

Article

Molecular-Scale Studies of Phosphorus Speciation and Transformation in Manure-Amended and Microdose-Fertilized Indigenous Vegetable Production Systems of Nigeria and Benin Republic

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Abstract: This study investigated the speciation, transformation and availability of P during indigenous vegetable production by employing a combination of chemical and spectroscopic techniques. The study focused upon sites in two ecozones of SSA, the Dry Savanna (Ina, Benin Republic) and Rainforest (Ilesha, Nigeria). Both sites were cultivated with two indigenous vegetable species; local amaranth (*Amaranthus cruentus* (AV)) and African eggplant (*Solanum macrocarpon* (SM)). The soils were treated with 5 t/ha poultry manure and urea fertilizer at the rate of 0, 20, 40, 60 and 80 kg N/ha. Soil samples were collected before planting and after harvest. Phosphorus *K-edge* X-ray absorption near-edge structure (XANES) spectroscopy was used to determine P speciation in these soils. Quantitative analysis showed that adsorbed and organic P were the two dominant P species in the manure amended Dry Savanna (DS) soils before planting and after harvest in soils cultivated with both AV and SM, with the addition of urea (40 kg N/ha) causing an increase in the organic P form in Dry Savanna soils cultivated with AV. Soils of the Rainforest (RF) cultivated with AV initially had large amounts of apatite P in the manure amended soils prior to planting which was transformed to adsorbed and organic P after harvest. Urea addition to the Rainforest soils shifted the dominant P species from organic P to adsorbed and apatite P, which is likely to limit P availability. Soils cultivated with SM had similar proportions of both organic and adsorbed P forms, with 40 kg N/ha addition slightly increasing the proportion of adsorbed P.

Keywords: Sub-Saharan Africa; phosphorus XANES; fertilizer microdosing; African leafy vegetables; synchrotron; sustainability; nutrient stewardship

1. Introduction

Soils of sub-Saharan Africa (SSA) are generally highly-weathered, sandy-textured, and are characterized by poor fertility [1], and low phosphorus (P) and nitrogen (N) levels [2]. These soil characteristics pose limits on agricultural productivity which in turn leads to food, household, and economic insecurity for West Africa [3-5]. With a rapid population growth rate of 3 to 5% [3], there is the need to sustainably intensify agricultural production to meet the food needs of this region. Accordingly, Integrated Soil Fertility Management (ISFM) is a package of agronomic recommendations designed to improve both soil fertility and agricultural yields. The ISFM incorporates the use of inorganic fertilizers and other organic amendments with improved crop varieties and crop rotations

[6,7]. One of the ISFM fertilization techniques is fertilizer microdosing; the precise application of relatively small quantities of fertilizer to plants near their planting holes, which reduces the volume of fertilizers farmers need to apply due to improved nutrient uptake efficiency. This technique has proven to be economical and efficient in a wide variety of agricultural systems [2,8]. Indigenous leaf vegetables such as *Amaranthus cruentus* and *Solanum macrocarpon* are an integral part of agriculture in SSA; they contribute to human nutrition, traditional medicine, and play a key role in rural economy and family subsistence [9-12]. This MicroVeg project is incorporating the fertilizer microdosing technique, to increase indigenous vegetable production in Nigeria and Benin Republic. Fertilizer microdosing has proven to increase yields in cereals across SSA but has been much less frequently utilized for vegetable crops.

The practice of microdosing is not without negatives. Since farmers are applying reduced rates of inorganic fertilizer and experiencing large yield increases, there is concern that repeated use of fertilizer microdosing may mine nutrients from the soil, reduce soil pH, and accelerate land degradation [13]. Specifically, in systems where microdosing uses urea instead of NPK fertilizer, nutrient mining of P may limit yields for microdosing. Phosphorus is essential for plant growth and development [14]. However, P is often strongly retained in the soil by chemical mechanisms such as adsorption to mineral surfaces, especially Al and Fe oxides [15,16] and precipitation as phosphate minerals with Al and Fe in acidic soils or with Ca and Mg in circumneutral soils [17]. As a result of these fixation reactions, plant-available P in the inorganic orthophosphate form (HPO_4^{2-} or H_2PO_4^-) is sparingly found in soil solution, and instead dissolved P is largely in the organic form [18], which can limit availability.

The application of manure to agricultural soils is beneficial in many ways: it provides essential macronutrients such as N and P [19-22], improves soil organic matter content [23], and helps to maintain soil quality [24]. However, manures can also alter the physical and chemical properties of the soil [25], with P minerals in the soil transforming into new phases even after short term manure application [26] and poses an environmental threat to surface waters if not properly managed. The bioavailability of P in manure and manure-amended soils varies with the type of manure and soil, the solubility of the P compounds in them, and the reaction products formed upon their application [20,27,28]. Therefore, understanding how the applied manure and nitrogen fertilizer influences the forms and plant availability of P is important in minimizing its negative effects to the environment [29] in any agricultural system.

X-ray absorption near-edge structure (XANES) spectroscopy has been used by many researchers in studying the speciation of P in soils [26,30-37]. This direct speciation technique is preferred because it is element specific and non-destructive [38] and it avoids bias in distinguishing different P species that can arise from chemical fractionation methods [39,40]. Typically, P species in soil samples can be identified and quantified using linear combination fitting (LCF), which requires a robust spectral library of P reference compounds [41,42]. The spectral features of different phosphate standard materials have been reported by several researchers [30,31,34,41,43-45] and can be used to determine overall P speciation in soils amended with organic or inorganic P fertilizer.

The objective of this study is to identify P species present in the soil before and after the cultivation of two indigenous vegetables (*Amaranthus cruentus* and *Solanum macrocarpon*) with the MicroVeg agronomic practices in two ecozones (Dry Savanna and Rain forest) of West Africa. This study utilizes a combination of both the direct solid-state speciation via synchrotron X-ray absorption spectroscopy (XAS) and indirect speciation via chemical analysis to determine linkages between availability and mobility of P and its speciation in soils.

2. Materials and Methods

The study sites were located in (a) Ina, Benin Republic ($9^{\circ} 95' N, 2^{\circ} 72' E$) which has an elevation of 380m, annual precipitation of 1073mm and a mean temperature of $26.5^{\circ}C$ and (b) Ilesha, Nigeria ($07^{\circ} 29' 21.9'' N 004^{\circ} 34' 17.3'' E$) which has an altitude of 372m, annual precipitation of 1386mm and a mean temperature of $25.5^{\circ}C$. Ina is in the Dry Savanna ecoregion, whereas Ilesha falls within the Rainforest ecoregion. Plots from both sites were cultivated with two indigenous vegetable species shown in Fig 1: Local amaranth (*Amaranthus cruentus*), and African eggplant (*Solanum macrocarpon*).



Figure 1. Photo of *Amaranthus cruentus* and *Solanum macrocarpon* growing in (a) Ilesha and (b) Ina.

