Puerto Rico 2019 Soil and Root Analyses by Depth

Contents

Introduction

The current model includes roots and 10 soil layers (as defined by hydrological models) up to 3 m. However, the behavior of roots within those layers and how they regulate P acquisition is not well constrained. One study found (citation?) that root phosphatase decreased with depth but actually increased by depth when root phosphatase is expressed per root length. Measuring root physical and physiological traits along a defined soil profile will enable a more complete understanding of root interactions with soil phosphorus availability across depths, providing data needed to parameterize the phosphorus model.

Methods

Sample Collection and Processing

On February 22nd - February 24th, 2019 we collected three soil cores per site up to a meter for soil phosphatase and bulk density. Soil phosphatase cores will also be processed for resin P and root biomass. Bulk density samples will also be processed for total P and organic P. We collected soil phosphatase cores using a 30 cm split core with a hammer and collected bulk density cores using a kit. We also collected a 30 cm core for paired root and soil phosphatase.

We returned 2/26/2019 and samples were to arrive on 2/27/2019. However, they arrived late on 2/28/2019.

Root and soil phosphatase: 2/28/2019 - 03/02/2019

Root and soil phosphatase was measured using a modified para-nitrophenyl phosphatase method. Approximately 0.5 g fresh wt of roots in 9 mls of 50 mM sodium acetate-acetic acid buffer was incubated for 1 hr in 200 rpm at 27 °C. Absorbance of the solution when terminated with 0.11 M NaOH was read at 400 nm and compared to a blank. Soil phosphatase was measured in a similar process except 1.0 g fresh soil was used with modified universal buffer. In addition, soil samples were terminated with 0.5 M NaOH and 0.5 M CaCl2.

Mycorrhizae: 2/28/2019 - 03/01/2019

Bulk density: 2/28/2019 - 03/03/2019 Bulk density samples were collected using a kit from AMR to ensure consistency. The diameter of each metal rings containing the soil was (**2.54 cm??**). Soil was removed from the metal rings and deposited into bulk density bags and oven dried at (110 °C).

Resin P: 03/01/2019 - 03/04/2019 Resin P was measuring using anion exchange membrane strips charged with sodium bicarbonate. Approximately 8 g fresh wt of soil and five strips were added to a sample cups with 80 mls diH2O and shaken vigorously (rpm) for 24 hours. Membrane strips were removed and washed with diH2O prior to shaking them with 50 ml of 0.25 M H2SO4 for 1 hr at lower speed. Phosphate concentration was measuring using the Lachat Quikchem.

Root picking for root biomass: 03/10/2019 - 03/26/2019 Roots from each depth: 0-5 cm, 7-12 cm, 12-14cm, 20-26cm, 33-47cm, and 58-88cm were picked for both transportive ($>$ 1 mm diameter) and absorptive ($<$ 1 mm diameter) roots.

Organic P: 4/15/2019 - 4/19/2019 Total soil organic phosphorus was measured using the Bowman extraction method, modified in Condron 1990. Briefly, 2 g of fresh soil was measured into a 50 ml falcon tube prior to adding 3 ml of 18 M H2SO4 added 1 ml at a time. 4 mls of diH2O was added in 1 ml increments followed by 48 mls of diH2O. Samples were vortexed in between each addition to ensure complete contact between acid, water, and soil. Samples were centrifuged then filtered. The filter was saved and added to the soil following the acid extraction for hte alkali extraction. Both the filter and the soil was shaken with 98 ml of 0.5 M NaOH for 2 hours. After centrifuging and filtering, both the acid extract and the alkali extract were read using the Lachat. **EVR organic P analysis needs to be redone**

Statistical analysis

For consistency, data are rank transformed prior to using two-way ANOVAs to discern differences among sites and depth.

To-do list

- graph organic p (& resin p) x sand:silt:clay
- combine depths to use for paired root&soil phosphatase and re-analyze
- multifactor analysis for soil phosphatase instead of pca
- fix depths so that they're consistent between graphs

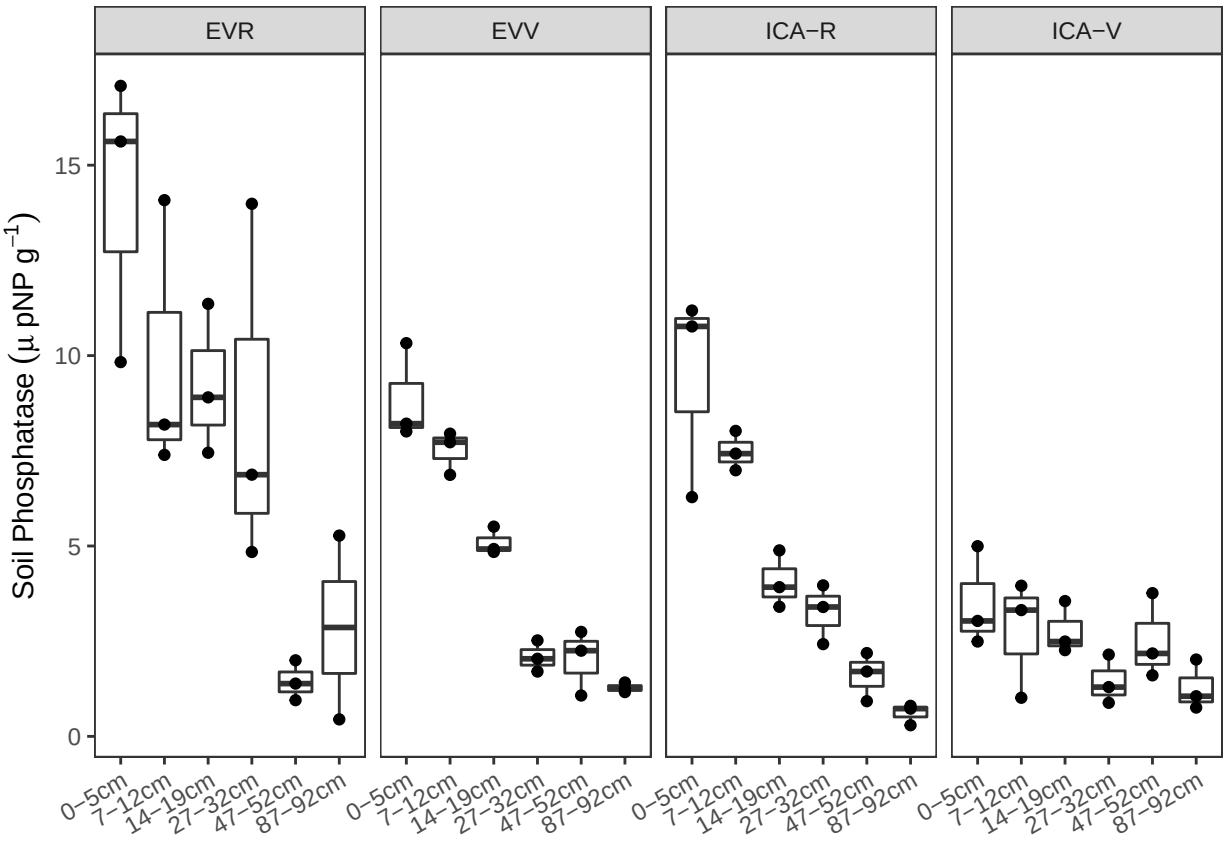


Figure 1: Fig. 1: Soil Phosphatase Boxplot by Depth

Soil Phosphatase

Soil phosphatase decreases with depth consistently among all sites,. Contrary to our hypothesis, soil phosphatase activity in ICA-R and ICA-V were lower than El Verde sites, despite previous results indicating much lower levels of available phosphorus. Interestingly, when ICA-R and ICA-V are combined as they were in a previous study, we see the same trend, where Icacos has higher phosphatase activity than EVR and EVV. In addition, previous results have suggested that Valley sites have lower phosphatase activity, perhaps due to surface runoff of phosphorus. However, here the differences between Ridge and Valley sites are not pronounced.

