# PHOSPHATASE HYDROLYSIS OF SOIL ORGANIC PHOSPHORUS FRACTIONS

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## Abstract

Despite the large amount of organic phosphate compounds in the soil environment, phosphorus remains a limiting factor for plant growth and development. Plant available inorganic phosphorus (Pi) is usually limited in highly weathered Ultisols. The high Fe, AL, and Mn contents in these soils enhance Pi retention and fixation. These metals are also known to form complexes with organic phosphorus (Po). Hydrolyzation of Po compounds is needed for Pi release. The ease with which the Po compounds are hydrolyzed depends on metal-Po complex strengths. Soils amended with poultry litter and inorganic nitrogen fertilizers in cropping systems may lead to the redistribution of Po and Pi concentrations and fractions amongst the metals and organic matter content. Our study was carried out to partition Po and Pi into various fractions, and to study the ease with which each Po fraction is hydrolyzed by various enzymes from different sources. Results from our study showed that the Al associated Po > Fe bound Po > Organic matter bound Po. The Pi was in the order of Fe bound > Al associated > organic matter. The ease with which the various enzymes hydrolyzed the Po was in the order of phytase > acid phosphatase > alkaline phosphatase.

## Introduction

- The chemical nature and associated reactivity of soil organic P will determine its mineralization rate.
- The predominance of inositol phosphates in soils has been linked to its fixation by Fe and Al oxides, and partly due to its association with the structural components (alkyl-C) of senescent plant materials, which limits its susceptibility to mineralization.
- Unlike inositol hexakisphosphate which is recalcitrant in the environment, orthophosphate diesters (e.g. DNA) are easily and rapidly mineralized in soil environments.
- Poultry litter and manure applications have not only created P crises in soils, but have also lead to a shift in P fractions.
- Enzymatic activities in soils have been used to evaluate soil liable organic P.
- Adding specific enzymes to each extract from a fractionation step will enable the characterization of organic P forms of significant plant available based on the assumption that specific enzyme will hydrolyze specific organic P compounds in the extracts.
- There is currently little and no comprehensive information available on the P forms and their responses to enzymatic hydrolysis in Alabama soils, yet this is urgently required to understand the biogeochemistry and potential ecosystem responses to complex organic polymers in these soils.

## Objective

The study was undertaken to assess the forms, distribution and enzyme labile fractions of P, and also to investigate the impact of management practices (tillage, cropping, manure and inorganic fertilizer applications) on the P forms.

## Materials and Methods

- Soils used in this study were collected in February of 2006, from sites that have been under experimental conditions since 1996, in an upland cotton production site at the Alabama Agricultural Experimental Station at Belle Mina, AL. with a hand probe (10 cm i.d.) from the 0 to 10 cm depth in four replicates.
- The chemical speciation of P in the soils was carried out using the detailed chemical fractionation scheme of Golterman, (1996) and Golterman et al. (1998).
- Enzyme hydrolyzation of organic P in Fe-P, Ca-P, TCA, $H_2SO_4$, and NaOH fractions was determined after neutralization of 1 mL of the supernatant to pH 5.0 for phytase from wheat germ, acid phosphatase; pH 2.5 for phytase from *Aspergillus ficuum;* and pH 10.4 for alkaline phosphatase to a final volume of 10 mL.
- The water fraction was not neutralized, in other to evaluate enzyme activities at the soil pH.