The experimental plots consisted of 1 control and 4 treatments: Manure only (T0), as control, Manure+20kg/ha urea (T20), Manure+40kg/ha urea (T40), Manure+60kg/ha urea (T60), and No manure+80 kg/ha urea (T80) using a randomized complete block design. Manure was applied at 5 t/ha, and it contained 20% C, 2.5% N, and 1.5% P by weight. Soil samples were collected before planting (BP) and after final harvest (AH i.e. after 3 cycles of harvesting the vegetable leaves) at the 0-20 cm depth using a soil auger. A sub-sample of a mixed composite sample was air-dried, ground to pass through a 2 mm sieve, packaged in airtight and sealed bags, and shipped to the University of Saskatchewan in April 2017. The soils were stored in air-tight vials at room temperature and analyzed for pH, total P and available P, estimated cation exchange capacity (eCEC) and P XANES analysis. Due to the limited volume of soil samples shipped, bulk density could not be measured. Soil pH was measured in triplicate in a 2:1 water:soil suspension (Carter and Gregorich, 2008), particle size analysis was done using the hydrometer method following dispersion with sodium hexametaphosphate [46]. Organic C was determined by combustion using a Leco C/N analyzer (Leco Corp., St. Joseph, MI), total P was measured colorimetrically with an auto-analyzer (Folio AA3) in triplicate following digestion using the $H_2SO_4 - H_2O_2$ acid block digestion method of Thomas et al. (1967). Available P and eCEC were determined using the Mehlich-III extraction procedure, as described by Carter and Gregorich (2008). This method can extract multiple elements of interest to this study and is known to be appropriate for tropical soils [46]. Available P was determined from the Mehlich-III extracted soil solution on an auto-analyzer and CEC was calculated by measuring the concentrations of exchangeable Ca^{2+} , Mg^{2+} , Na^+ , and K^+ cations in vacuum filtered Mehlich-III solutions on a Microwave Plasma Atomic Absorption Spectrometer (MP-AES 4100, Agilent Technologies). Estimated CEC was then calculated as the sum of exchangeable base cations.

2.1. Phosphorus K- Edge XAS

Prior to XANES analysis, all soil samples were finely ground, homogenized and spread as a thin film (area $2\text{ cm} \pm 0.5\text{ cm}$) on graphite tape and then carefully mounted on a sample holder. Phosphorus K-edge XANES data was collected at the Soft X-ray Micro-characterization beamline (SXRMB) of the Canadian Light Source (CLS), Saskatoon, Canada. Measurements were performed with an InSb (III) double-crystal monochromator with an energy resolution of 0.5 eV in partial fluorescence mode (PFY) using a 4-element solid-state detector (Bruker Optics) windowed to the phosphorus K emission line. The beamline energy was calibrated after every beam fill using a $ZnPO_4$ reference

compound. The acquisition time for the spectrum varied between 2s to 5s, and two to three spectra were taken and merged for each sample depending on the total P concentration in the sample. All spectra were baseline corrected with a linear pre-edge (energy region: 2115.5–2140.5 eV) and edge-normalized (energy region: 2180.5–2200.5 eV) to a step height of 1.0 using the software package ATHENA (Ravel & Newville, 2005).

Linear Combination Fitting (LCF) as used by Peak (2002), Prietzel et al. (2013), Kar et al. (2017) and Hamilton et al. (2018) was used to fit the spectra of the unknown composition from the soil samples against the spectra of carefully selected reference materials. The LCF approach assumes that the spectrum from a heterogeneous soil sample is an average of all the P species in the soil. All available standard spectra were included as potential components in the LCF of each sample, but a maximum of four standards per sample was allowed in the fits. Fits were accepted when the sum of the percentages of included standards were in the range of 90% to 110%, but then normalized to 100% for comparison.

Two samples that had very different bulk XANES speciation were also imaged using the tender x-ray microprobe of the SXRMB beamline using a 4-element Vortex detector, a $10 \times 10 \mu\text{m}$ incident beam spot size with an overall $0.5 \times 0.5 \text{ mm}$ dimension, and a constant incident energy of 7200 eV to capture the regions of interest between Si and Fe. Soil samples were loaded as a dried, ground, thin layer on carbon tape. X-ray fluorescence map data analysis was processed using the SMAK software package (Sam Webb, Stanford Synchrotron Radiation Lightsource) to subtract the inline beamline I_0 and generate tricolour images to visualize co-locations of elements of interest in the samples.

2.2. Statistical analysis

The physico-chemical analysis data were analyzed using Statistical package of Social Science (SPSS Inc.) at $P \leq 0.05$ level. The significance of treatments and time effects as well as interaction of treatments and time effects was analyzed using repeated measure analysis of variance and determined significant at $P \leq 0.05$ probability levels.

3. Results

3.1. Soil physico-chemical analysis

Particle size analysis results showed that soils in both ecoregions were coarse textured, having sand content as high as 81% in Ina and 85% in Ilesha. However, the RF soils had more fine texture particles than those in DS, with clay contents as high as 33% in some samples (Tables 1,2). The soils in the DS were slightly basic to neutral in pH (6.1 to 7.0) (Table 1) while the RF soils ranged from acidic, 3.5 to slightly basic, 6.7 (Tables 2). Generally, for soils cultivated with AV, the pH reduced over time in both the DS and RF ecoregions (Tables 1 and 2). For the soils cultivated with SM, the pH significantly increased after harvest in all treatments at the DS site (Tables 1, Fig 4a) while in the RF, the pH significantly decreased over time except in the T80, i.e. the samples with heavy urea addition (Table 2, Fig 5b).

The DS soils had a significantly less total P than the RF soils. The maximum total P measured in the DS was $347.25 \text{ mg kg}^{-1}$ (Table 1), versus $1775.50 \text{ mg kg}^{-1}$ in the RF (Table 2). At both sites, the available P was a small fraction of the total P, with only 3.3% and 11% of the total P being available in the DS and RF sites, respectively (Tables 1 and 2). In the soils cultivated with AV, total and available P reduced after harvest compared to before planting at both sites (Figs 3b and 5a), while in the soils cultivated with SM, total P reduced after harvest in all treatments as available P increased at the DS site (Fig 4b, Table 1). However, in the RF, for soils cultivated with SM, total P increased in all treatments except T40 (Table 2, Fig 5a), albeit within the margin of error, while available P had a different trend, increasing in all treatments except T20 at the RF site for soils cultivated with SM (Table 2, Fig 5a).

In the *DS*, eCEC reduced with time in all treatments except for *T80* in the soils cultivated with *AV*, while the eCEC increased with time for all treatments in the soils cultivated with *SM* (Table 1). In the *RF*, the eCEC reduced with time in all treatments in soils cultivated with *AV* (Table 2), and in the soils cultivated with *SM*, eCEC increased in *T0* and *T40* and reduced in *T20* and *T80* (Table 2). The changes in eCEC positively correlated with the changes in pH (Tables 1 and 2).

3.2. Yields

The *RF* had higher vegetable yields than the *DS* for both vegetable species (Figs 2a and 2b). For the *AV*, the *T40* had the highest yield in the *RF*, while the *T60* gave the highest yield in the *DS* (Fig 2a). For the *SM*, the *T60* gave the highest yield in both ecoregions (Fig 2b). However, for economic profitability, the *T40* was considered the recommended rate.

