ORGANIC PHOSPHORUS FORMS IN THE EVERGLADES WETLAND SOILS

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INTRODUCTION

The importance of phosphorus (P) availability in regulating the productivity and diversity of wetlands is well recognized, though the forms and dynamics of P in such ecosystems remain less known. This is due to the difficulty in identifying and quantifying P compounds in the complex matrices of wetland soils. Recent development of techniques such as ^{31}P Nuclear Magnetic Resonance (NMR) spectroscopy, allows to identify P compounds in the environment by their chemical functionality. NMR spectroscopy is now used for characterizing organic forms of P in soils and sediments, however, there is limited information on its adaptation for high organic matter and low P soils such as those encountered in the Everglades. In this study we examined various optimization methods including P extraction methods and NMR acquisition parameters to improve the identification of organic P forms, and made several refinements to help standardize this technique for use in wetland systems.

Objective: To modify and refine current methods for optimizing the ^{31}P-NMR method to quantify organic P forms in flocs and soils from Everglades Stormwater Treatment Areas (STAs) and Water Conservation Area WCA-2A. This will allow us to understand the nature and diversity of functional P forms found in wetland soils, which is related to their availability and stability.

MATERIALS AND METHODS

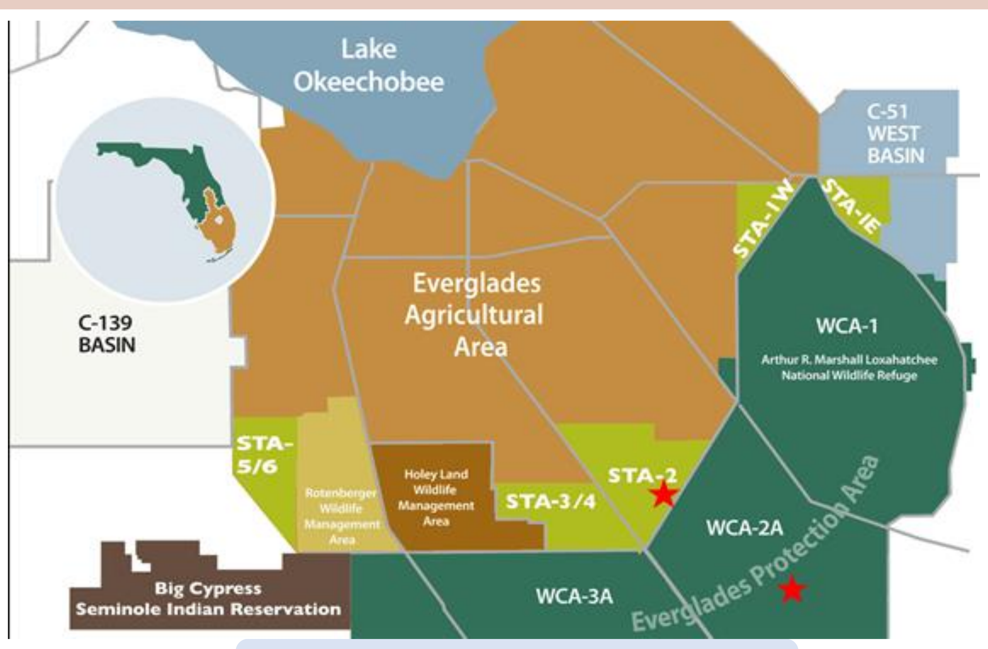

Figure 1. Study sites

Study sites

- **Ordway Preserve Site** (Gainesville, FL, samples collected from 0-10cm depth). This was used as a reference site. Site location is not shown above.
- **Everglades Stormwater Treatment Area STA-2 Cell-1** (also called Emerged Aquatic Vegetation - EAV cell, consists of cattails, *Typha* spp.)
- **Everglades Water Conservation Area** WCA - 2A (U-3 site, an open-water slough in an unenriched area of hardwater marsh, dominated by calcareous periphyton mats comprised of calcium-precipitating cyanobacteria and diatoms)

These sites provide the gradient of low (WCA-U-3) and high (STA-2 Cell-1) phosphorus concentration sites.

