minerals[37] in response to the depletion of P or Ca in the soil solution,[5] or; 2) direct solubilisation

59   of CaP minerals by a combination of rhizosphere acidification , and absorption of P and Ca from

60   the soil solution.[38-41]

3

61　　The presence of CaP minerals in soils is commonly inferred via sequential fractionation

62　　schemes (e.g. Hedley et al.[42] or Williams et al.[43]). However, the forms of P indicated by these

63　　schemes cannot differentiate between P species soluble in each chemical extract, and the

64　　schemes may introduce artefacts of chemical formation during the tests.[44-46]. Mineral solubility

65　　models can indicate the presence of a controlling mineral phase,[1,2,47] but are limited in their

66　　assessment of some mineral or sorbed phases.[3,4,46,48]

67　　Synchrotron based X-ray absorption near edge structure (XANES) spectroscopy is a powerful

68　　technique for speciating inorganic P in soils. While the approach has limitations (such as the

69　　weakness of the identification of organic P species, statistical limitations of data analysis such as

70　　linear combination fitting (LCF), and detection limit difficulties),[46,49-51] XANES has enabled

71　　different mineral and sorbed species of soil P to be identified directly, as well as providing some

72　　abundance estimates .[46,50]

73　　We previously reported on the depletion of P from six alkaline Vertisols as they were

74　　incrementally acidified, during which the concentration of P extracted increased sharply near pH

75　　6.[52] Subsequently, P extractions of three of the soils at static pH levels suggested dissolution of

76　　distinct CaP species.[53] Using XANES on one of those soils, McLaren et al.[36] showed that CaP

77　　species were removed with the BSES extraction, however a high detection limit for P at the

78　　beamline used in that study prevented a more detailed analysis. Most XANES investigations

79　　have been conducted on soils with relatively high P concentrations (e.g. > 800 mg P kg^{-1}) or with

80　　the addition of P fertiliser or P-rich amendments as the similar spectral features of CaP species

81　　and the poor spectral quality at P concentrations close to the detection limit of the beamline

82　　limits the accuracy of LCF.[44,51]　Recently, Klysubun et al.[54] reported P K–edge XANES spectra

83　　collected at low total P concentrations (~50 mg kg^{-1}) at beamline 8 (BL8) of the Synchrotron

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84   Light Research Institute (SLRI, Thailand). This enabled identification of P species[55] at

85   concentrations that may be well suited to identifying CaP in P depletion investigations. More

86   recent synchrotron facilities, such as SLRI, have the capability to analyse soil P in unspiked

87   situations, while still being able to observe treatment effects that may be difficult to detect in

88   high resolution μ-XANES facilities.[56]

89   The current study uses bulk P K-edge XANES spectroscopy at BL8 of the SLRI to investigate

90   the speciation of P in three alkaline Vertisols as P was partially depleted using anion exchange

91   membranes (AEM), or combined AEM and cation exchange membranes (ACEM), while

92   maintaining pH within narrow ranges.[53] We hypothesise that P K-edge XANES spectroscopy

93   will show that: 1) several forms of both CaP and sorbed-P species are present in each soil and

94   that some sorbed P is preferentially depleted at the initial soil pH (pH_i); and 2) some sorbed P is

95   preferentially depleted ahead of CaP.

96   METHODS

97   Soil description, chemical analysis and P depletion.

98   The soil samples have been described in detail elsewhere (see supporting information, SI).[52]

99   Briefly, soil samples (0-10 cm) were collected from sites used for cereal and oilseed cropping

100   with fertilizer inputs of <10 kg P ha^{-1} y^{-1} for at least 30 y. Soil characterisation analyses (Table

101   1) were performed as detailed in Rayment and Lyons[57]. Reserve-P was calculated as the

102   difference between Colwell-P and BSES-P.[33] Organic P was measured as the difference in the

103   concentration of P extracted from a 1:50 (g:mL) sample with 0.5 M H_2SO_4 for 16 h before and