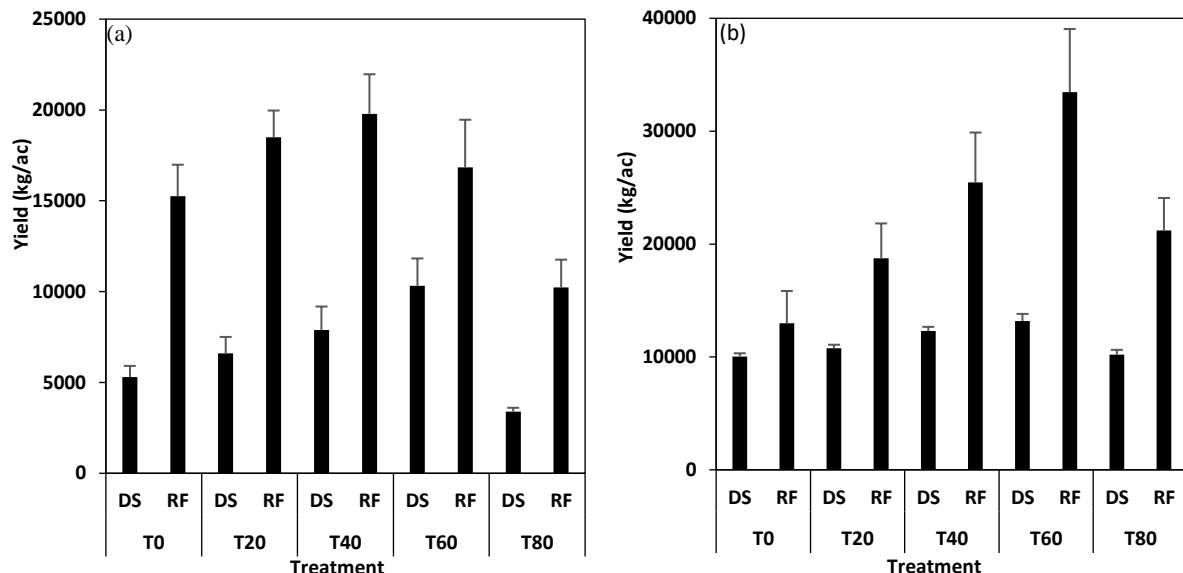


Figure 2. Average treatment yields of (a) *Amaranthus cruentus* and (b) *Solanum macrocarpon* in the Dry Savanna (DS) and Rainforest (RF) sites. A = *Amaranthus cruentus*, S = *Solanum macrocarpon*. T0 = 0kg Urea + manure treatment, T20 = 20 kg N + manure, T40 = 40 kg N + manure, T60 = 60 kg N +manure, T80 = 80 kg N + manure.

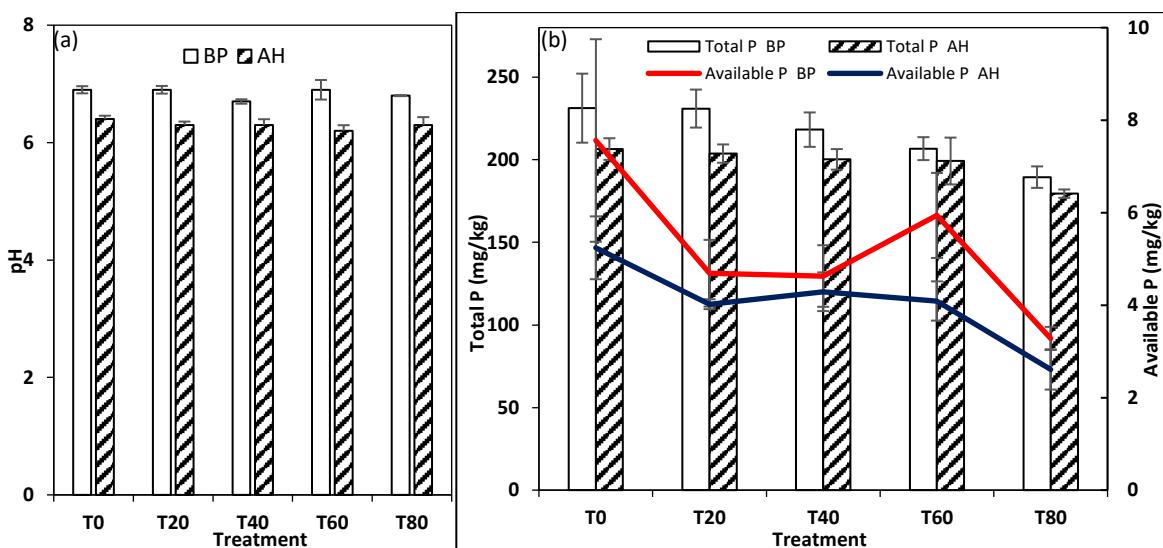


Figure 3. Effects of fertilizer treatments on (a) pH (b) total P and available P measured before planting (BP) and after harvest (AH) of *Amaranthus cruentus* cultivated on Dry Savanna soils.

Table 1. Physico-chemical properties of the INA (Dry Savanna) soils cultivated with *Amaranthus cruentus* and *Solanum macrocarpon* (n = 4).

| Treatment | pH | | Total P | | Available P | | eCEC | | Org C | | Sand | | Clay | | Silt | | Texture | | | | | | | | | | | | | | | | | | | |
|------------------------------------|--------|-----|---------|--------|-------------|------|------|------|-------|------|------|----|------|-----|------|----|---------|----|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|
| | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ----- (mg kg ⁻¹) ----- | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Amaranthus cruentus</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| T0 | 6.9 | 6.4 | 231.25 | 206.5 | 7.56 | 5.24 | 42.8 | 40.9 | 0.81 | 0.89 | 75 | 78 | 13 | 11 | 13 | 11 | SL | SL | | | | | | | | | | | | | | | | | | |
| T20 | 6.9 | 6.3 | 231 | 203.75 | 4.69 | 4.02 | 45.1 | 38.5 | 0.82 | 0.8 | 74 | 77 | 14 | 11 | 13 | 11 | SL | SL | | | | | | | | | | | | | | | | | | |
| T40 | 6.7 | 6.3 | 218.25 | 200.25 | 4.63 | 4.29 | 39.3 | 38.9 | 0.73 | 0.82 | 75 | 76 | 11 | 11 | 14 | 13 | SL | SL | | | | | | | | | | | | | | | | | | |
| T60 | 6.9 | 6.2 | 206.75 | 199.25 | 5.94 | 4.09 | 50.7 | 40 | 0.86 | 0.81 | 76 | 76 | 13 | 13 | 11 | 11 | SL | SL | | | | | | | | | | | | | | | | | | |
| T80 | 6.8 | 6.3 | 189.5 | 179.5 | 3.28 | 2.61 | 36.9 | 40.2 | 0.82 | 0.8 | 76 | 80 | 11 | 13 | 13 | 8 | SL | SL | | | | | | | | | | | | | | | | | | |
| <i>Solanum macrocarpon</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| T0 | 6.2 | 6.7 | 347.3 | 273.8 | 4.05 | 5.79 | 49 | 64 | 1.09 | 1.14 | 75 | 75 | 13 | 14 | 13 | 11 | SL | SL | | | | | | | | | | | | | | | | | | |
| T20 | 6.2 | 6.7 | 321.5 | 280.3 | 4.1 | 6.15 | 48 | 61 | 1.04 | 1.14 | 76 | 79 | 11 | 5 | 13 | 16 | SL | LS | | | | | | | | | | | | | | | | | | |
| T40 | 6.1 | 6.7 | 291 | 264 | 3.28 | 5.12 | 47 | 57 | 1.02 | 1.1 | 75 | 81 | 11 | 7.5 | 14 | 11 | SL | LS | | | | | | | | | | | | | | | | | | |
| T60 | 6.2 | 6.8 | 278.5 | 232.8 | 5.91 | 4.53 | 52 | 60 | 1.03 | 1.06 | 74 | 77 | 13 | 6.3 | 14 | 16 | SL | LS | | | | | | | | | | | | | | | | | | |
| T80 | 6.1 | 7 | 239 | 236.5 | 2.95 | 3.06 | 40 | 51 | 0.98 | 0.94 | 72 | 80 | 14 | 5 | 14 | 15 | SL | LS | | | | | | | | | | | | | | | | | | |
| Vegetables | 0.199 | | <0.001 | | 0.846 | | | | 0.004 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Treatments | 0.247 | | 0.005 | | 0.041 | | | | 0.244 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Time | 0.555 | | 0.009 | | 0.665 | | | | 0.082 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Vegetables * Treatments | 0.431 | | 0.013 | | 0.473 | | | | 0.255 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Vegetables * Time | <0.001 | | 0.157 | | 0.111 | | | | 0.411 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Treatments * Time | 0.093 | | 0.367 | | 0.281 | | | | 0.015 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Vegetables * Treatments * Time | 0.246 | | 0.166 | | 0.159 | | | | 0.199 | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Table 2. Physico-chemical properties of the ILESHA (Rainforest) soils cultivated with *Amaranthus cruentus* and *Solanum macrocarpon* (n = 4).