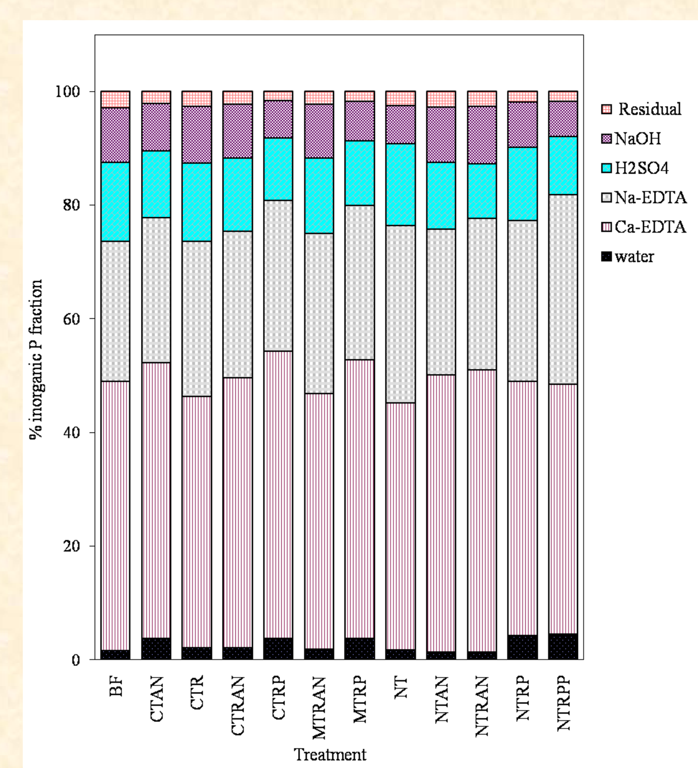

Fig. 1. Distribution of inorganic P in soil.

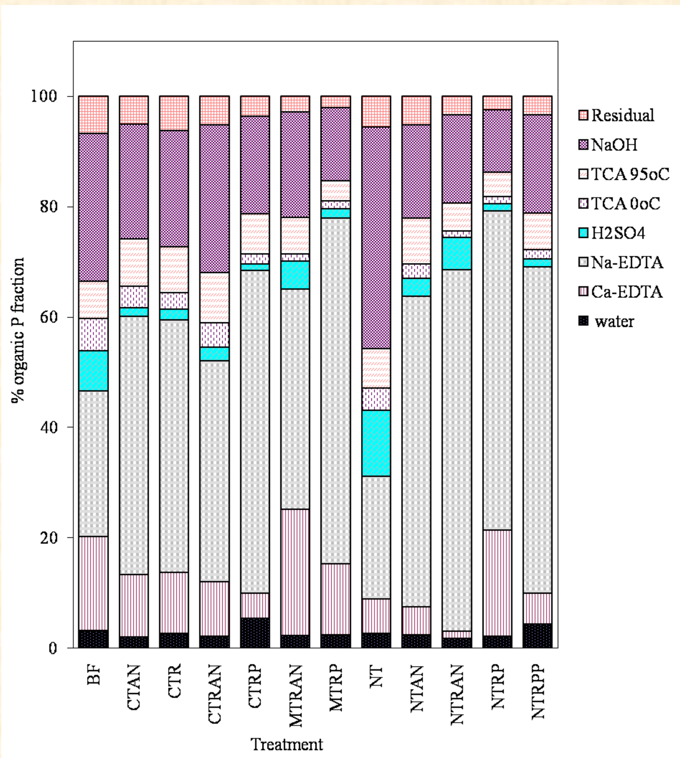

Fig. 2. Distribution of organic P in soil.

Table 1. Response of Po fractions in soils to tillage systems, conventional till (CT), no-till (NT), mulch till (MT), bare fallow (BF), and rye cropping (R) and fertilizers amendment [poultry litter (P, PP), and ammonium nitrate (AN)].

