

Supporting Information to

Phosphorus stress-induced changes in plant root exudation could potentially facilitate uranium mobilization from stable mineral forms.

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Section 1. Preparation of soil inoculum

Rhizosphere soils were collected from fully grown plants in the field during the active growing stage in August. Plants were carefully uprooted, and the bulk soil attached to the roots was removed by tapping gently to reveal individual roots. The soil closely adhering to the roots were then collected by gently tapping the roots on aluminum foil. The soil- water ratio of 1:2 was selected to prepare soil slurry so as to provide enough soil particles in the inoculum. The inoculum was limited to 1 ml to prevent addition of excess P from the soil slurry which could confound the results. The amount of P nutrient supplied by the slurry was below 0.1 ppm

Phosphorus analysis.

The roots and shoots were placed into individual 250 mL covered beakers and oven dried at 50°C to a constant weight to obtain dry plant tissue mass. The oven dried plant tissues were acid digested on a hotplate using HNO₃ and H₂O₂ following EPA Method 3050B, Section 7. The resulting digestate and DDI digestion beaker rinse were filtered through 0.2 µm polypropylene syringe filters and the filter was rinsed with approximately 5 mL of 2% HNO₃. The filtered digestate samples were diluted as appropriate for analysis of phosphorus via inductively coupled mass spectrometry (ICP-MS, Thermo Scientific XSeries 2).

Section 2. GC-MS parameters

GC analysis provides a consistent, well resolved separation of derivatized polar compounds from complex sample matrix. Also, hydroponics solution, due to the presence of high amount of nutrient salts, is generally not amenable for HPLC analysis without any pre-treatment and desalting. The separation of metabolites was obtained on a DB-5 MS capillary column (30 m length, 0.25 mm internal diameter, 0.20 μ m film thickness) and analyzed using a transmission quadrupole mass detector (Agilent 5975 C series). The initial oven temperature of 60⁰ C was held for 1 min, ramped at 10⁰ C to a final temperature of 300⁰C and then held for 7 min. The carrier gas used was helium, which was maintained at a constant pressure of 76.53 kPa. The samples were introduced into the GC using split injection rates of 1:20 and 1:80 and scanned using a scanning range of 100-360 amu, achieving 2.66 scans s⁻¹. An in-house mass spectral library supplemented with Fiehn Lib (G1676AA; Agilent Technologies, Wilmington, DE, USA) was used to identify the spectral peaks. Peak deconvolution was conducted using AMDIS with a minimum match factor of 70 relative to the retention index (RI) library. The integrated signal for each metabolite was normalized with that of the internal standard ribitol.

Section 3. Modified hoagland solution for different P treatment

Full nutrient solution (KH₂PO₄); 200mg L⁻¹ Ca as CaNO₃, 234mg L⁻¹ K as KNO₃, 48mg L⁻¹ Mg as MgSO₄ and 140 mg L⁻¹ N as NH₄NO₃, KNO₃ and CaNO₃, 30mg L⁻¹ P as KH₂PO₄

FePO₄ treatment; 200mg L⁻¹ Ca as CaNO₃, 234mg L⁻¹ K as KNO₃, 48mg L⁻¹ Mg as MgSO₄ and 140 mg L⁻¹ N as NH₄NO₃, KNO₃ and CaNO₃, 60mg L⁻¹ P as FePO₄

Ca₃(PO₄)₂ treatment; 200mg L⁻¹ Ca as CaNO₃, 234mg L⁻¹ K as KNO₃, 48mg L⁻¹ Mg as MgSO₄ and 140 mg L⁻¹ N as NH₄NO₃, KNO₃ and CaNO₃, 60mg L⁻¹ P as Ca₃(PO₄)₂