| Treatment | pH | | Total P | | Available P | | eCEC | | Org C | | Sand | | Clay | | Silt | | Texture | | | | | | | | | | | | | | | | | | | |
|------------------------------------|-----|-----|---------|--------|-------------|-------|------|-----|-------|------|------|------|------|----|------|----|---------|-----|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|
| | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | BP | AH | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ----- (mg kg ⁻¹) ----- | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Amaranthus cruentus</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| T0 | 6.7 | 6.3 | 1193.3 | 705.25 | 76.26 | 22.55 | 35.8 | 156 | 3.4 | 2.49 | 69.4 | 70.8 | 17 | 15 | 14 | 14 | SL | SL | | | | | | | | | | | | | | | | | | |
| T20 | 6.6 | 6.1 | 1442.8 | 950.5 | 105.1 | 22.04 | 247 | 39 | 3.8 | 4.21 | 69.3 | 65.5 | 19 | 20 | 12 | 14 | SL | SCL | | | | | | | | | | | | | | | | | | |
| T40 | 6.6 | 6 | 1775.5 | 841.25 | 195.14 | 41.73 | 303 | 217 | 3.4 | 3.57 | 70.8 | 68.9 | 15 | 16 | 14 | 16 | SL | SL | | | | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|--------------------------------|--------------|--------------|--------------|------------------|-------|--------------|------|------|-----|------|------------------|------|-----|-----|----|----|-----|-----|
| T60 | 6.3 | 5.5 | 710 | 530.25 | 64.41 | 27.81 | 150 | 128 | 2.2 | 2.48 | 74.8 | 73 | 8.8 | 13 | 16 | 14 | SL | SL |
| T80 | 5.8 | 5.8 | 603.25 | 409 | 54.85 | 16.39 | 186 | 104 | 2.1 | 2.25 | 73.6 | 74.7 | 8.8 | 8.8 | 18 | 17 | SL | SL |
| <i>Solanum macrocarpon</i> | | | | | | | | | | | | | | | | | | |
| T0 | 5.6 | 5.3 | 892 | 1164.8 | 54.48 | 64.97 | 172 | 218 | 3 | 3.49 | 63.2 | 56.5 | 19 | 26 | 18 | 18 | SL | SCL |
| T20 | 5.1 | 4.6 | 944.5 | 965.5 | 52.96 | 35.34 | 176 | 157 | 3.6 | 2.92 | 53.5 | 51.4 | 30 | 33 | 16 | 15 | SCL | SCL |
| T40 | 4.8 | 4.8 | 796.75 | 766 | 81.56 | 84.69 | 169 | 207 | 2.8 | 2.53 | 81.1 | 71.9 | 7.6 | 15 | 11 | 13 | LS | SL |
| T60 | - | 5.1 | - | 1152.5 | - | 73.19 | - | 254 | - | 3.78 | - | 67.7 | - | 23 | - | 9 | - | SCL |
| T80 | 3.5 | 4.3 | 421.75 | 431.75 | 18.18 | 26.68 | 91.7 | 61.5 | 1.8 | 1.86 | 79.9 | 85 | 10 | 5 | 10 | 10 | SL | LS |
| Vegetables | 0.001 | | 0.030 | | | 0.110 | | | | | 0.304 | | | | | | | |
| Treatments | | 0.043 | | <0.001 | | 0.095 | | | | | <0.001 | | | | | | | |
| Time | | 0.120 | | 0.171 | | 0.021 | | | | | 0.001 | | | | | | | |
| Vegetables * Treatments | | 0.010 | | 0.012 | | 0.020 | | | | | 0.011 | | | | | | | |
| Vegetables * Time | | 0.033 | | 0.005 | | 0.016 | | | | | 0.053 | | | | | | | |
| Treatments * Time | | 0.034 | | 0.454 | | 0.510 | | | | | 0.010 | | | | | | | |
| Vegetables * Treatments * Time | | 0.065 | | 0.150 | | 0.319 | | | | | 0.079 | | | | | | | |

T0 = 0kg Urea + manure treatment, T20 = 20 kg N + manure, T40 = 40 kg N + manure, T60 = 60 kg N +manure, T80 = 80 kg N + manure, BP = Before planting, AH = After harvest. SL = Sandy Loam, LS = Loamy Sand, SCL = Sandy Clay Loam. Values are significantly different at P < 0.05.

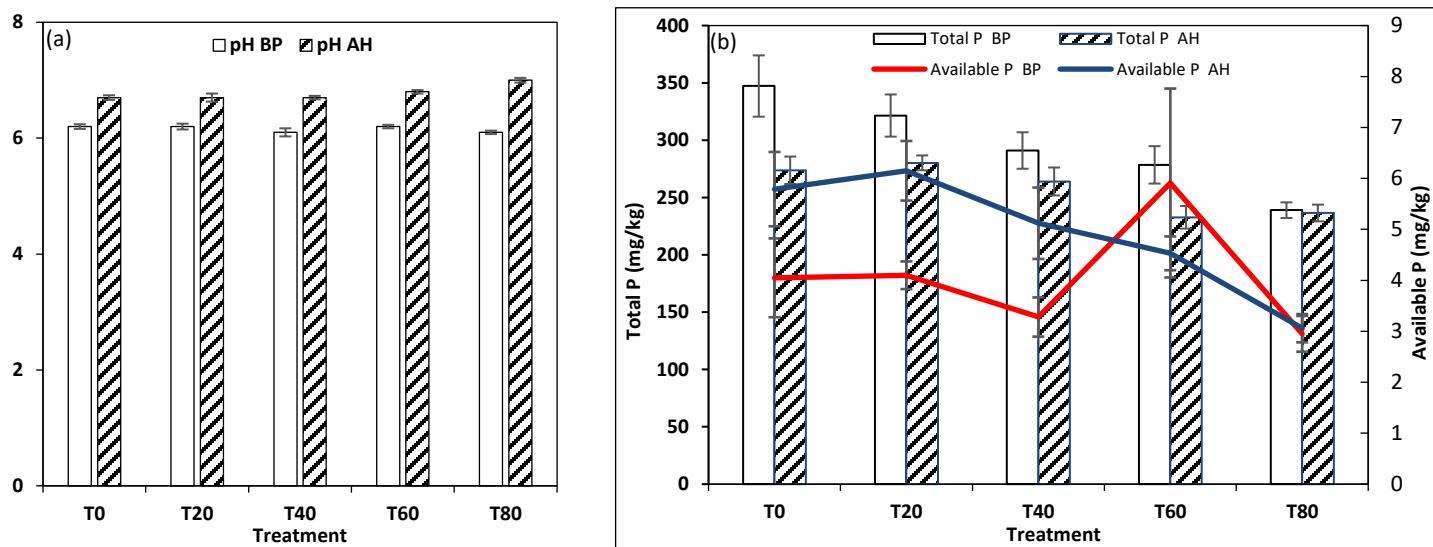


Figure 4. Effects of fertilizer treatments on (a) pH (b) total P and available P measured before planting (BP) and after harvest (AH) of *Solanum macrocarpon* cultivated on Dry Savanna soils.