| Contrast | water | CaEDTA | NaEDTA | $H_2SO_4$ | TCA 0°C | TCA 95°C | NaOH | Residual |
|---|---|---|---|---|---|---|---|---|
| Tillage system | | | | | | | | |
| BF vs CTRP | 8.70 vs 25.7*** | 46.4 vs 22.1 ns | 72.1 vs 279 ns | 19.8 vs 5.86 ns | 15.8 vs 9.01 ns | 18.5 vs 34.3*** | 73.1 vs 84.7 ns | 18.5 vs 17.3 ns |
| BF vs NTRP | 8.70 vs 14.5 ns | 46.4 vs 131*** | 72.1 vs 392** | 19.8 vs 8.80 ns | 15.8 vs 9.01 ns | 18.5 vs 29.8** | 73.1 vs 76.6 ns | 18.5 vs 16.4 ns |
| BF vs NTRPP | 8.70 vs 24.8*** | 46.4 vs 31 ns | 72.1 vs 329* | 19.8 vs 8.34 ns | 15.8 vs 9.50 ns | 18.5 vs 37.0*** | 73.1 vs 98.9 ns | 18.5 vs 18.6 ns |
| BF vs MTRP | 8.70 vs 20.2** | 46.4 vs 103** | 72.1 vs 504*** | 19.8 vs 14.0 ns | 15.8 vs 11.7 ns | 18.5 vs 29.3** | 73.1 vs 107* | 18.5 vs 16.6 ns |
| BF vs CTRAN | 8.70 vs 6.67 ns | 46.4 vs 30.2 ns | 72.1 vs 122 ns | 19.8 vs 7.44 ns | 15.8 vs 13.5 ns | 18.5 vs 27.5 ns | 73.1 vs 81.6 ns | 18.5 vs 15.7 ns |
| BF vs MTRAN | 8.70 vs 13.6 ns | 46.4 vs 131*** | 72.1 vs 230 ns | 19.8 vs 29.1 ns | 15.8 vs 7.66 ns | 18.5 vs 38.3*** | 73.1 vs 110* | 18.5 vs 16.4 ns |
| BF vs NTRAN | 8.70 vs 8.5 ns | 46.4 vs 6.80 ns | 72.1 vs 320* | 19.8 vs 28.4 ns | 15.8 vs 5.41 ns | 18.5 vs 24.8 ns | 73.1 vs 78.2 ns | 18.5 vs 16.4 ns |

*, **, ***, Significant at $P \leq 0.05$, 0.01, and 0.001 probability levels respectively. ns (not significant); Po (organic P).

Table 2. Trend distribution of Pi fraction in soil.

| Trend | water | CaEDTA | NaEDTA | $H_2SO_4$ | NaOH |
|---|---|---|---|---|---|
| Linear | 0.001*** | 0.24 ns | 0.01** | 0.99 ns | 0.95 ns |
| Quadratic | 0.01** | 0.97 ns | 0.26 ns | 0.90 ns | 0.59 ns |
| Cubic | 0.001*** | 0.001*** | 0.001*** | 0.01** | 0.03** |
| Quatic | 0.01** | 0.03** | 0.01** | 0.05* | 0.54 ns |

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001 probability levels respectively. ns (not significant).

Table 3. Trend distribution of Po fraction in soil

| Trend | water | CaEDTA | NaEDTA | $H_2SO_4$ | TCA 0°C | TCA 95°C | NaOH | Residual |
|---|---|---|---|---|---|---|---|---|
| Linear | 0.02** | 0.40 ns | 0.01** | 0.39 ns | 0.02** | 0.07 ns | 0.16 ns | 0.38 ns |
| Quadratic | 0.99 ns | 0.42 ns | 0.62 ns | 0.19 ns | 0.27 ns | 0.37 ns | 0.05* | 0.05* |
| Cubic | 0.003*** | 0.78 ns | 0.48 ns | 0.01** | 0.67 ns | 0.001*** | 0.59 ns | 0.67 ns |
| Quatic | 0.001*** | 0.07 ns | 0.63 ns | 0.20 ns | 0.27 ns | 0.19 ns | 0.002*** | 0.63 ns |

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001 probability levels respectively. ns (not significant).