104   after ignition at 550 °C for 1 h.[58] The oxalate tests were performed in duplicate, and all other

105   tests were performed in triplicate. Concentrations of molybdate-reactive P were measured using

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106   the malachite green method of Motomizu et al.[59] Inorganic carbon measured previously on these

107   soils was < 0.04% of soils 1 and 2, and not detected in soil 3.[52]

108   Total P was measured by portable X-ray fluorescence (PXRF) spectroscopy using a Bruker

109   Tracer IV SD according to McLaren et al.[60]. However, the PXRF signal for P in soil 3 was

110   outside the calibration range. Therefore, total P in soil 3 was measured by *aqua regia* digestion

111   followed by inductively coupled plasma optical emissions spectroscopy (See SI).[36,61]

112   **Table 1.** Initial soil pH, Colwell-P, reserve-P, Total P, molar Ca: P ratio of the BSES extract, and

113   oxalate extractable Al and Fe.

Soil	pH	Colwell-P	Reserve-P	Organic P	Total P	BSES	Oxalate	
						Ca:P	Al	Fe
	1:5w		——— mg/kg ———				— mg/kg —	
1	8.1	96	306	164	788	12.7	782	911
2	8.4	50	289	130	806	21.9	1833	990
3	7.8	79	6534	1749	8922	2.9	1473	2000

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115   Particle size distributions were measured using the pipette method after dispersing the soil in

116   sodium hexametaphosphate.[62] The clay activity ratio was calculated as the ECEC divided by %

117   clay to provide an indication of clay mineralogy.[63,64] Sample mineralogy was quantitatively

118   measured by X-ray diffraction (XRD)[65] (see SI).

119   Phosphorus was depleted from each soil in 18 h steps, for 288 h (16 cycles, soils 1 and 2) or

120   306 h (17 cycles, soil 3).[53] Briefly, P was extracted with AEM in the Cl⁻ form,[66] and from soil 3

121   with AEM plus CEM (K⁺ form), in 1:80 soil:water suspensions (see SI). The proportion of

122   reserve-P that was extracted with AEM in soil 3 was low, whereas a larger proportion of reserve-

6

123 P was extracted with ACEM. Therefore, the results of the latter are focused on in the current

124 study.

125 Phosphorus reference materials.

126 Sixteen reference materials were selected from different mineral and sorbed P phases to

127 represent a range of mineral, sorbed and organic P species (see SI).

128 Phosphorus K-edge XANES spectroscopy.

129 All samples and standard compounds were analysed by bulk XANES spectroscopy at the P K -

130 edge on BL8 of the SLRI (1.2 GeV) in Nakhon Ratchasima, Thailand, in a He filled chamber. In

131 the photon energy region for P K-edge analysis, the photon flux was approximately $2x10^{10}$

132 photon s^{-1} at 100 mA using an InSb(111) crystal.[54] The beam size at the target was 14 mm x

133 1 mm. A 13-element Ge detector was used to collect spectra in fluorescence yield mode.

134 Radiation damage was not evident as the spectra had good reproducibility.[44] At least two spectra

135 were acquired for each reference material, and three for each unknown sample.

136 Data processing and statistical analyses.

137 The concentration of P remaining in each P-depleted soil sample was calculated as the

138 difference between the total P in the untreated soil and that extracted with AEM or ACEM. The

139 concentration extracted was the cumulative total P eluted from the membranes and the P

140 remaining in solution after each extraction.