Summary Statistics

```
## # A tibble: 24 x 6
## # Groups:   Site [?]
##    Site  Depth       n  mean    sd    se
##    <fct> <fct>   <int> <dbl> <dbl> <dbl>
##  1 EVR   0-5cm       3 14.2  3.84  2.21
##  2 EVR   14-19cm     3  9.24 1.98  1.14
##  3 EVR   27-32cm     3  8.57 4.80  2.77
##  4 EVR   47-52cm     3  1.45 0.526 0.304
##  5 EVR   7-12cm      3  9.89 3.65  2.11
##  6 EVR   87-92cm     3  2.86 3.41  1.97
##  7 EVV   0-5cm       3  8.85 1.28  0.741
```

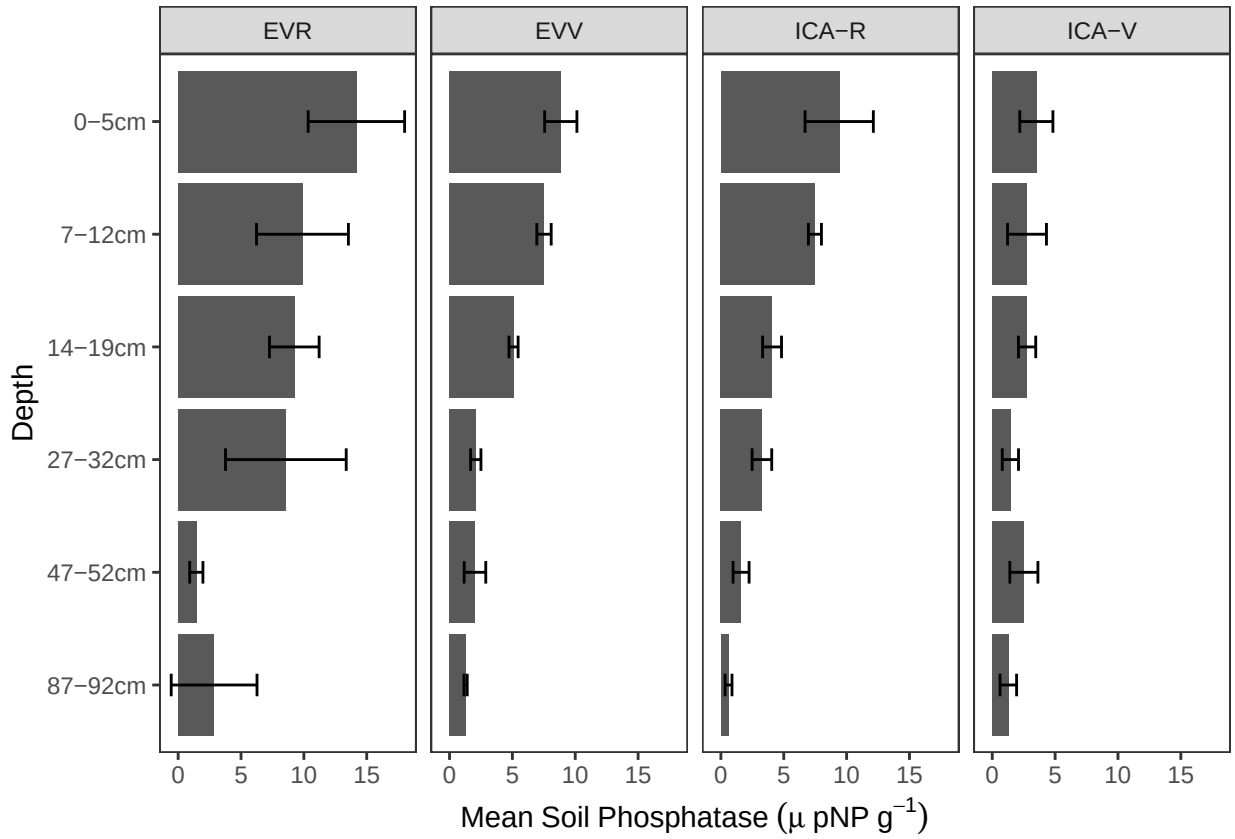


Figure 2: Fig. 2: Mean Soil Phosphatase by Depth

```
##  8 EVV   14-19cm     3  5.09 0.364 0.210
##  9 EVV   27-32cm     3  2.09 0.411 0.237
## 10 EVV   47-52cm     3  2.02 0.858 0.496
## # ... with 14 more rows
```

ANOVA

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## Site         3   5764  1921.2   17.29 9.49e-08 ***
## Depth        5  15436  3087.1   27.79 4.21e-13 ***
## Site:Depth  15   4566   304.4    2.74  0.00413 **
## Residuals   48   5333   111.1
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Combined Icacos Plot

Paired Root and Soil Phosphatase

Root and soil phosphatase was analyzed using a two-way repeated measures MaNOVA to determine whether phopshatase activity depended on whether it was measured from roots or soil and to discern differences in activity by site and depth. Root phosphatase and soil phosphatase, when standardized to soil volume were