Section 4. Ethyl chloroformate (ECF) derivatization protocol

The derivatization reaction was carried out in glass tubes without any closures. In the first step of this reaction, 75 μL ethanol-pyridine mixture (4ml:1ml v/v) was added to 75 μL of the samples. The reaction mixture was then vortexed for 5 seconds to create an emulsion. Then, 75 μL of the derivatization reagent, chloroform and ECF (1350 μL :150 μL), was added and the samples were vortexed for 15 seconds. Next, 75 μL of 1 M NaOH was added for pH adjustment and the mixture was vortexed for 30 seconds, followed by a second addition of 75 μL aliquot of chloroform-ECF (1350 μL :150 μL) and vortexing for 30 seconds. The final mixture was treated with 75 μL of 3 M HCL and vortexed for 5 seconds, and aliquots of 1 μL of the organic phase were injected into the GC-MS. The initial oven temperature was held at 80 $^{\circ}\text{C}$ for 2 min, then increased to 260 $^{\circ}\text{C}$ at the rate of 10 $^{\circ}\text{C min}^{-1}$. Helium was used as the carrier gas at a constant flow rate of 1.2 mL min $^{-1}$ through the column. The split ratio was 1:5, and the scanning range was set to 100-400 amu, while the temperature of the injection port was set to 270 $^{\circ}\text{C}$ and the solvent delay was set at 5 min. The quantity of specific amino acids and organic acids was measured using calibration curves plotted for standard amino acids and organic acids at five different concentrations.



Image S1. Healthy root system of *Andropogon virginicus* grown in half strength hoagland solution that contained all macro and micro nutrients for 40 days

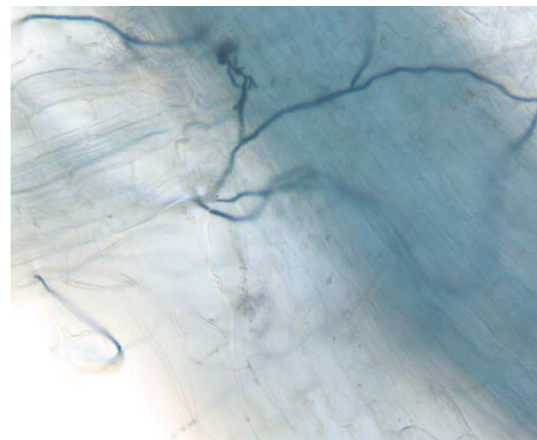


Image S2. Staining of root segment of *Andropogon virginicus* collected from field (Clemson, SC) with intercellular non-septate hyphae, characteristic of arbuscular mycorrhizal fungi.

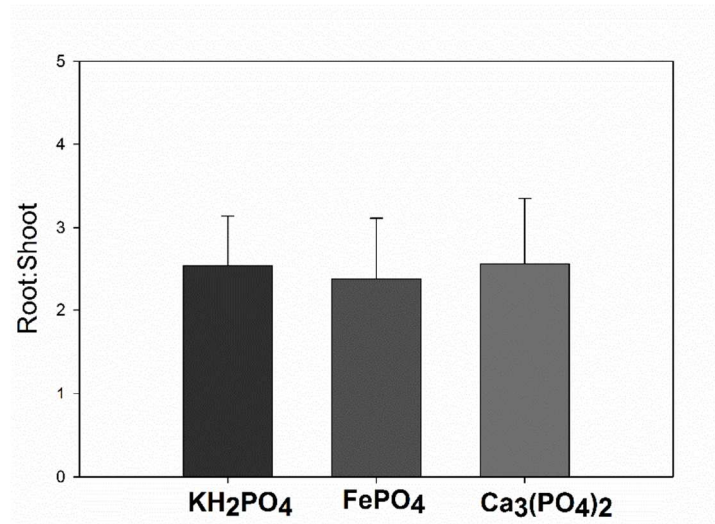


Figure S1. Effect of phosphorus treatment on root to shoot weight ratio (R: S) of *Andropogon virginicus*