141 The XANES data were processed using Athena.[67] Replicate spectra were aligned and merged,

142 the pre-edge and post-edge were normalised from zero to one, and then calibrated to the P K-

143 edge (E_0, 2145.5 eV). A short list of reference compounds of interest for each sample was

144 selected based on visual assessment of prominent spectral features. The P species likely present

145 in the unknown samples were determined through a sequence of LCF procedures (see SI). Linear

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146   combination fitting was performed repeatedly for each unknown sample, each time excluding

147   reference materials that received negative weightings, until only components with positive

148   weights remained. The final LCF was performed between 2147.5 and 2175.5 eV (see SI). The

149   sum of weights was then normalised to 1, and the percentage of the weight assigned for each P

150   species was multiplied by the total soil P in the sediment in order to calculate an indicative

151   concentration (mg kg^{-1}). The normalised data of each spectra and fit was exported from Athena,

152   and the figures were constructed using R (version 3.1.0).[68]

153   RESULTS

154   Soil solution chemistry and physical characteristics.

155   All soils were alkaline. Concentrations of Colwell-P were greatest in soils 1 and 3. The

156   concentration of reserve-P, organic P and total P were an order of magnitude greater in soil 3

157   than in soils 1 and 2, and soil 3 had a lower Ca:P ratio (Table 1). Soil 1 contained the lowest

158   concentrations of oxalate extractable Al and Fe (Table 1) and the clay activity ratio (see SI). The

159   XRD analyses indicated and quartz were common components of the soils, with hematite and

160   apatite and feldspars (albite/anorthite, orthoclase/sanidine) also notable (see SI).

161   Soil pH manipulation and P extraction.

162   In all soils, the amount of P extracted with AEM or ACEM increased with each decrease in

163   soil pH (Table 2). The cumulative concentration of P depleted at pH 5.5 exceeded the

164   concentration of BSES-P (Table 2). The proportion of total P in the pH 5.5 sediments compared

165   to that in the untreated soils ranged from 25 – 49 % across all soils (Table 2).

166   **Table 2.** The concentration of total P in each soil after depletion at each pH level.

Soil	P remaining (mg kg^{-1})

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	Untreated	pH$_i$	pH 6.5	pH 5.5
1	788	586	460	351
2	806	719	589	395
3	8922	6861	3527	2219

167

168   Bulk P K-edge XANES spectroscopy.

169   Phosphorus K-edge XANES spectroscopy on the P reference materials revealed a prominent

170   white line energy peak (ii in Figure 1), representing excitation of 1s electrons and subsequent

171   fluorescence as the K orbital is filled, and constructive and destructive interference at higher

172   energy levels from neighbouring atoms.[46] Notwithstanding variation observed in the literature

173   between spectra collected for the same nominal material,[51] the CaP reference materials (Figure 1,

174   a and b) display the features commonly associated with CaP minerals, i.e. a post white line

175   shoulder (iii), and a secondary peak (iv).[51] The shoulder feature is commonly associated with Ca

176   content, and the secondary peak with the degree of crystallinity.[69] The secondary peak (iii) in

177   brushite was at a slightly lower energy than the other CaP minerals, as commonly observed.[51,70]

178   The increased intensity in the wide energy band (v) is attributed to oxygen oscillation.[51] The

179   spectrum of calcite-P had similar features to that observed for CaP minerals. The other sorbed P

180   species, ferrihydrite-P, hematite-P, humic-P, and smectite-P, had similar, generally featureless

181   spectra. Similarly, phytate lacked distinct spectral features although there was some change in

182   the white line energy peak (ii) and oxygen oscillation peak (v), which were broader compared to

183   the sorbed P species. The intensity pre-edge (-1 to -5 eV relative to E$_0$) of ferrihydrite-P$_3$ was

184   slightly elevated, though not to the degree of $FePO_4.4H_2O$, nor was this higher than the samples.

185   Amongst the sorbed-P species in Figure 1a, the intensity of ferrihydrite-P was slightly greater in

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186   the valley between (iii) and (iv), while humic-P was slightly lower in the oscillation range (v).