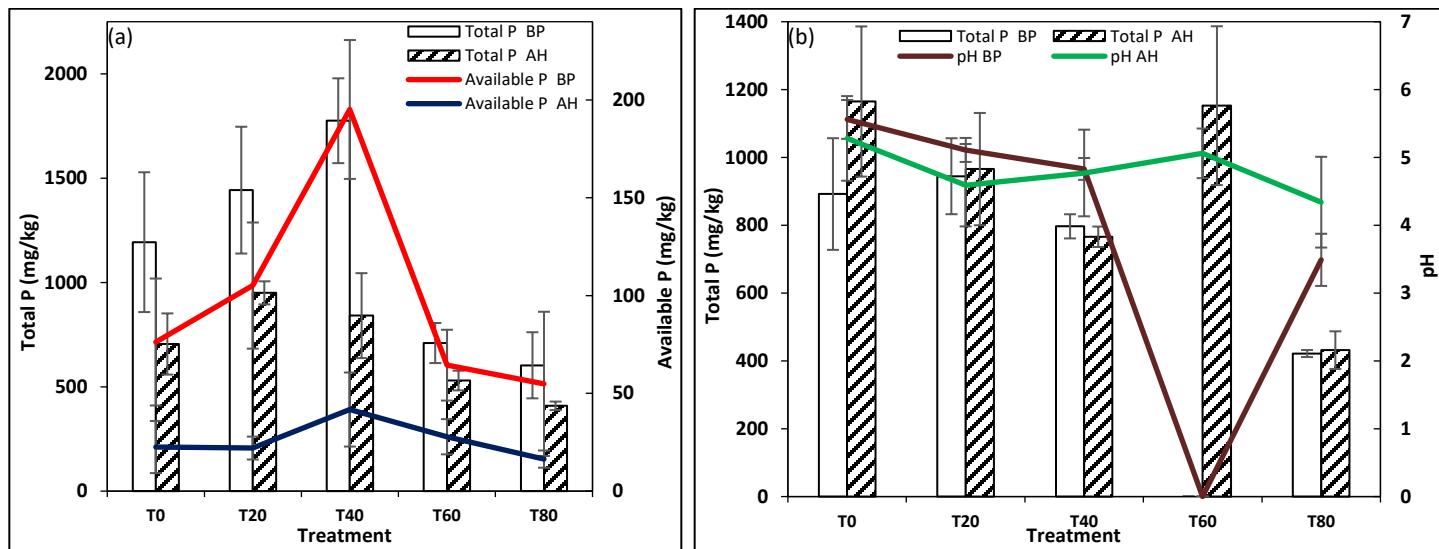


Figure 5. Time and treatment effects on (a) Total P and Available P of Rainforest soils cultivated with *Amaranthus cruentus* and (b) on pH and Total P of Rainforest soils cultivated with *Solanum macrocarpon*.

3.3. P XANES Analysis

For this study, we chose to compare the control soils, T0, with the T40 treatments as this represented the MicroVeg fertilizer recommendation in both ecoregions. The XANES spectra for all the DS soils were almost identical in shape and energy position. Visual inspection of the sample spectra presented in Fig 6a shows features that are characteristic of organic and adsorbed phosphates such as a post-white line peak at 2171.3 eV (Zuo et al., 2015). The LCF results of the DS in the AV control, AT0, showed that adsorbed P (64.9 %) and organic P (35.1 %) were the dominant P species (Table 3). Upon harvest however, both P forms slightly reduced by 1% and 2% respectively, and apatite accounting for the remaining 2.9 % of P present in the soil. In the soils amended with 40 kg N/ha of Urea (AT40), the P forms present prior to planting was adsorbed P (67.9%) and organic P (32.1 %); after harvest, however, the adsorbed P was reduced by 27.2 % while the organic P increased by 26.1 % (Table 3, Fig 6b).

Table 3. Proportion of P species in the amended soils from linear combination fitting (standard error of the fit in parentheses).

| Treatment | Goodness of fit | | | % ----- | | |
|---------------------|-----------------|----------|-----------|------------|--------------------|--------------------|
| | R factor | χ^2 | Organic P | Adsorbed P | Apatite P | |
| Ina (Dry Savanna) | AT0 | BP | 0.00162 | 0.2089 | 35.1 (± 1.4) | 64.9 (± 0.5) |
| | | AH | 0.00202 | 0.2530 | 33.1 (± 1.5) | 63.9 (± 0.5) |
| | AT40 | BP | 0.00179 | 0.2382 | 32.1 (± 1.5) | 67.9 (± 0.5) |
| | | AH | 0.0038 | 0.3863 | 58.3 (± 1.9) | 40.7 (± 0.6) |
| Ilesha (Rainforest) | ST0 | BP | 0.00231 | 0.2472 | 51.6 (± 1.5) | 48.4 (± 0.5) |
| | | AH | 0.00211 | 0.2224 | 49.1 (± 1.4) | 47.8 (± 0.5) |
| | ST40 | BP | 0.00233 | 0.2493 | 51.3 (± 1.5) | 48.7 (± 0.5) |
| | | AH | 0.00233 | 0.2529 | 49.8 (± 1.5) | 50.2 (± 0.5) |

A = *Amaranthus cruentus*, S = *Solanum macrocarpon*. T0 = 0kg Urea + manure treatment, T20 = 20 kg N + manure, T40 = 40 kg N + manure, T60 = 60 kg N +manure, T80 = 80 kg N + manure, BP = Before planting, AH = After harvest.

In the DS soils cultivated with SM, the LCF showed an almost equal distribution of organic P (51.6 %) and adsorbed P (48.4 %) (Fig 6a,b) as the P forms present prior to planting in ST0. After harvest, the adsorbed P and organic P reduced slightly to 47.8 % and 52.7% respectively, and there was the occurrence of some apatite P (3.1 %) (Fig 6a,b). In the ST40 treatment, adsorbed and organic P had even distribution as the dominant P form, accounting for 49 % and 51% respectively of P forms in the soil before planting and having no significant change after harvest.