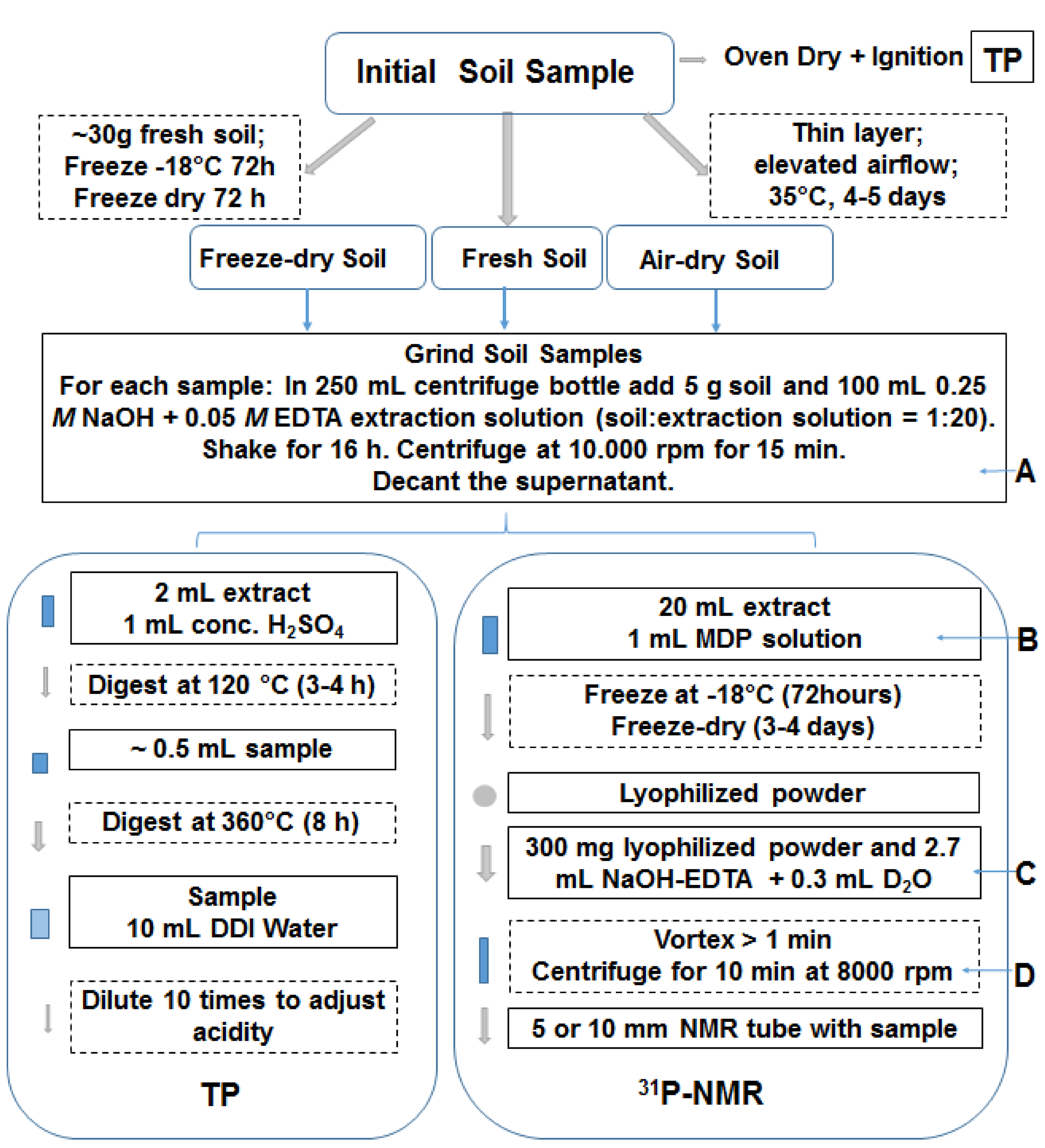

Figure 2. Sample preparation scheme for ^{31}P-NMR analysis of soil and floc samples. TP = Total phosphorus.