187   The remaining spectra were not included in the LCF (Figure 1c) after a preliminary fit displayed

188   distinctive features that differed from the unknown samples (Figure 2). These included

189   fluctuations in intensity between the shoulder (iii) and secondary (iv) peaks, and a distinctive

190   pre-edge feature (i) characteristic for $FePO_4.4H_2O$.[50]

191   *Figure 1 near here*

192   **Figure 1.** Normalised spectra of P reference materials analysed. a) spectra fitted to samples in

193   LCF, b) spectra used in LCF though not fitted to any samples, and c) spectra exhibiting features

194   distinctly different to the unknown samples so not included in LCF. Dashed lines indicate

195   spectral features at: i, 2143 eV (pre-edge); ii, 2146.5 eV (white line); iii, 2148.7 eV (shoulder);

196   iv, 2157 eV (secondary peak); and v, 2163 eV (oscillation).

197   The LCF provided good fits across the broader energy range even though they were performed

198   on the post-white line energy range (Figure 2). In soils 1 and 2, the secondary peak (iii) became

199   most distinct at pH 6.5. The signal to noise ratio in soils 1 and 2 decreased with P depletion at pH

200   6.5 as the sorbed P became less important (Tables 4 and 5). The shoulder and secondary peak

201   were most prominent in the untreated, the pH_i and the pH 6.5 treatments of soil 3. The shoulder

202   (iii) and secondary peaks (iv) were absent in the pH 5.5 extraction of all 3 soils, whereas the

203   white line energy peak (ii) and broad oscillation peak (v) were still present.

204   *Figure 2 near here*

205   **Figure 2.** Normalised P K-edge XANES spectra of untreated soil samples and soil residues after

206   acidification to three levels of soil pH (pH_i, pH 6.5 and pH 5.5) and removal of P using AEM

207   (soils 1 and 2) or ACEM (soil 3). Linear combination fits using standards are detailed in Tables

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208 4, 5 and 6 for the three soils. The fit to AEM pH 5.5 for soil 2 is the spectrum of smectite-P, the

209 only non-negative reference material in the LCF. Dashed lines indicate spectral features at ii,

210 2146.5 eV (white line); iii, 2148.7 eV (shoulder); iv, 2157 eV (secondary peak); and v, 2163 eV

211 (oscillation).

212   The LCF identified smectite-P as the dominant component in the untreated sample of soil 1,

213 along with apatite and the more soluble brushite (Table 3). Both CaP minerals and sorbed-P

214 contributed to the concentration of P extracted at pH_i (Table 3). Linear combination fitting

215 revealed that brushite was absent after extraction at pH 6.5, and that apatite had become the

216 dominant remaining component with an increasing indicative concentration in soil 1. In the most

217 depleted sample at pH 5.5 of soil 1, no CaP minerals were identified, while the indicative

218 concentration indicated an increase in sorbed P and the presence of humic associated P (Table 3).

219 **Table 3.** Linear combination fitting components for each treatment: LCF weight, LCF R-factor,

220 delta-R, indicative concentration of P in each phase (mg kg^{-1}), and ranked importance of the

221 reference compounds to the LCF for soil 1.

Soil	Sample		Apatite	Brushite	Humic-P	Smectite-P	R-factor
1	untreated	weight	0.188	0.091		0.766	0.03727
		delta-R	0.00296	0.00040		0.08150	
		mg kg^{-1}	142	69		578	
		rank	2	3		1	
	pHi	weight	0.329	0.105		0.637	0.02351
		delta-R	0.00983	0.00057		0.06092	
		ppm	180	57		349	
		rank	2	3		1	

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		Apatite	Brushite	Smectite-P	R-factor
pH6.5	weight	0.589		0.456	0.03148
	mg kg^{-1}	259		201	
	rank	1		2	
pH5.5	weight		0.461	0.518	0.05821
	mg kg^{-1}		165	186	
	rank		1	2	

222

223   [A]Rank determined by delta R when at least 3 reference compounds were included in the LCF,
224   otherwise determined by weight assigned by LCF or sole component.