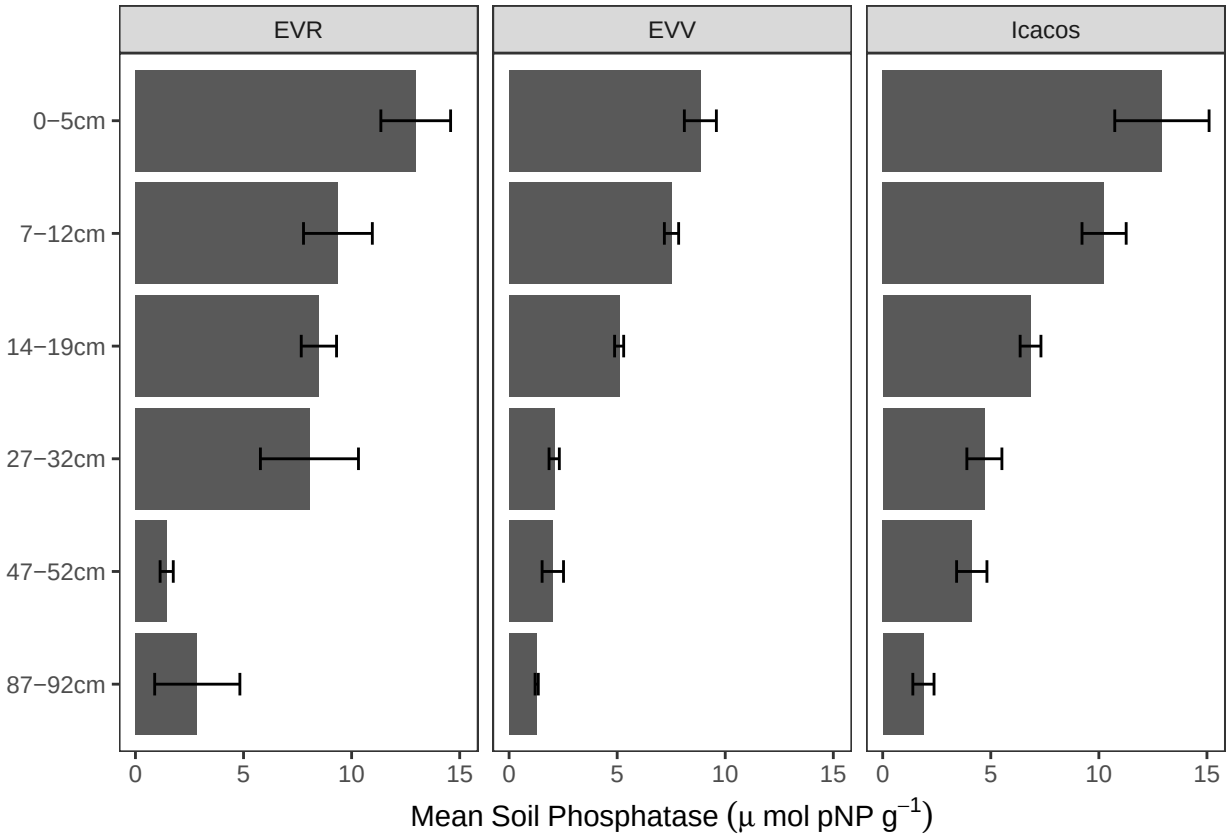


Figure 3: Fig. 3: Combined Icacos plot

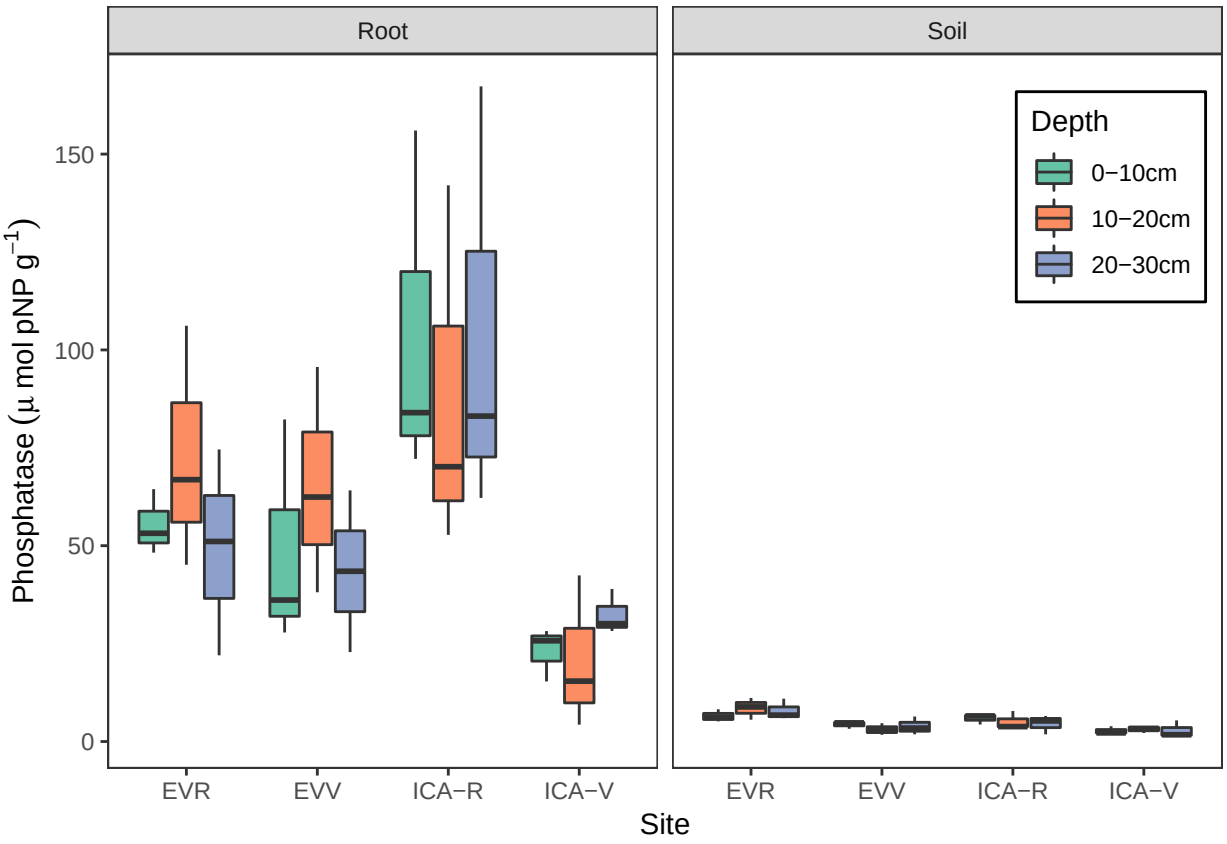
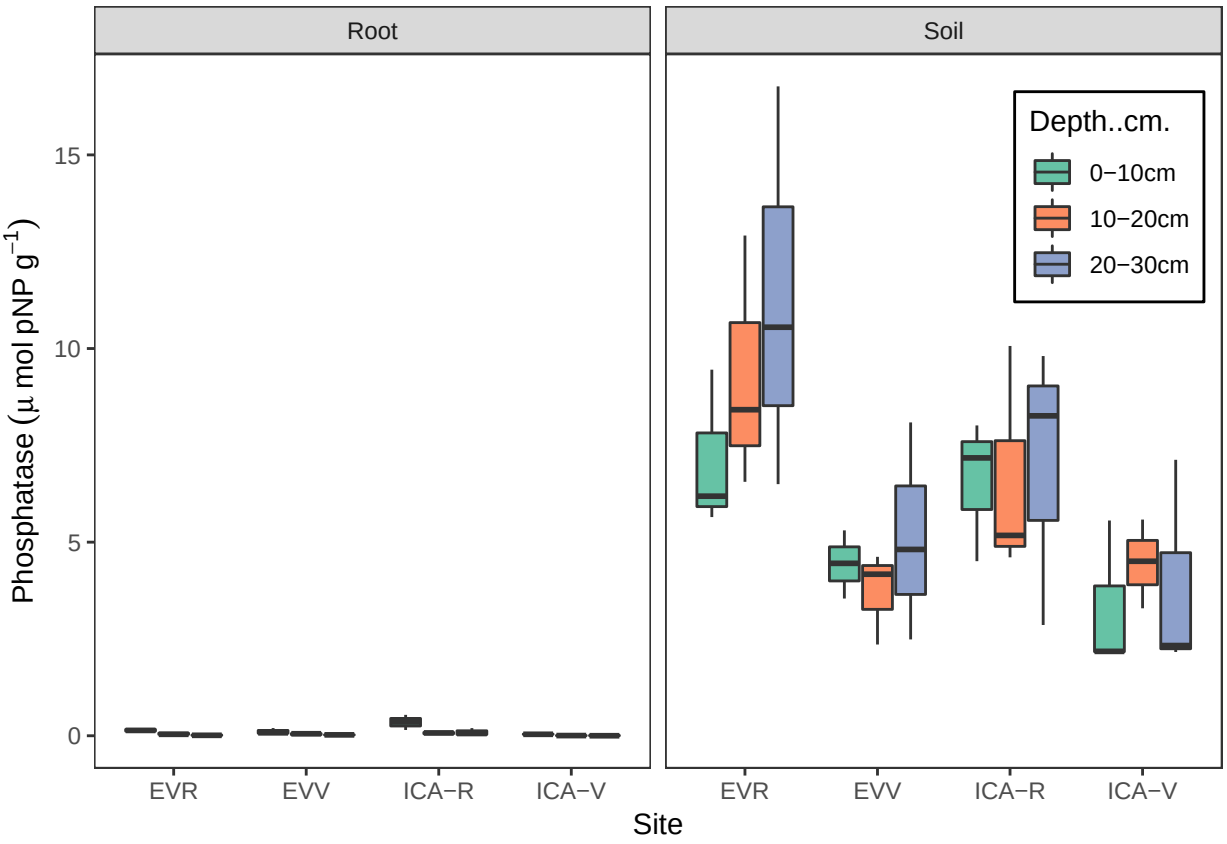


Figure 4: Fig. 4: Root and Soil Phosphatase from 0-30cm

drastically different, with greater soil phsophatase than root phosphatase. Both differed among sites ($p < 0.05$), but not by depth ($p = 0.98$).




```
## # A tibble: 24 x 7
## # Groups:   Site, Pase_Type [?]
##    Site  Pase_Type Depth       n  mean     sd     se
##    <fct> <fct>     <fct>   <int> <dbl>  <dbl>  <dbl>
##  1 EVR   Root      0-10cm      3 55.3   8.31   4.80
##  2 EVR   Root      10-20cm     3 72.7  30.9   17.9
##  3 EVR   Root      20-30cm     3 49.2  26.3   15.2
##  4 EVR   Soil      0-10cm      3  6.50  1.57   0.909
##  5 EVR   Soil      10-20cm     3  8.51  2.78   1.61
##  6 EVR   Soil      20-30cm     3  7.88  2.67   1.54
##  7 EVV   Root      0-10cm      3 48.7  29.3   16.9
##  8 EVV   Root      10-20cm     3 65.4  28.9   16.7
##  9 EVV   Root      20-30cm     3 43.5  29.2   16.9
## 10 EVV   Soil      0-10cm      3  4.33  0.933  0.539
## # ... with 14 more rows
```

Two-way repeated measures MANOVA

```
##
## Error: ID
##           Df Sum Sq Mean Sq
## Pase_Type  1  50.41   50.41
##
## Error: ID:Site
##           Df Sum Sq Mean Sq
## Pase_Type  1   8924    8924
```