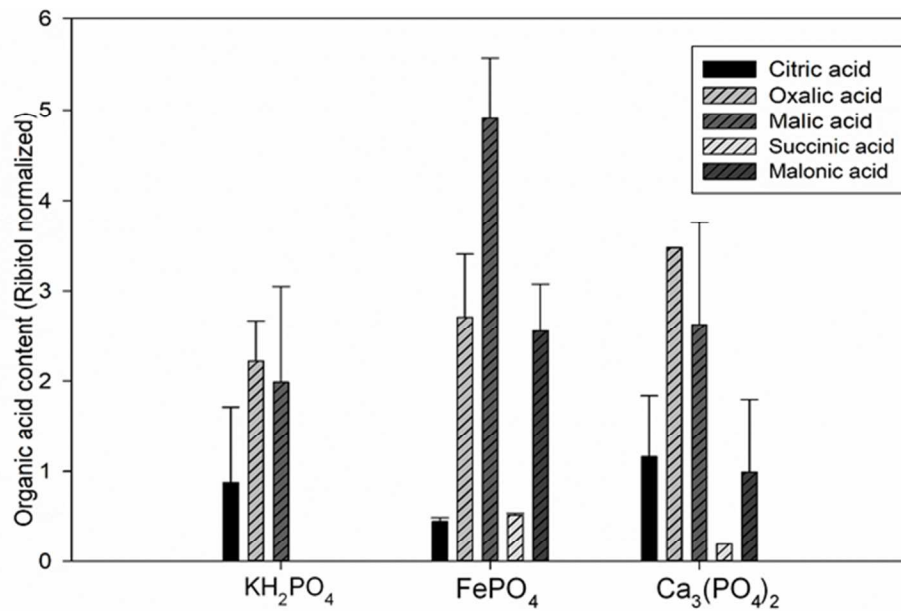


Figure S2. Abundance of major five organic acids in the root exudates collected from sand attached to root surface (rhizosphere soil). The data represent the normalized values with respect to ribitol concentration of $100 \mu\text{g ml}^{-1}$.