225   The depletion of P from soil 2 resulted in similar changes to the identified components as

226   occurred in soil 1. Smectite-P was the dominant component in the untreated sample, along with

227   apatite and brushite (Table 4). All forms of soil P identified by LCF in the untreated sample

228   contributed to the concentration of P extracted at pH_i. Brushite was not identified after extraction

229   at pH 6.5 for soil 2, in which apatite was the dominant component with an increased indicative

230   concentration. Extraction of P with AEM at pH 5.5 left smectite-P as the only component

231   identified by LCF (Table 4).

232   **Table 4.** Linear combination fitting components for each treatment, LCF weight, LCF R-factor,

233   delta-R, indicative concentration of P in each phase (mg kg^{-1}), and ranked importance of the

234   reference compounds to the LCF for soil 2.

Sample		Apatite	Brushite	Smectite-P	R-factor
untreated	weight	0.427	0.067	0.589	0.0373
	delta-R	0.02125	0.00021	0.05369	
	mg kg^{-1}	318	50	438	
	rank	2	3	1	

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pH$_i$	weight	0.442	0.068	0.572	0.0475
	delta-R	0.02267	0.00001	0.04457	
	mg kg^{-1}	294	45	380	
	rank	2	3	1	
pH 6.5	weight	0.694		0.405	0.0464
	mg kg^{-1}	372		217	
	rank	1		2	
pH 5.5	mg kg^{-1}			395	
	rank			1	

235

236   The high concentration of P in the untreated sample of soil 3 was dominated by apatite, with

237   smectite-P and OCP also identified by LCF (Table 5). Depletion with ACEM at pH$_i$ extracted P

238   from all phases, leaving the sorbed-P as the least important component, while there was an

239   anomalous increase in the indicative concentration of OCP (Table 5). As with soils 1 and 2, the

240   more soluble CaP phase (OCP) was removed by extraction at pH 6.5. Also, depletion of P at pH

241   5.5 removed all CaP minerals, and in this most P depleted sample there was an apparent increase

242   in the indicative concentration of sorbed P. A minor component of brushite was also identified in

243   the pH$_i$ extraction with AEM in soil 3 (indicative contribution of 78 mg kg^{-1}), verifying the

244   components identified at the Australian synchrotron.[36]

245   **Table 5.** Linear combination fitting components for each treatment, LCF weight, LCF R-factor,

246   delta-R, indicative concentration of P in each phase (mg kg^{-1}), and ranked importance of the

247   reference compounds to the LCF for soil 3.

Sample	Apatite	OCP	Humic-P	Smectite-P	Ferrihydrite -P	R-factor

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untreated	weight	0.863	0.044		0.144		0.0154
	delta-R	0.02903	0.00008		0.00113	
	mg kg^{-1}	7326	374		1222	
	rank	1	3		2	
pH$_i$	weight	0.822	0.123		0.034		0.0029
	delta-R	0.03463	0.00051		0.00010	
	mg kg^{-1}	5761	862		238	
	rank	1	2		3	
pH 6.5	weight	0.781			0.221		0.0088
	mg kg^{-1}	2749			778	
	rank	1			2	
pH 5.5	weight			0.679		0.233	0.0314
	mg kg^{-1}			1652		567
	rank			1		2

248

249   DISCUSSION

250   Release of P into solution.

251   We identified both sorbed P and CaP phases in each of the untreated soils, highlighting the

252   complexity of assessing P mobility in these soils. The depletion of P at pH$_i$ suggests that the

253   release of P to the soil solution could occur through the process of slow replenishment of labile

254   pools from either sorbed[71] or mineral[36] phases.[72,73] Additional P was extracted at pH 6.5 and pH

255   5.5, which suggests that rhizosphere acidification could be an important process for P uptake in

256   these soils. Rhizosphere acidification of up to 2.7 pH units has been reported for various crops[74]

257   and is feasible in these soils with moderate pH buffering capacity.[52] Truog,[34] considered that P