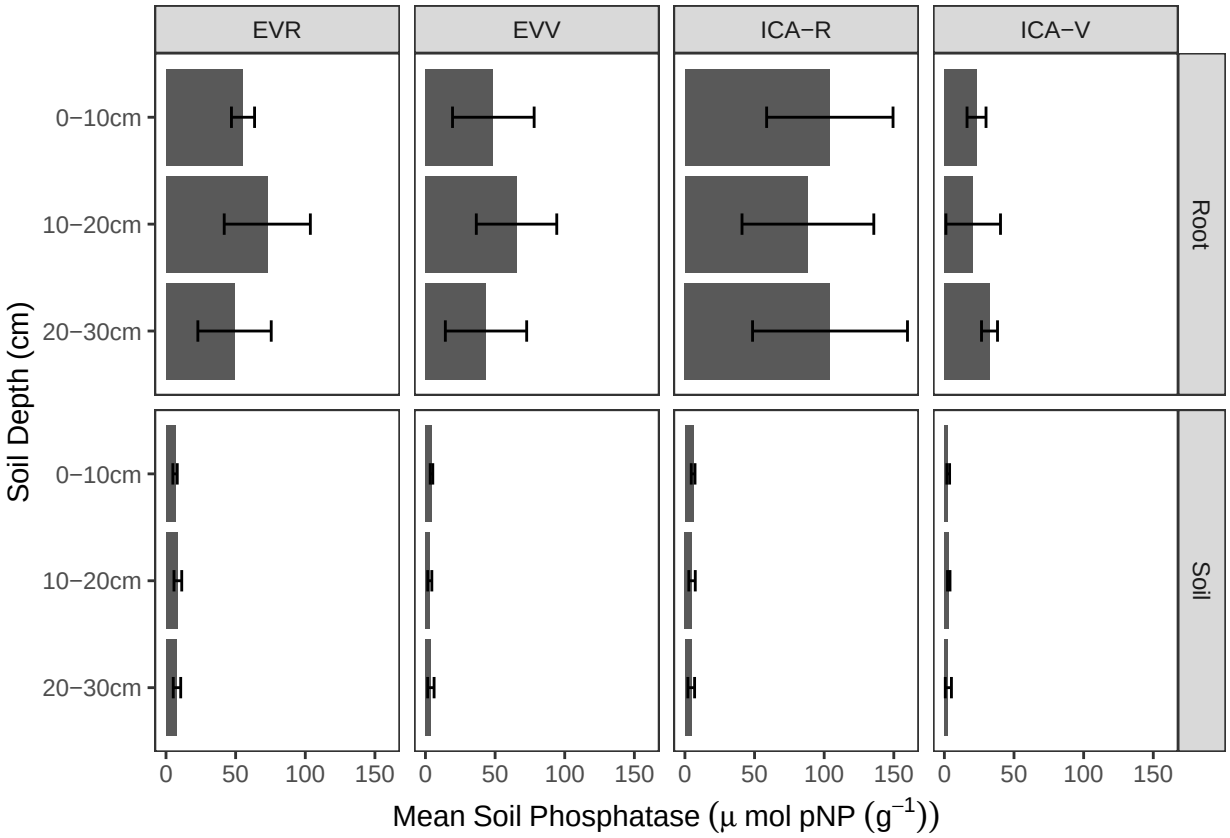


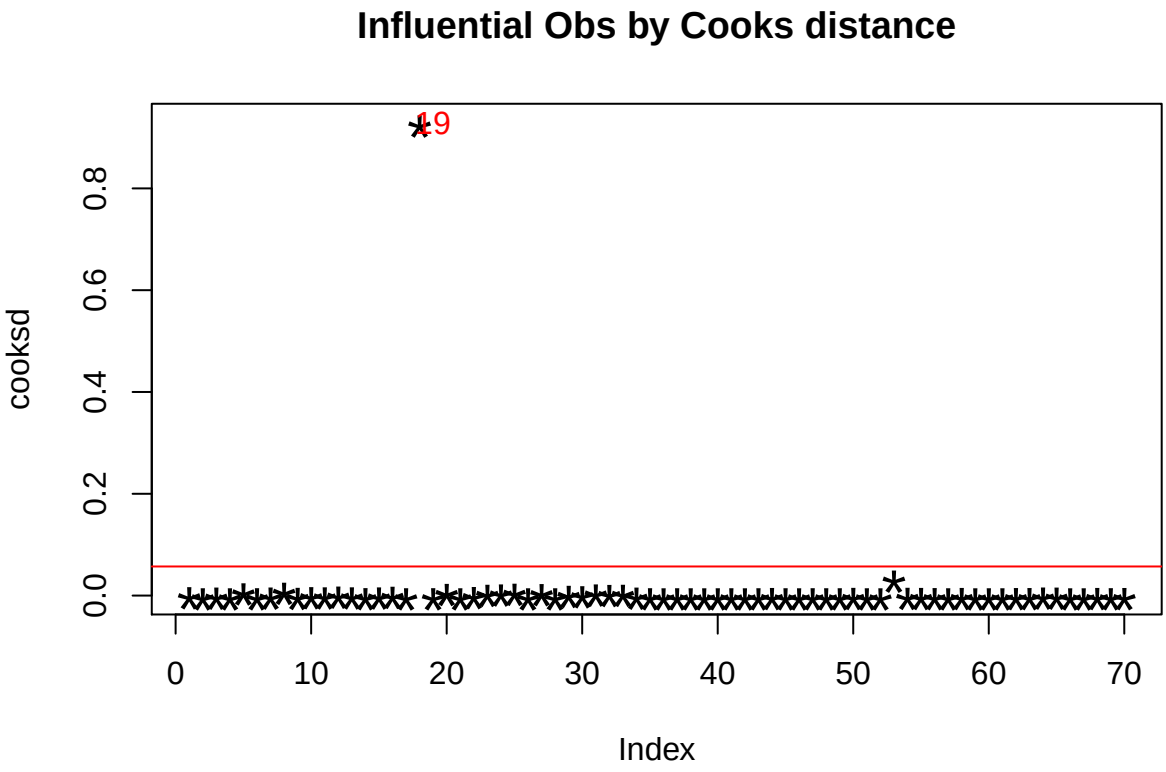
Figure 5: Fig. 5: Mean Root and Soil Phosphatase 0-30cm

```
## Site       2   1884     942
##
## Error: ID:Depth
##           Df Sum Sq Mean Sq
## Pase_Type  1  26.57   26.57
## Site       1  69.00   69.00
##
## Error: ID:Site:Depth
##                Df Sum Sq Mean Sq
## Pase_Type       1 139.86  139.86
## Site            3 245.82   81.94
## Depth           1  32.27   32.27
## Pase_Type:Site  1   3.53    3.53
##
## Error: Within
##                      Df Sum Sq Mean Sq F value  Pr(>F)
## Pase_Type             1  14505   14505 181.036 1.3e-15 ***
## Site                  3   1000     333   4.158  0.0125 *
## Depth                 2      3       1   0.017  0.9834
## Pase_Type:Site        3    715     238   2.973  0.0445 *
## Pase_Type:Depth       2     78      39   0.484  0.6203
## Site:Depth            6    128      21   0.266  0.9492
## Pase_Type:Site:Depth  6    410      68   0.853  0.5380
## Residuals            36   2884      80
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Resin P

An outlier test using Cooks Distance indicates that sample 19 (EVV Location 1: 0-5 cm) is a data point that significantly skews statistical models. In this case, sample 19 is 4 times the mean, suggesting that it should not be included. Phosphorus availability differed among sites ($p < 0.001$), but not by depth. Generally, El Verde sites contained more phosphorus than in Icacos sites and there weren't any consistent patterns of phosphorus availability by depth.

Outlier Test



Summary Statistics

```
## # A tibble: 24 x 6
## # Groups:   Site [?]
##    Site  SoilDepth     n  mean     sd     se
##    <fct> <fct>     <int> <dbl>  <dbl>  <dbl>
##  1 EVR   0-5cm         3 0.5   0.261  0.150
##  2 EVR   14-19cm       3 0.237 0.0306 0.0176
##  3 EVR   27-32cm       3 0.253 0.163  0.0940
##  4 EVR   47-52cm       3 0.503 0.388  0.224
##  5 EVR   7-12cm        3 0.56  0.356  0.206
##  6 EVR   87-92cm       3 0.545 0.262  0.185
##  7 EVV   0-5cm         3 0.315 0.0495 0.0350
##  8 EVV   14-19cm       3 0.237 0.0208 0.0120
##  9 EVV   27-32cm       3 0.485 0.276  0.195
## 10 EVV   47-52cm       3 0.393 0.232  0.134
## # ... with 14 more rows
```

ANOVA