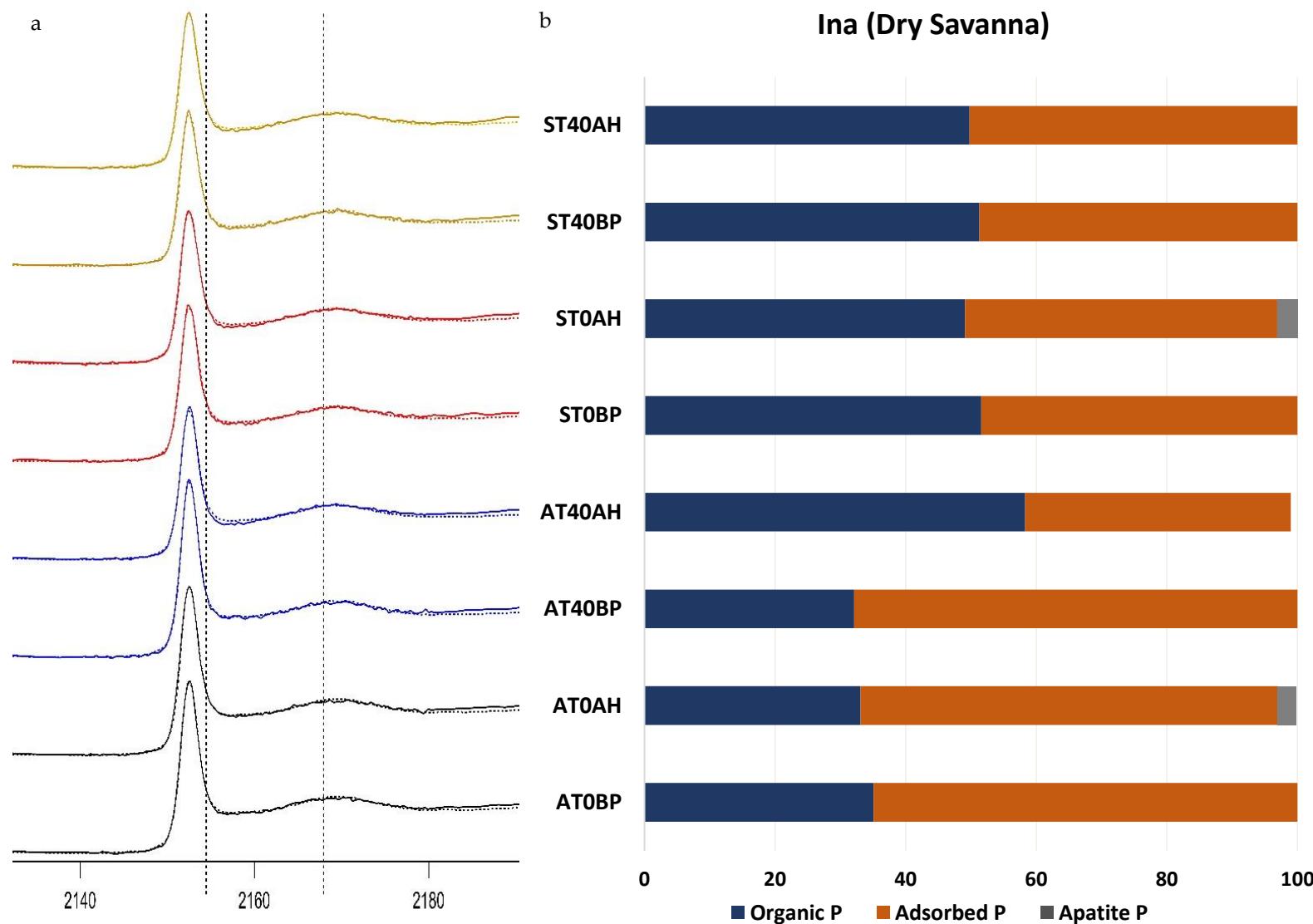


Figure 6a: Normalized P K-edge X-ray Absorption Near Edge Structure (XANES) spectra showing the Linear Combination Fitting (LCF) results of Dry Savanna (Ina) soils cultivated with *Amaranthus cruentus* (A) and *Solanum macrocarpon* (S) amended with poultry manure alone (T0), and that amended with poultry manure and 40 kg N/ha of Urea (T40). BP means before planting, AH means after harvest.

Figure 6b: Proportion of P compounds found in the LCF of soils in figure 6a.

In the *RF*, visual inspection of the sample spectra presented in Fig 7a,b show features that are characteristic of Ca phosphates such as apatite a post-edge shoulder near 2157.5 eV, and a post-white line peak at 2164.7 [47] as prominent in the soils cultivated with *AV*. LCF results showed apatite and adsorbed P to be the P forms present in *AT0* before planting, with both P forms accounting for 81 % and 18.9 % of P forms respectively (Table 3, Fig 6a,b). Upon harvest, there was a major change in the P forms found in the soil, organic and adsorbed P were the dominant P forms found after harvest, accounting for 62.9 % and 21.5 % of P forms respectively while apatite accounted for 14.7 % of the P forms present (Table 3, Fig 7a,b). In *AT40*, prior to planting, organic P accounted for 47.7 % of the P present, while adsorbed and apatite P accounted for 42.1 % and 8.1 % of the P forms present respectively (Table 3, Fig 7a,b). After harvest, there were notable changes in the P forms present, as apatite P more than tripled and accounted for 32.9 % of P forms, adsorbed P decreased to 27.5 % while organic P saw a reduction to 37.5 % of the P forms present in the soil (Fig 7a,b).

Also in the Rainforest soils cultivated with *SM*, the LCF showed adsorbed P and organic P as the dominant P forms in *ST0* before planting, at 51.4 % and 47.6 % respectively (Table 3, Fig 7a,b). After harvest (*ST0AH*), there were changes in the P speciation as organic P increased to 60.1 %, while adsorbed P decreased to 38.5% (Table 3, Fig 7a,b). In *ST40* treated soils, organic P accounted for 57.6 % of the P present in the soil prior to planting while adsorbed P accounted for 40.5%. After harvest, organic P reduced slightly to 50.9 % while adsorbed P increased, accounting for 49.1 % of the P species present (Fig 7a,b).