258   soluble in dilute H_2SO_4 is readily available because the pH of saturated carbonic acid, as may be

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259 found near the root tip, is approximately 3.7. This is well below the approximate threshold of pH

260 6 where large increases in resin-extractable P were measured during a process of incremental

261 acidification.[52] In addition, the mobilisation of soil P by plants would be enhanced by the co-

262 removal of Ca, as shown by the increased concentrations of P removed with ACEM.[53] The

263 presence of organic acids (e.g. citrate) in the rhizosphere would also influence dissolution of CaP

264 minerals, as previously shown for brushite.[41] Both acidification due to proton extrusion and the

265 presence of organic acids are likely to affect P mobility of P in the rhizosphere of alkaline soils,

266 and the potential interaction is an area where research may be advanced.

267   Mineral P.

268   The acidification of soils to pH 6.5 and 5.5 resulted in the preferential extraction of CaP and

269 sorbed P phases. Increased dissolution of CaP was expected, as acidification from the initially

270 alkaline levels increases the solubility of CaP minerals.[5] The preferential depletion of the two

271 identified CaP phases was detected by the combination of XANES and the acidification

272 experiment, and would not have been detected using more standard chemical extractions.[34,46] The

273 identification of the two CaP phases of different solubility in each soil explains the increased

274 concentration of P that was extracted from soils with the addition of acid.[53] The less soluble

275 apatite may represent native CaP minerals that have low solubility at the high pH levels in these

276 soils.[5] The high rank assigned to apatite in soil 3 was supported by the Ca:P ratio in the BSES

277 extract in soil 3. This ratio is relatively close to that in CaP minerals, and much lower than

278 reported for other alkaline soils.[5,37] This suggests that CaP minerals may be the dominant forms

279 of P that comprise reserve-P in this soil.[75,76] The more soluble CaP phase in each soil, and

280 perhaps others not identified by LCF such as calcite-P or amorphous CaP, may represent

281 fertiliser reaction products that form intermediate CaP minerals that slowly transforms to less

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282   soluble forms.[77-79] The presence of more soluble CaP phases showed that at least a portion of the

283   reserve-P could be more available in the rhizosphere in alkaline soils than previously

284   considered.[33]

285   The increased concentration of CaP minerals as P was depleted from the untreated sample to

286   pH_i and pH 6.5 in soils 1 and 2 may reflect the limitations of LCF (see SI), or re-precipitation by

287   CaP. As it is unlikely that the reference materials can truly represent all components in a soil,

288   and LCF is not sensitive to minor components, errors in estimating the indicative concentrations

289   are likely.[49,75] For instance, the absence of amorphous CaP, β-TCP, monetite or calcite-P from

290   the LCF does not preclude their presence, nor can we observe their depletion. Alternatively, an

291   artefact may have been introduced during the extractions, where increased Ca in solution may

292   have precipitated P before the AEM could remove it from suspension.[80]

293   Sorbed P.

294   The identification of P sorbed to clay is an important novel finding of this research, and lends

295   support to recent re-analyses of sorption data indicating the importance of clays to P sorption.[10]

296   The change in sorbed species as P was extracted indicated that the depletion of the sorbed-P

297   phases involved more complexity than the single species (smectite-P) identified by LCF in the

298   untreated soils. The soils were dominated by the clay fraction with a high proportion of smectite ,

299   so sorption to clay surfaces is likely.[6,10,81] The preferential extraction of sorbed-P that resulted in

300   it becoming less dominant at pH 6.5 in soils 1 and 2 indicated that at least a portion of the sorbed

301   P was more readily extracted and therefore more available than the mineral CaP phases and

302   remaining sorbed phases.[82] It is likely that the extraction of this P represents P bound

303   electrostatically or by monodentate bonds.[10,83] Clays such as these Vertisols with coatings of Fe-

304   hydroxides have high P sorption capacity,[84] and may involve synergistic effects with desorption

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305   of Ca from association with (oxy/hydr)oxides in soils as pH decreases can result in decreased P

306   sorption.[6] Release of Ca associated with (oxy/hydr)oxides may explain some of the Ca that

307   yielded the high Ca:P ratios in the BSES extracts.