```
##                Df Sum Sq Mean Sq F value   Pr(>F)
## Site            3 1.1170  0.3723   8.290 0.000169 ***
## SoilDepth       5 0.1614  0.0323   0.719 0.612821
## Site:SoilDepth 15 0.7333  0.0489   1.088 0.393261
## Residuals      45 2.0213  0.0449
```

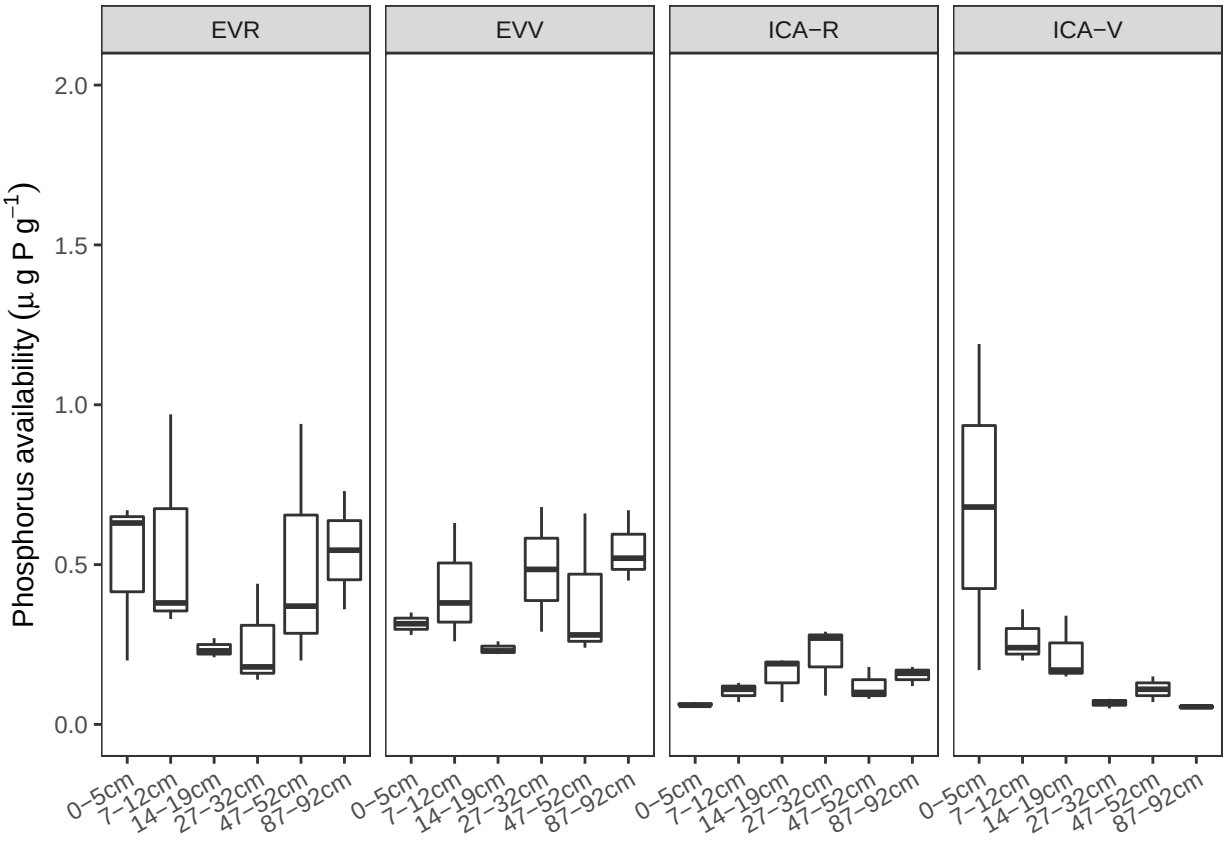


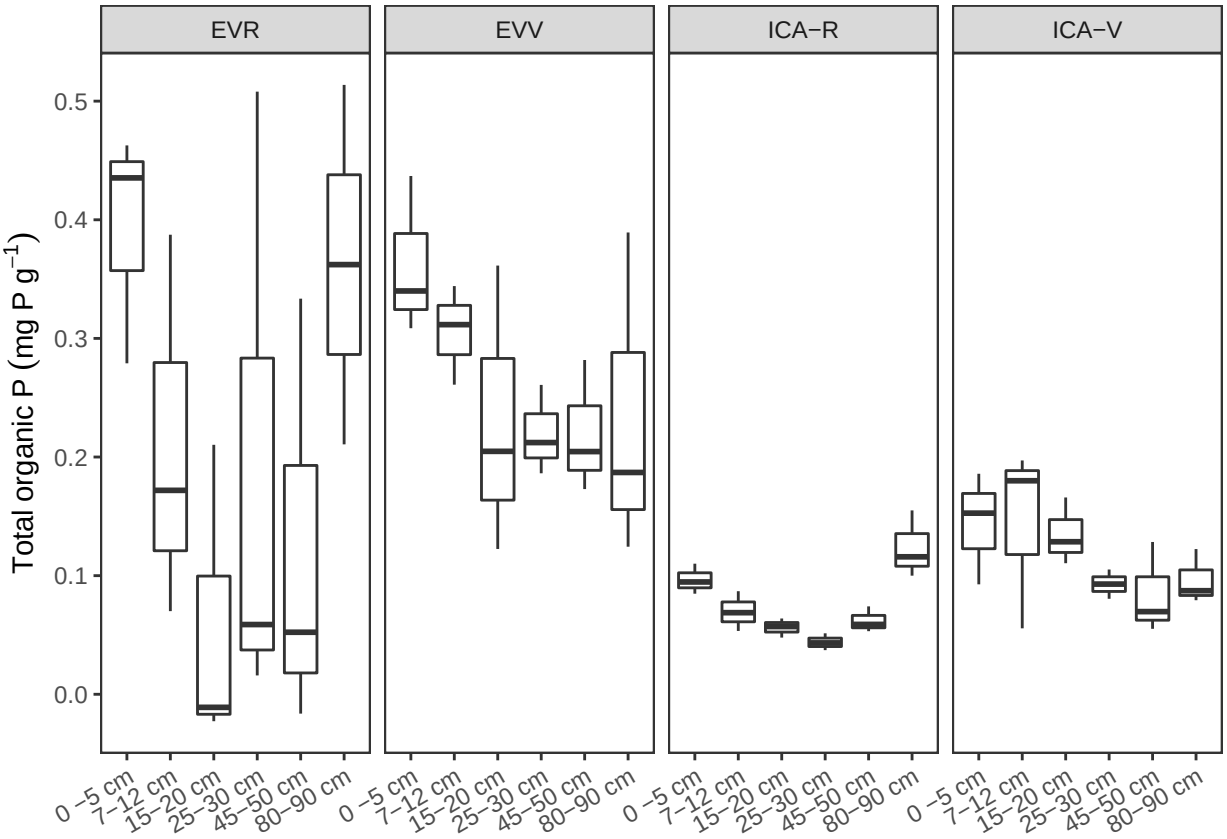
Figure 6: Fig. 6: Phosphorus availability as measuring by resin P

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 3 observations deleted due to missingness
```

Organic P

Unlike resin P, there are stark differences in organic phosphorus content among the sites ($p < 0.001$) and depths ($p < 0.01$). In particular, both El Verde sites have significantly higher concentrations than in the Icacos sites. There was no significant interaction between site and depth in determining organic phosphorus, and the boxplot suggests that organic phosphorus tends to decrease until 25-30 cm before increasing slightly.

Summary Statistics




```
## # A tibble: 24 x 6
## # Groups:   Site [?]
##    Site  Depth        n   mean     sd     se
##    <fct> <fct>    <int>  <dbl>  <dbl>  <dbl>
##  1 EVR   0 -5 cm      3 0.392  0.0991 0.0572
##  2 EVR   15-20 cm     3 0.0589 0.131  0.0758
##  3 EVR   25-30 cm     3 0.194  0.273  0.157
##  4 EVR   45-50 cm     3 0.123  0.185  0.107
##  5 EVR   7-12 cm      3 0.210  0.162  0.0935
##  6 EVR   80-90 cm     3 0.362  0.214  0.152
##  7 EVV   0 -5 cm      3 0.362  0.0669 0.0386
##  8 EVV   15-20 cm     3 0.230  0.121  0.0701
```

```
##  9 EVV   25-30 cm     3 0.220  0.0378 0.0218
## 10 EVV   45-50 cm     3 0.220  0.0560 0.0323
## # ... with 14 more rows
```