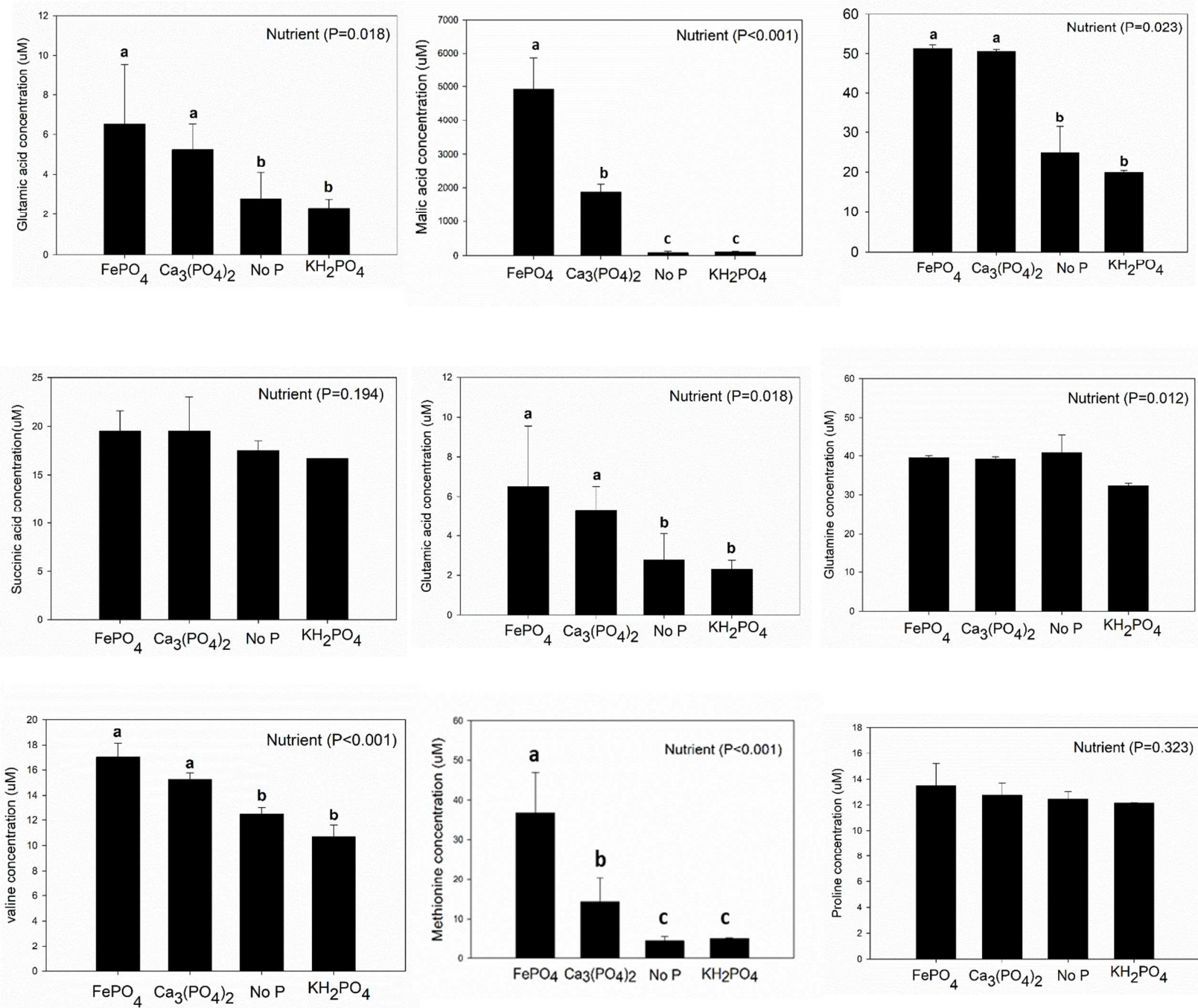


Figure S3. Average concentration of identified organic acids and amino acids in the root exudates of *Andropogon virginicus* exposed to P treatments in hydroponic media for 72h. Plant exposed to four treatments; KH_2PO_4 , FePO_4 , $\text{Ca}_3(\text{PO}_4)_2$ and no P. Different letters stand for statistical differences at $P \leq 0.05$ (Tukey's HSD multiple comparison at $p \leq 0.05$).

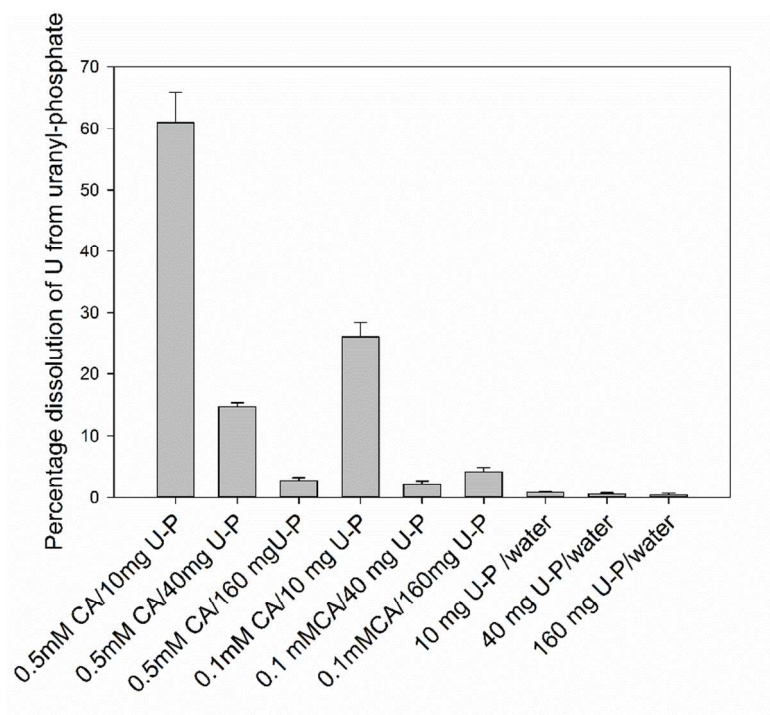


Figure S4; Dissolution of U from uranyl-phosphate using different concentration of citric acid and uranyl-phosphate

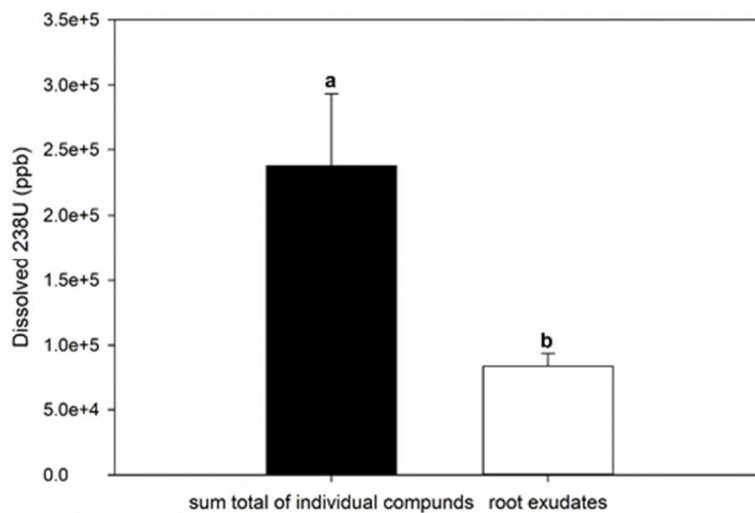


Figure S5. Comparison between observed U dissolution with root exudate matrix and expected dissolution with all individual ligands together based on two dissolution experiments.