308   After the more labile forms of sorbed-P were removed, the concentration remaining in the

309   most P depleted samples at pH 5.5 represent forms more resistant to extraction with the

310   technique used. In so far as the approach mimicked plant uptake of P, the remaining

311   concentrations were also probably resistant to plant uptake.[66] Where the remaining P was

312   identified as smectite-P or ferrihydrite-P, it may represent occluded P, or bidentate bound P.[85]

313   Where the remaining P was identified as humic-P, it may represent ternary complexes, or may be

314   an artefact of the extraction process.[86] In soils 1 and 3, the LCF identified additional sorbed-P

315   phases. This appearance of new phases in a more depleted soil may well occur, as a phase needs

316   to comprise approximately 10-15% of the P to be identified by LCF.[49] The higher concentration

317   of oxalate extractable Fe in soil 3, compared to soils 1 and 2, supports the identification of

318   ferrihydrite–P.[5] This Fe associated P may be strongly sorbed in micropores of crystalline

319   (oxy/hydr)oxides,[87] resulting in it being present after depletion of other P phases. Due to the

320   limitations of LCF, and the absence of extra reference spectra of P sorbed to phases (such as

321   different clays, gibbsite or goethite), it is possible that other sorbed-P species were present in the

322   soil, and may play a role in P mobility at low pH.[10,49] Further investigation into the role of sorbed

323   P should be a subsequent focus of work using the combination of approaches put forward in this

324   study in addition to plant-based studies.

325   The identification of humic-P by LCF in soils 1 and 3 at pH 5.5 may also be due to relative

326   resistance to extraction at lower the pH level. Humic associated P (as opposed to the P sorbed to

327   humic substances reference material) has been identified with solution ^{31}P-NMR in alkaline

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328   Vertisols.[88] However, as the humic-P reference material XANES spectrum is broad and without

329   other defining features, its identification may represent a range of organic or possibly even

330   inorganic P species.[46,89] The forms of P identified in the most depleted samples may alternatively

331   be an artefact of the extraction process, whereby some repartitioning of dissolved P may have

332   occurred during the extractions with sorption to pH dependent surfaces or the formation of

333   ternary complexes.[86,90-92] This warrants further detailed investigation.

334   Implications for P testing and interpretation in alkaline Vertisols.

335   A number of studies[40,74,93,94] suggest that release of reserve-P by temporary rhizosphere

336   acidification is within the conditions observed in these soils, and may provide the mechanism by

337   which at least some of the P taken up by plants is accessed. Our results support previous studies

338   that the contribution of acid-soluble P to crop nutrition can be important in alkaline soils,[19,23-27]

339   as the depletion of the approximate equivalent concentration of BSES-P at pH 5.5 with a root

340   analogue occurred within the range achievable by rhizosphere acidification.[34,74] This supports the

341   use of the BSES test to estimate plant available reserve-P,[23,34] but does illustrate that the BSES-

342   P test does not discriminate between the more readily available and less readily available forms

343   of reserve-P.

344   ASSOCIATED CONTENT

345   Supporting Information.

346   Details of the XRD methodology, P depletion, XANES reference materials, XANES

347   spectroscopy settings, and LCF procedure. Results of the PSA, clay activity ratio and

348   quantitative XRD. This information is available free of charge via the internet at

349   http://pubs.acs.org.

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350   AUTHOR INFORMATION

351   Corresponding author

352   * Email kanderss@myune.edu.au; telephone +61 2 6773 3497; fax +61 2 6773 3238.

353   Author Contributions

354   The manuscript was written with contributions by all authors. All authors have given approval to

355   the final version of the manuscript.

361   Notes

362   The authors declare no competing financial interest.

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