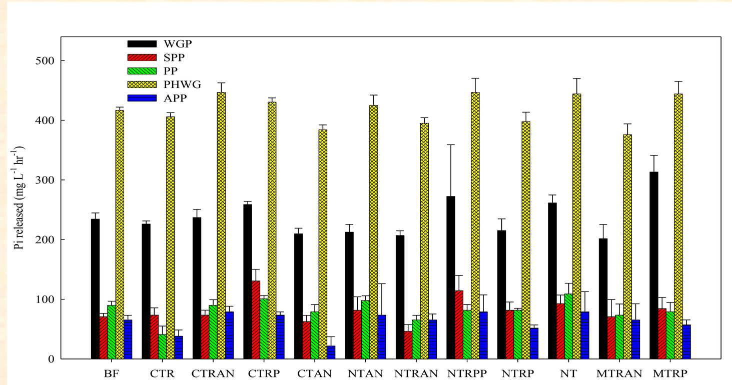

Fig. 5. Enzymatic hydrolysis of Na-EDTA soluble organic phosphorus.

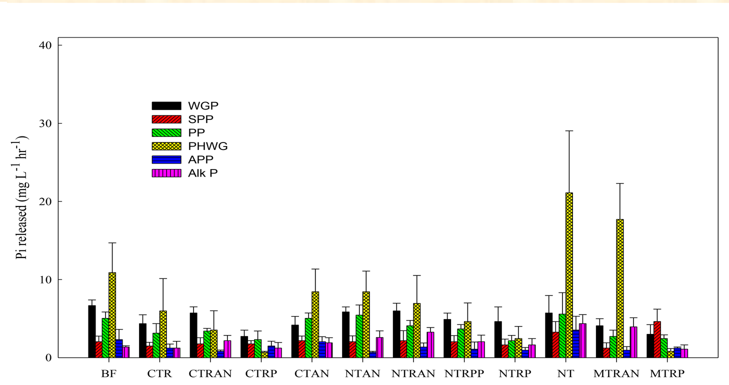

Fig. 3. Enzymatic hydrolysis of water soluble organic phosphorus.

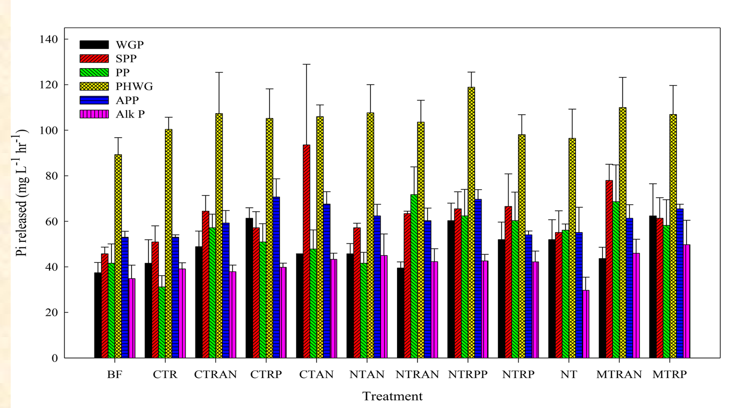

Fig. 7. Enzymatic hydrolysis of NaOH soluble organic phosphorus.

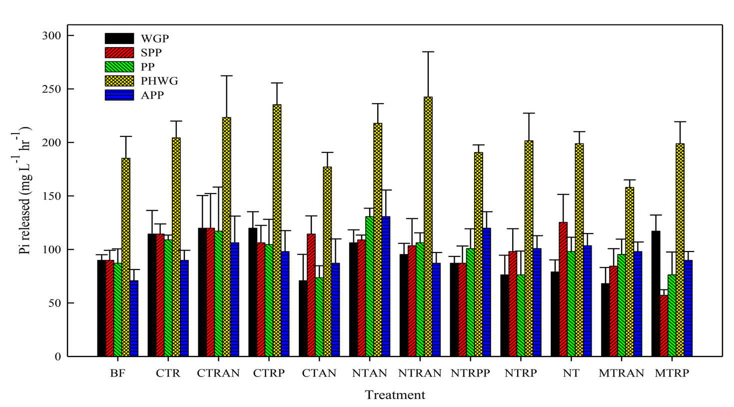

Fig. 4. Enzymatic hydrolysis of Ca-EDTA soluble organic phosphorus.