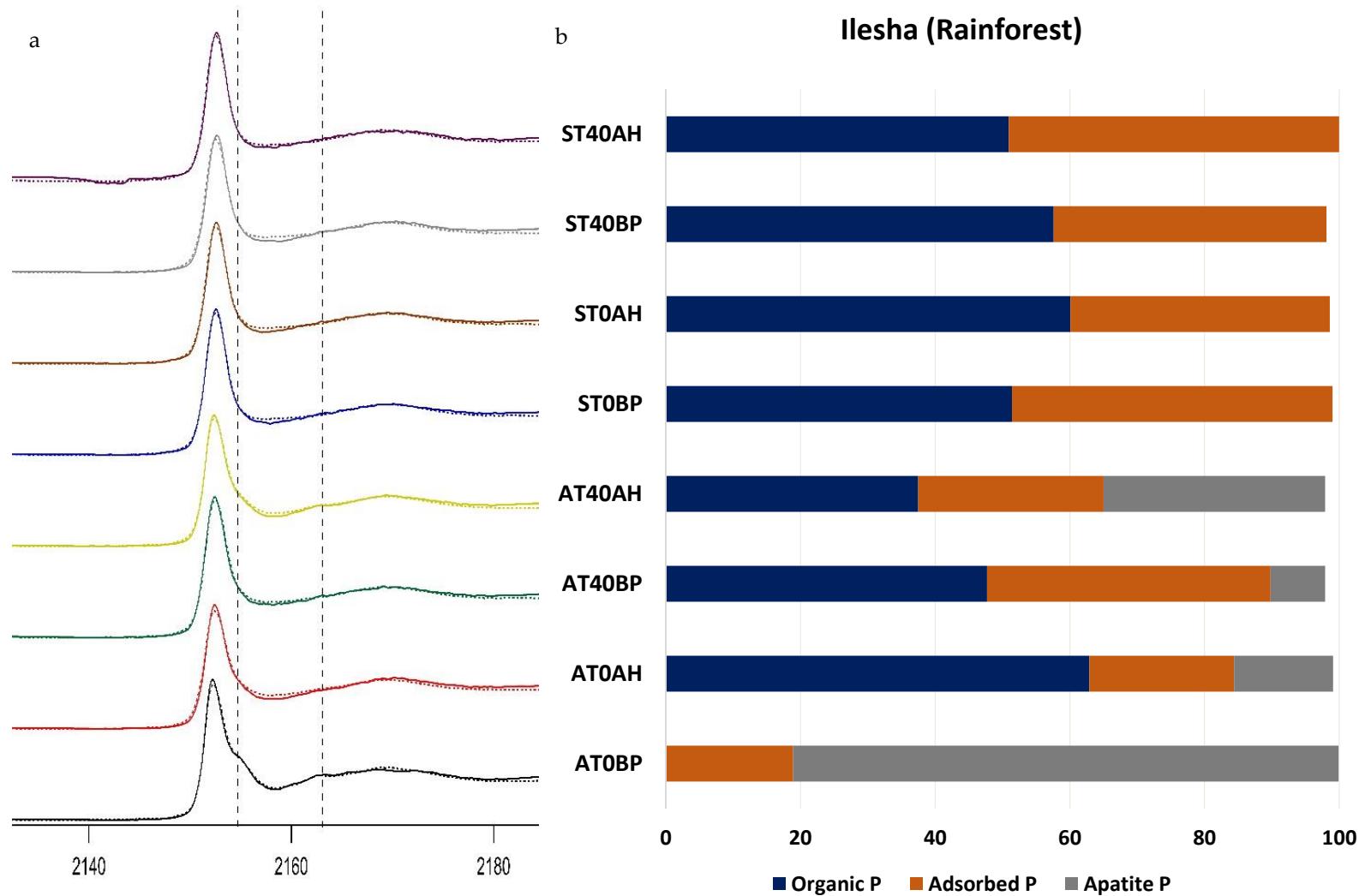


Figure 7a. Normalized P K-edge X-ray Absorption Near Edge Structure (XANES) spectra showing the Linear Combination Fitting (LCF) results of Rainforest (Ilesha) soils cultivated with *Amaranthus cruentus* (A) and *Solanum macrocarpon* (S) amended with poultry manure alone (T0), and that amended with poultry manure and 40 kg N/ha of Urea (T40). BP means before planting, AH means after harvest

Figure 7b. Proportion of P compounds found in the LCF of soils in figure 7a.

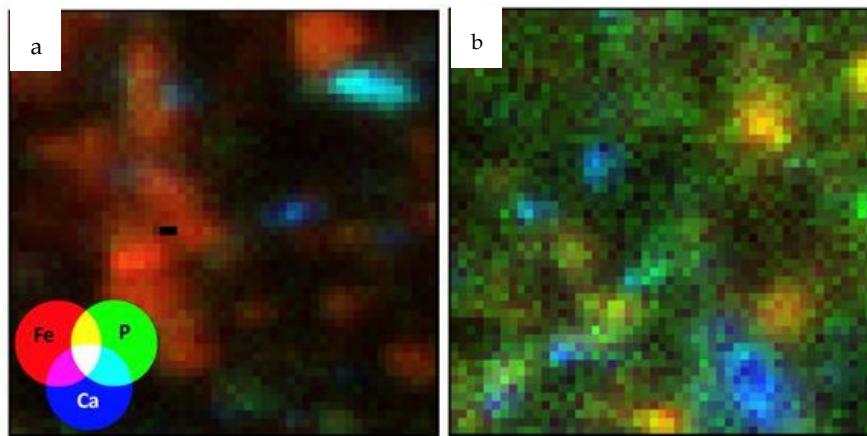


Figure 8. A tri-color map showing the distribution of phosphorus across samples (a) *RF_AT0Bp* and (b) *RF_AT40Ah*.

3.4. X-ray Fluorescence Imaging

Further investigation into the *RF* samples *AT0Bp* and *AT40Ah* on a microprobe showed that the *AT0Bp* samples have concentrated discrete particles that contain high levels of both Ca and P (turquoise “hotspots” in Fig 7a) which is consistent with the high apatite content observed by LCF analysis of the bulk XANES for this sample. In contrast, the *AT40Ah* image shows P that is both correlated with Fe (yellow regions) and spread diffusely throughout the entire sample. This is what would be expected based upon the LCF model that described the sample with P adsorbed on iron oxides and P of organic origin.

4. Discussion

The *RF* soils were generally more acidic and had higher P content than the *DS* soils (Tables 1 and 2). It is possible that the presence of Fe (as evident from the soil acidity) or higher clay content in the *RF* soils allows the binding and retention of more P. The concentrations of extracted soil P forms were affected by the interaction of treatment and time but the interaction between these two factors showed inconsistent effects for all P forms (Tables 1 and 2). In the *DS*, total P significantly reduced with increasing rates of urea addition (Figs 3b and 4b), the *T0* had significantly higher total and available P than the *T40* treatment in soils cultivated with both *AV* and *SM* (Table 1), but the decline in total and available P was more in the *T0* than in the *T40* (Table 1), suggesting that urea addition slowed down P mining in these soils. In the *RF*, soil pH declined with increasing rates of urea addition (Fig 5b), the significant reduction in the soil pH of the *T0* and *T40* treatments in soils cultivated with *AV* are correlates with increment in total P (Table 2). This suggests that urea addition is contributing to soil acidification in these treatments, and simultaneously increasing the soils total P content. There was also a significant decline in available P in both samples at harvest (Table 2). For soils cultivated with *SM*, the significant increment in the total and available P content of the soils over time in the *T0* treatment suggests that the manure added to the soil raised the soils P content over time, whereas, the decline in the total P in the urea addition (i.e. *T40* treatment), and the significant increment in available in that sample, suggests that urea addition causes P mining in this soil.

The LCF results suggested the adsorption of P to mineral phases of Fe, organic P, and Ca phases (in some of the *RF* samples). These results are in line with other studies [26,31,33] that observed a dominance of adsorbed P in manure-amended soils and highlighted the role of Fe in the chemistry of P in manure-amended soils. In the *DS*, the marginal shift in the dominant P species in the *AT0*, from 64.9 % adsorbed P and 35.1 % organic P (Table 3, Fig 6b) before planting to 63.9 % adsorbed P and 33.1 % organic P, and the occurrence of apatite P (2.9 %) after harvest (Table 3, Fig 6a) is also accompanied by a reduction in the pH, total and available P after harvest (Table 3, Fig 3a-b). This reduction in both the adsorbed and organic P species and the formation of apatite suggests a slow but gradual conversion of P into mineral (apatite) P, which may become soluble as pH decreases by rhizosphere acidification [48]. Apatite can dissolve under acidic conditions that are achievable by rhizosphere acidification and be broken-down to adsorbed P [49] which is plant accessible in alkaline and neutral soils because acidification increases solubility of Ca-P minerals, making Ca-P and sorbed P phases to be preferentially extracted [49].