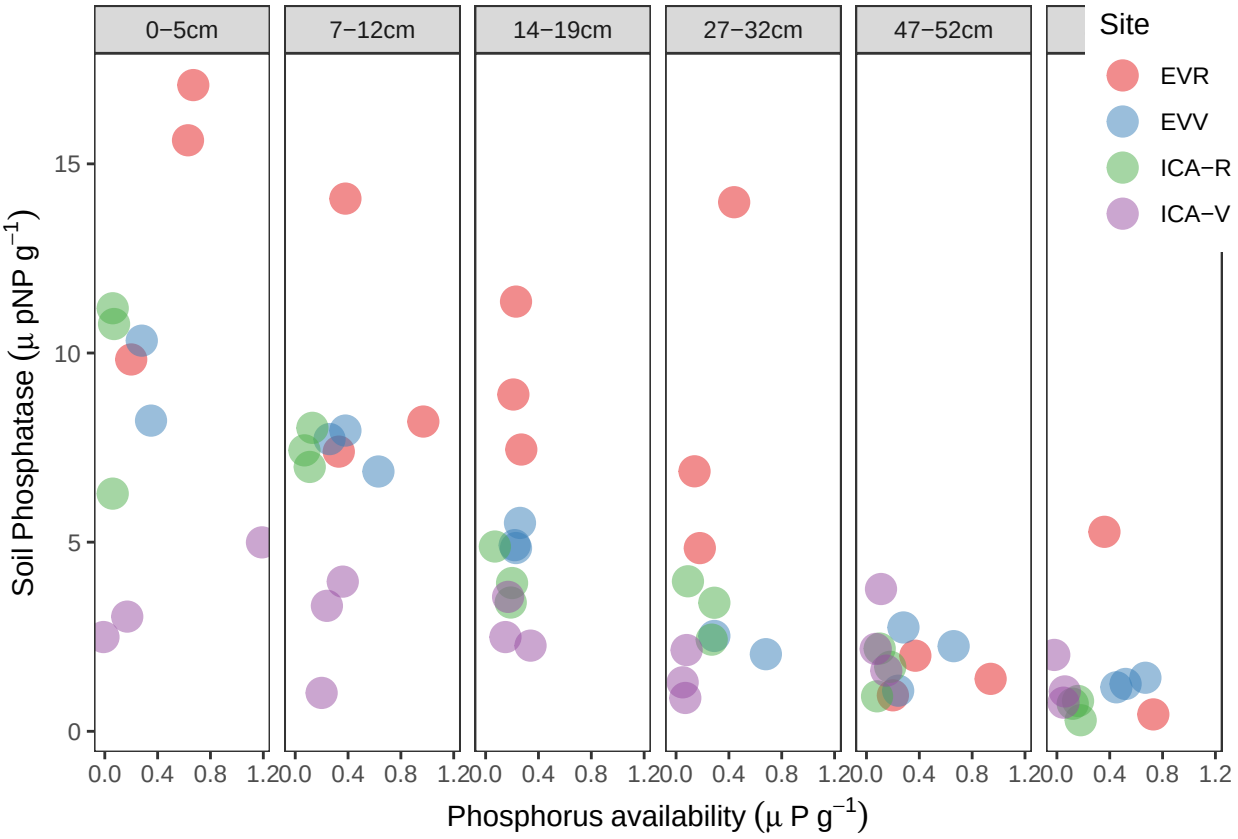
ANOVA

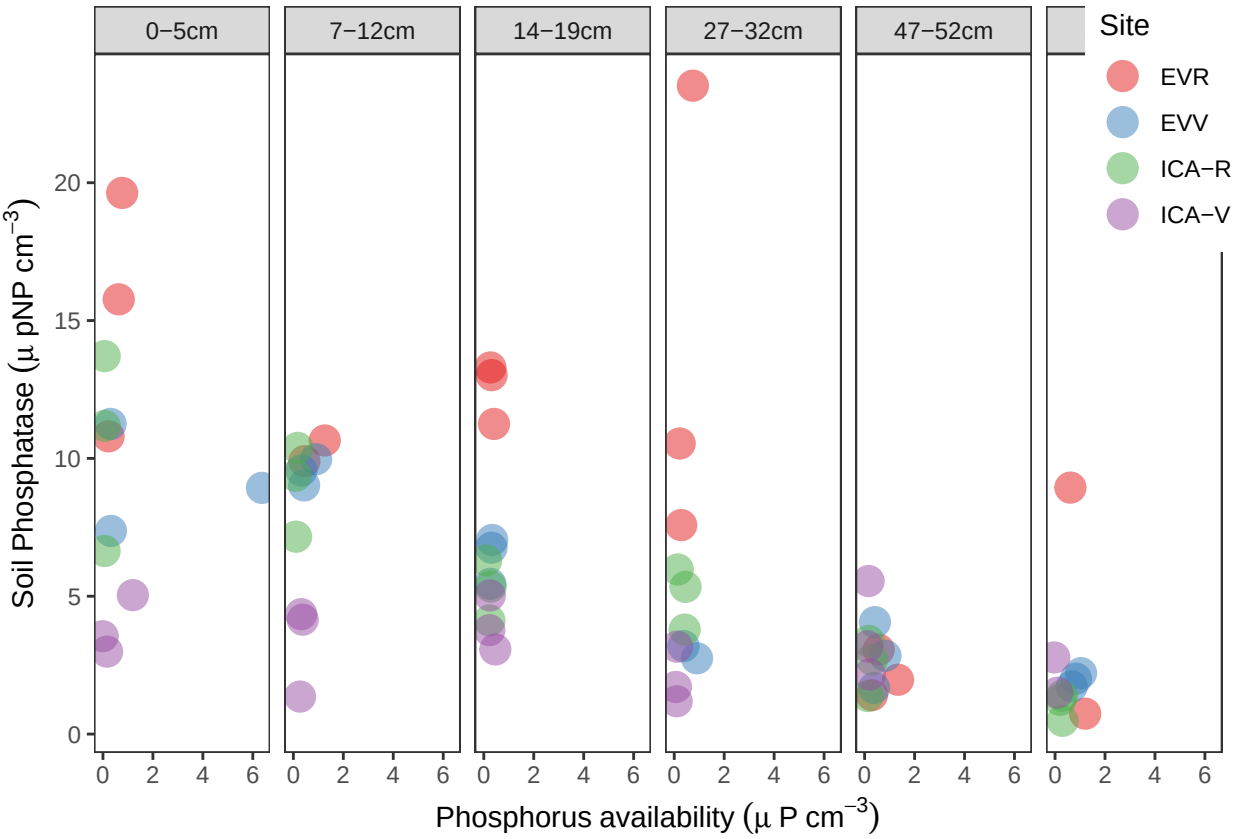
```
##             Df Sum Sq Mean Sq F value  Pr(>F)
## Site         3  11798    3933  18.153 5.2e-08 ***
## Depth        5   4565     913   4.214 0.00296 **
## Site:Depth  15   4335     289   1.334 0.21990
## Residuals   48  10399     217
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Correlations

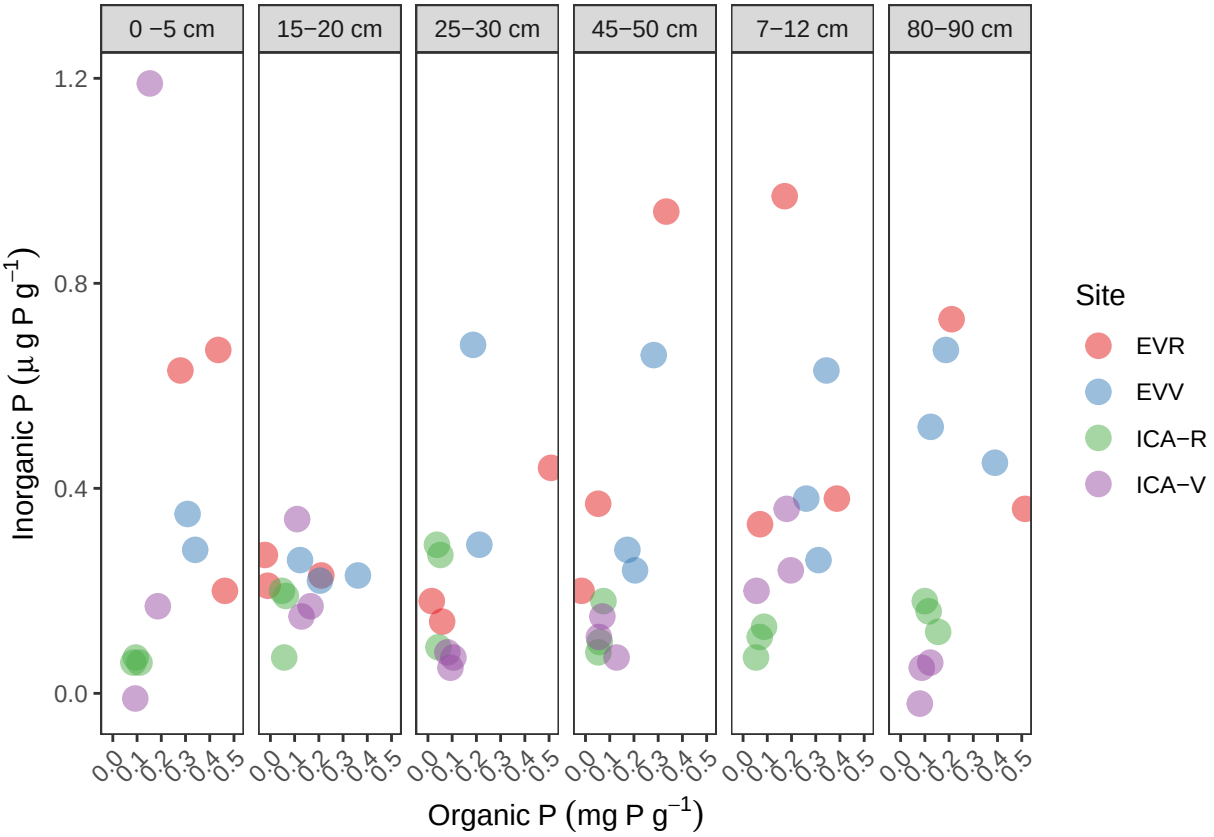
Resin P x Soil Phosphatase

The relationship between resin P and soil phosphatase depends on site and depth. As depth increases, the separation among sites diminishes until after 27-32 cm where there is no differenc among sites in soil phosphtase and resin P availability. In general, the Icacos sites tend to have lower soil phosphatase and resin P availability, within each depth. Given similar levels of phosphorus, El Verde sites consistently have higher soil phosphatase activity.