Table 4. Linear regression expressions relating Po fraction (Y) to total Po (X) in soil

| Fraction | Linear regression equation | $R^2$ | Level of significant |
|---|---|---|---|
| Water | Y = 0.63x – 0.62 | 0.30 | *** |
| Ca_EDTA | Y = 1.1x – 1.4 | 0.21 | ** |
| Na_EDTA | Y = 1.8x – 2.4 | 0.84 | *** |
| $H_2SO_4$ | Y = -0.17x + 1.4 | 0.01 | NS |
| TCA 0°C | Y = -0.05x + 1.1 | 0.003 | NS |
| TCA 95°C | Y = 0.02x + 21 | 0.21 | ** |
| NaOH | Y = 0.04x + 67 | 0.12 | * |
| Residual | Y = 0.0008x + 16.8 | 0.02 | NS |

*, **, *** Significant at $P \leq 0.05$, 0.01, 0.001; respectively. NS, (not significant); Po (organic P).

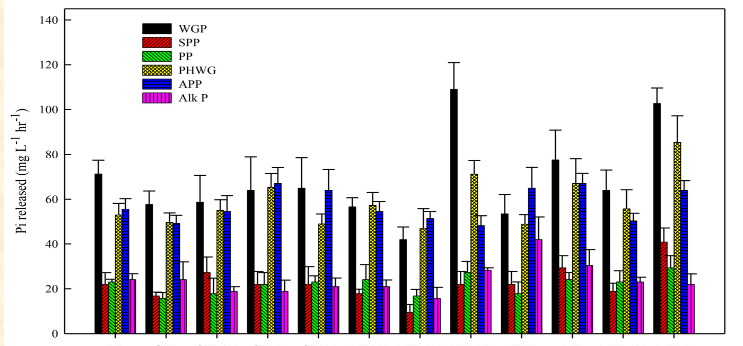

Fig. 6. Enzymatic hydrolysis of $H_2SO_4$ soluble organic phosphorus.

## Results

- The water soluble fraction was among the least distributed fraction and accounted for less than 5% of the total soil Pi (Fig. 1).
- The Ca-EDTA fraction was the most dominant fraction in the soil irrespective of treatment (Fig 1). This fraction which represents Fe associated Pi accounted for more than 43% of the total Pi in the soil.
- The water extractable Po was the least fraction accounting for less than 5% of the total soil Po (Fig. 2).
- The Ca-Al bound Po was the most abundant fraction with greater than 40% of the total Po with the exception of the control (BF) and NT soils where this fraction was less than 40% (Fig. 2).
- Approximately 15-26% of the total Po was in the NaOH fraction with the NT soils having 40% of its Po this fraction.
- Contrast analysis shows that water fraction Po in soils treated with poultry litter was significantly different from control irrespective of treatment (Table 1).
- A linear relationship was also observed for Po concentrations within each fractions and total Po except in the $H_2SO_4$, cold TCA, and residual fractions (Table 4).
- Tables 2 and 3 shows the significance of trend distribution of Pi and Po in this soil.
- Wheat germ phytase (PHWG) activity was the highest in all fractions except in the $H_2SO_4$ fraction where acid phosphatase (WGP) activity was highest (Figs. 3, 4, 5, and 6).
- This may suggest that phytic acid was the major form of hydrolyzable Po in the these fractions.

## Conclusions

- Soil P fractions gives an idea of the soil P supplying capacity to plants and the ease with which P can be leached to surface and ground water.
- Elevated level of water, Fe and Al inorganic P was observed in soils treated with poultry litter.
- The bulk of the soil Po and Pi was associated with Fe and Al oxides.
- The order in which Po fractions is dominant in this soil is Na-EDTA > NaOH > Ca-EDTA > hot TCA ≥ Residual ≥ cold TCA ≥ $H_2SO_4$.
- Bioavailable Po in soils can be investigated by using phosphate-releasing enzymes.
- Simple monoester P may have been the major hydrolysable P in the $H_2SO_4$ fraction.

### References

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