In the *DS* soils amended with manure and 40 kg N/ha, the dominant P form shifted from 67.9 % adsorbed P and 32.1 % organic P to 40.7 % adsorbed P and 58.3 % organic P, indicating a significant transformation of P over time from the adsorbed form to the organic form. This is also accompanied by a reduction in pH, total and available P (Table 1, Fig 3a-b). The shift in the P phase in *AT40* from adsorbed P to organic P may be due to the microbial immobilization of the available P in the soil into the organic P phase (i.e. from adsorbed P – solution P – organic P). The accompanying reduction in the soil pH may be caused by the production of organic acids by the plant roots and/or soil microorganisms, or a product of acidification from the process of nitrification of ammonium [34] in soils treated with urea.

The same speciation of adsorbed and organic P in the *AV* cultivated soils was also observed in the P K-edge XANES results of the *DS* soils cultivated with *SM*. In *ST0*, both organic and adsorbed P are the dominant P species and accounting for 51.6 % and 48.4 % of P species respectively (Table 3, Fig 6b) prior to planting, however upon harvest, there was a slight reduction in both P species, possibly because of its conversion into apatite P, hence its occurrence with an abundance of 3.1% after harvest (Fig 6a,b). The gradual shift in the P pool from adsorbed and organic P towards apatite P (Fig 7a,b) is consistent with the findings of Kar et al. (2017), who reported the gradual conversion of adsorbed P into apatite P and Dendougui and Schwedt (2004), who noted the strong affinity of organic P to Ca minerals. This observed shift in P speciation may be due to precipitation of P with Ca^{2+} in the high CEC *ST0Ah* soil of the *DS* (Table 1) leading to a slow transformation of P into the mineral phase in this soil as the soil pH increased (Table 1, Fig 4a), increasing available P while reducing total P (Fig 4b). The *ST0Ah* LCF spectra however did not have a well pronounced shoulder feature around the 2154 to 2158 eV typical of Ca phosphates [44] possibly due to the low P concentration in the soil.

In the *ST40* treatment, there was only a marginal change in the P speciation prior to planting and at harvest, with adsorbed and organic P accounting for 40.5 % and 57.6 % of total P respectively before planting and 49.1 % and 50.9% after harvest (Fig 6a,b) although the pH and available P increased as the total P reduced (Table 1, Fig 4a,b). This decrease in total P as the soil pH and P

availability increases suggests P mining from the soil by the vegetables, hence explaining the optimum yield from the ST40 treatments (Figs 2). It is possible that as the soil microbes gets access to the N from the urea, they mineralize the organic P in the soil, making them more plant available, hence the reduction in the proportion of the organic P and total P while available P increased (Table 1). This could cause a potential increase in P susceptibility to loss by leaching or runoff in this soil.

In the *RF*, the major shift in the dominant P species in the *AT0* treatment from 81% apatite P (possibly present due to poultry manure amendment, which is known to be high in Ca-P phases [43,51,52]) and 18.9 % adsorbed P before planting to organic P (62.9 %), adsorbed P (21.5 %) and 14.7 % apatite P (Table 3, Fig 7b) after harvest strongly suggests that the dominant P cycling process here is microbial mediated immobilization. The apatite P can be expected to weather slowly due to organic acid exudates from soil microbes and/or plant roots, and then P becomes more plant available and thus taken up out of the soil. It is also possible that some of the P is lost via leaching/runoff as the P is transformed from the mineral phase into the adsorbed/organic phase, hence accounting for the reduced total and available P over time (Table 3).

In the *RF AT40* treatment, prior to planting, organic P (47.7 %) adsorbed P (42.1 %) and apatite P (8.1 %) were present. Upon harvest, the dominant P forms shifted to organic P (37.5 %) and apatite P (32.9 %), while adsorbed P decreased to 27.5 % (Table 3, Fig 7b). This suggests a conversion of organic P to the mineral P phase; this is possibly a microbial mediated process of mineralization that makes P become more plant available. However, the XANES LCF shows that mineralized P rapidly form precipitates with Ca, thereby reducing the availability of P in the soil (Table 3). Also, the huge reduction in the total P after harvest (Table 2) suggests that some of the P may be lost to the environment through erosion and runoff. Also, in the *RF*, the P K-edge XANES for *ST0* soils showed adsorbed and organic P as the only two P species present in the soils, both accounting for 47.6 % and 51.4 % of the P species respectively before planting (Table 3, Fig 6b), with a small change in the P phase occurring after harvest as organic P increased by 8.7 % while adsorbed P reduced by 9.1 %. Explanations for this include (a) either some of the adsorbed P is desorbed and then becomes immobilized by the soil microbes, or (b) as the manure remains in contact with the soil, it begins to get slowly immobilized by the soil microbes. The latter sounds more feasible, given that the soil pH slightly decreased between planting and harvest, which would favor P immobilization over P desorption. However, in the *ST40* treatments, organic P (57.6 %) and adsorbed P (40.5%) were the initial P forms (Table 3, Fig 7b), whereas organic P reduced to 50.9 % after harvest while the adsorbed P increased to 49.1 %. This shift in P phase suggests that as N becomes more available to plants and microbes in the soil, some of the organic P in the soil becomes mineralized and increased in availability (Table 2). As availability increases, some of the P may adsorb to Fe minerals given the low soil pH (Table 2) [50].

5. Conclusions

This study investigated the solid-phase speciation of P in manure amended and urea fertilized soil of the Rainforest ecozone of Nigeria and Dry savanna ecozone of Benin Republic under cultivation with two indigenous vegetable species; local amaranth (*Amaranthus cruentus*) and African eggplant (*Solanum macrocarpon*) using P K-edge XANES spectroscopy. The quantitative analysis of

the XANES spectra of the Dry Savanna soils of Ina, Benin Republic under cultivation by the local amaranth (*AV*) and the African eggplant (*SM*) showed that adsorbed P and organic P were the two dominant P species in the manure amended soils before and after planting. In the manure amended soils of the Dry Savanna, the formation of apatite after harvest as pH and total P reduced shows the gradual conversion of the organic and adsorbed P into mineral P (Ca-P) which may become soluble and dissolve under acidic conditions. It is however difficult to conclude on how N addition affects P speciation and transformation, as the addition of 40 kg N/ha caused an increase in organic P form in soils cultivated with *AV*, and an increase in adsorbed P in soils cultivated with *SM*.

In the Rainforest soils of Ilesha Nigeria, XANES analysis showed the presence of apatite P in the manure amended soils cultivated with *AV* which is then transformed via microbial immobilization into organic P. When N is added to this soil (i.e. *AT40*), XANES analysis showed a shift in the dominant P species from organic P before planting to adsorbed P and apatite P, most likely through a combination of mineralization and precipitation processes. In the soils cultivated with *SM*, organic and adsorbed P were the two dominant P species in both the manure amended (*ST0*), and the manure amended + urea fertilized soils (*ST40*). However, at harvest, there was a slow immobilization of P from the adsorbed phase into the organic phase in the manure amended soils (*ST0*), with N addition reversing this effect and mineralizing the P pool from the organic P to adsorbed P. We conclude that the N addition to the soils of both ecoregions causes P to cycle differently, thereby suggesting that we may be mining P from these soils.

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