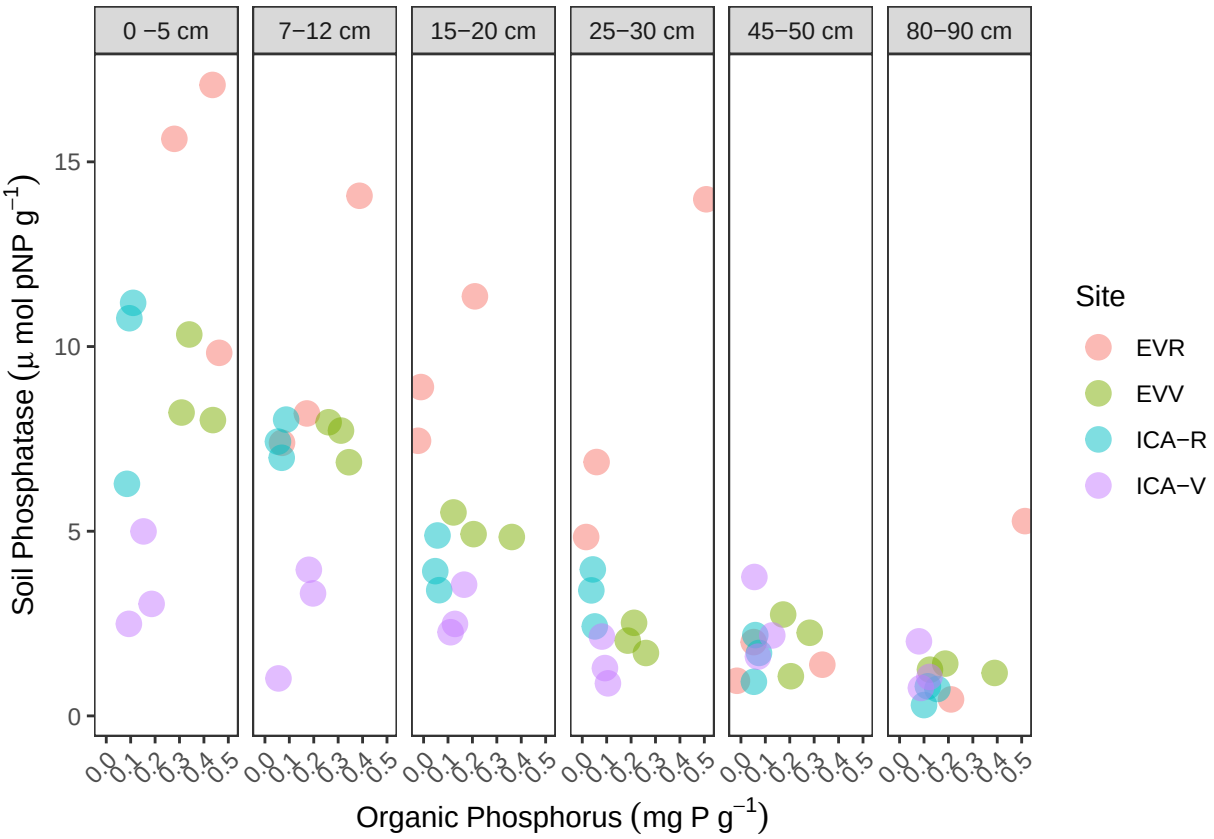

0−5cm
7−12cm
14−19cm
27−32cm
47−52cm
Site
EVR
EVV
ICA−R
ICA−V
Soil Phosphatase (μ pNP cm−3)
Phosphorus availability (μ P cm−3)
0
5
10
15
20
0
2
4
6

Organic P x Resin P



Organic P x Soil Phosphatase

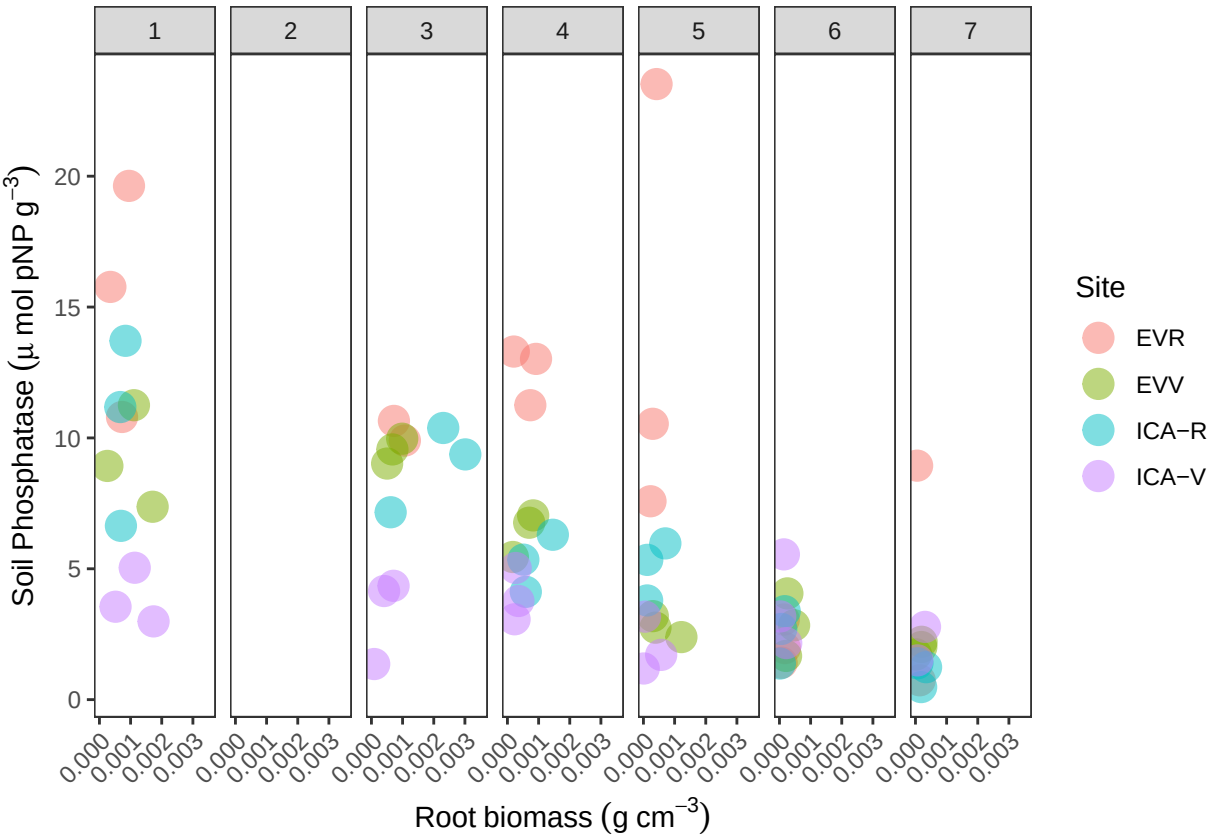
The relationship between organic P and soil phosphatase mirrors that of resin P, where soil phosphatase and organic phosphorus consistently decline with depth and the differences between sites disappear after 25-30 cm in depth. For a given organic phosphorus concentration, there is generally higher soil phosphatase in El Verde Ridge. El Verde Valley and Icacos Ridge appear to have similar soil phosphatase activity, despite Icacos Ridge having consistently lower amounts of organic phosphorus. Icacos Valley tends to have both lower organic phosphorus content and soil phosphtatase.



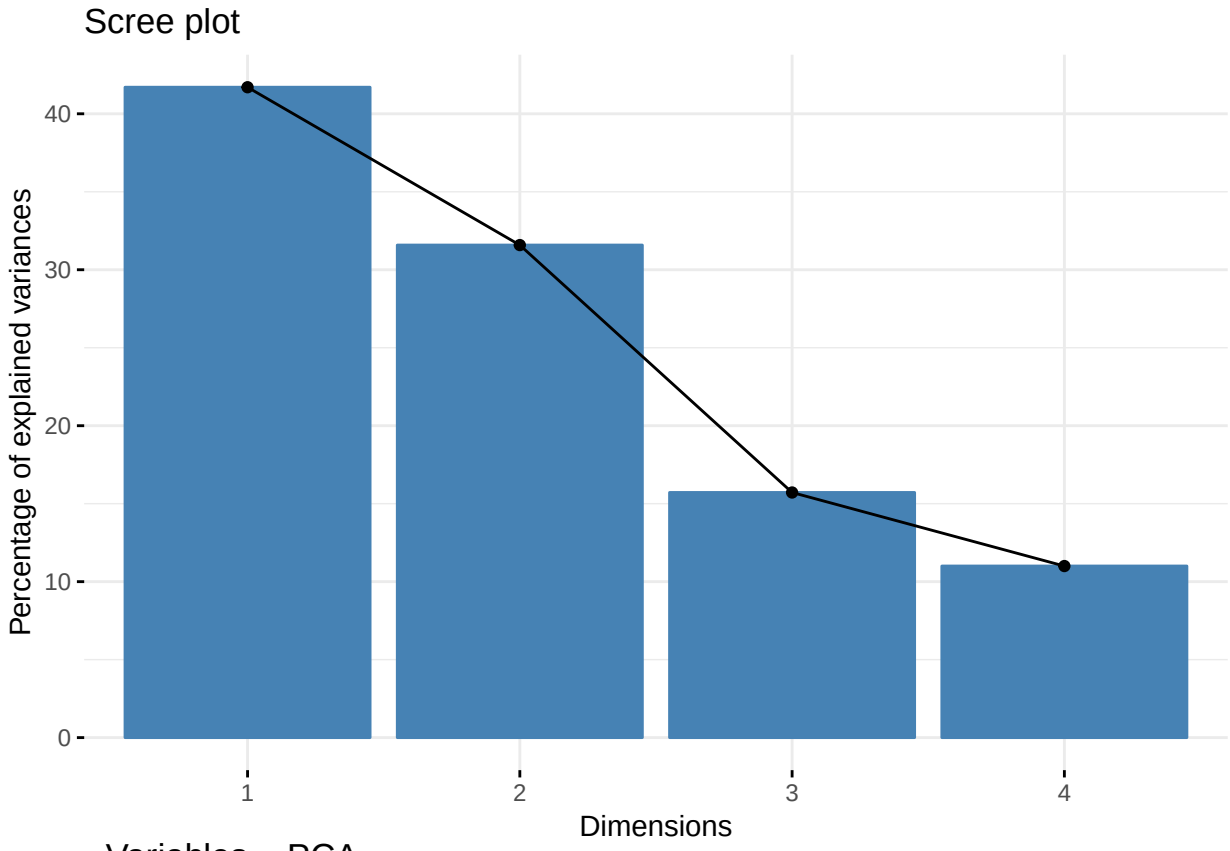
Root Biomass x Root Phosphatase

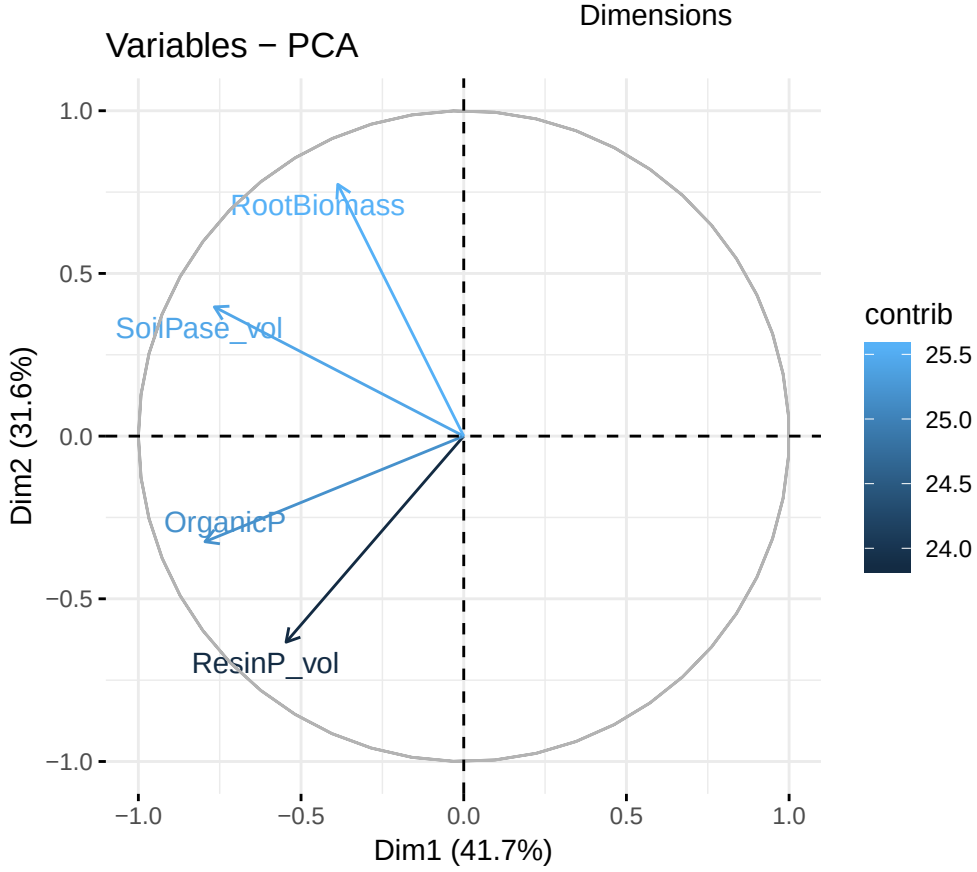
As expected, root biomass decreass with depth. Root biomass does not appear ot differ significantly among sites, consistently remaing within 0.001 g/cm3 - 0.002 g/cm3. However, soil phosphatase varies among sites, where despite similar amounts of root biomass; El Verde Ridge is highest in soil phosphatase activity, followed by El Verde Valley and Icacos Ridge, with Icacos Valley having the lowest amounts.

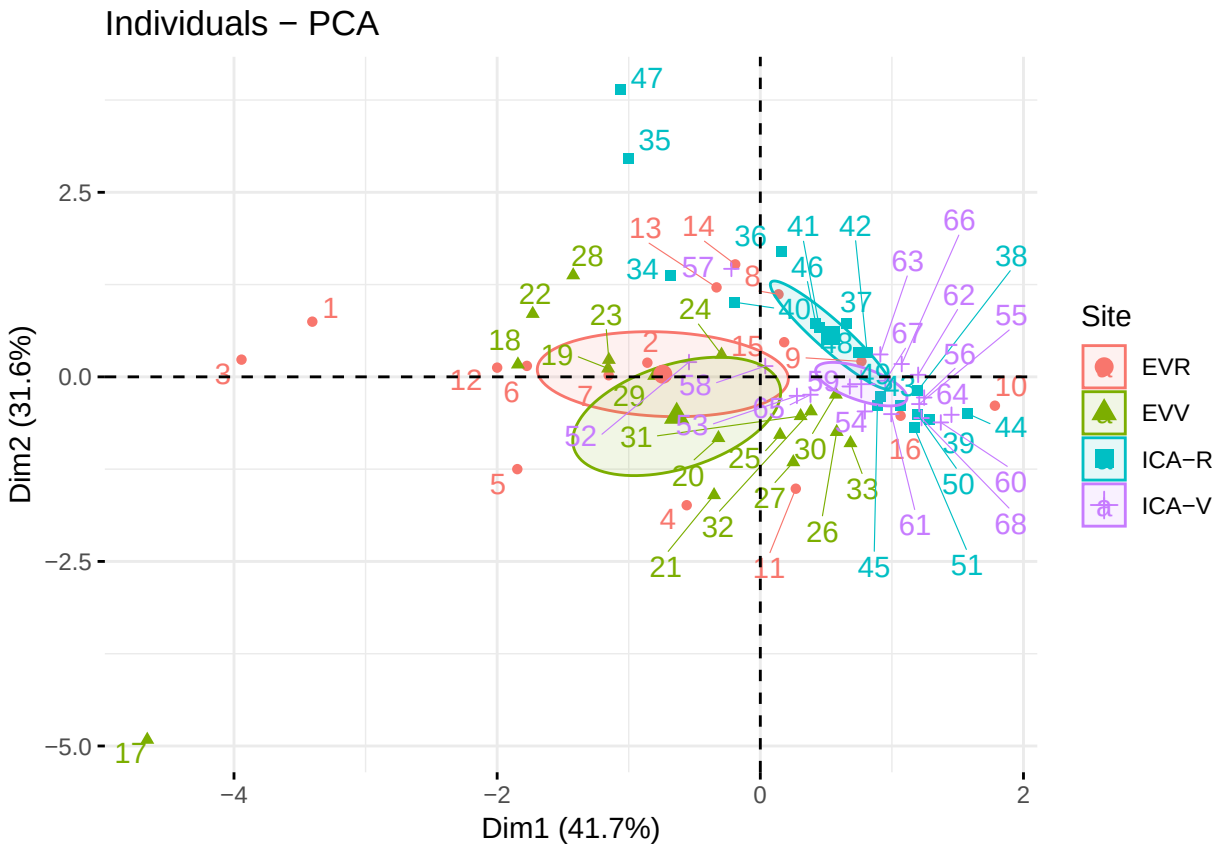
Need to remove 5-7 cm depth (facet #2)


1
2
3
4
5
6
7
Soil Phosphatase (µ mol pNP g^{-3})
0
5
10
15
20
0.000
0.001
0.002
0.003
Root biomass (g cm^{-3})
Site
EVR
EVV
ICA−R
ICA−V

Principal Component Analysis






Individuals − PCA
Dim2 (31.6%)
Dim1 (41.7%)
2.5
0.0
−2.5
−5.0
−4
−2
0
2
Site
EVR
EVV
ICA−R
ICA−V