Acknowledgements

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RESULTS

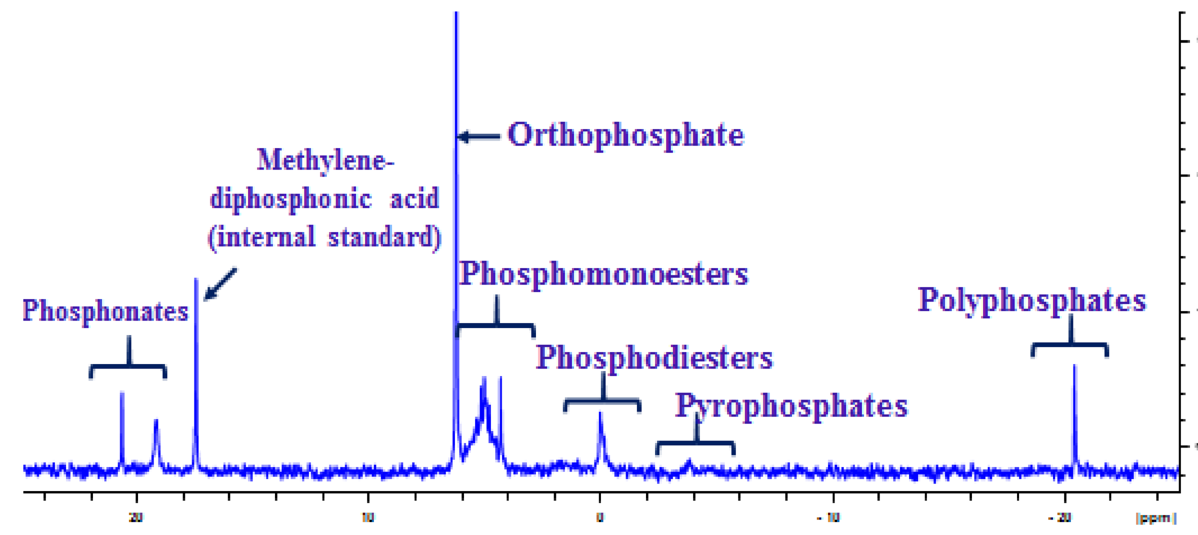
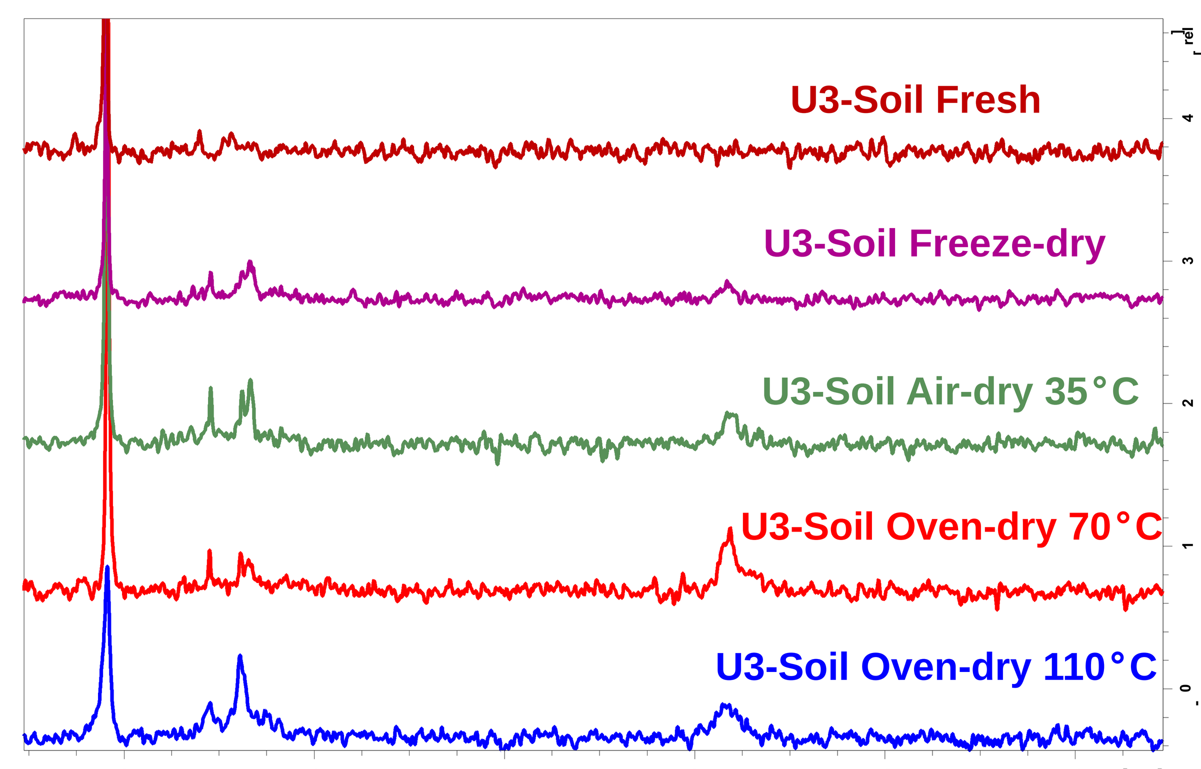


Figure 3. Example of solution ^{31}P spectrum showing commonly identified peaks. Ordway Preserve Site (0-5cm soil).

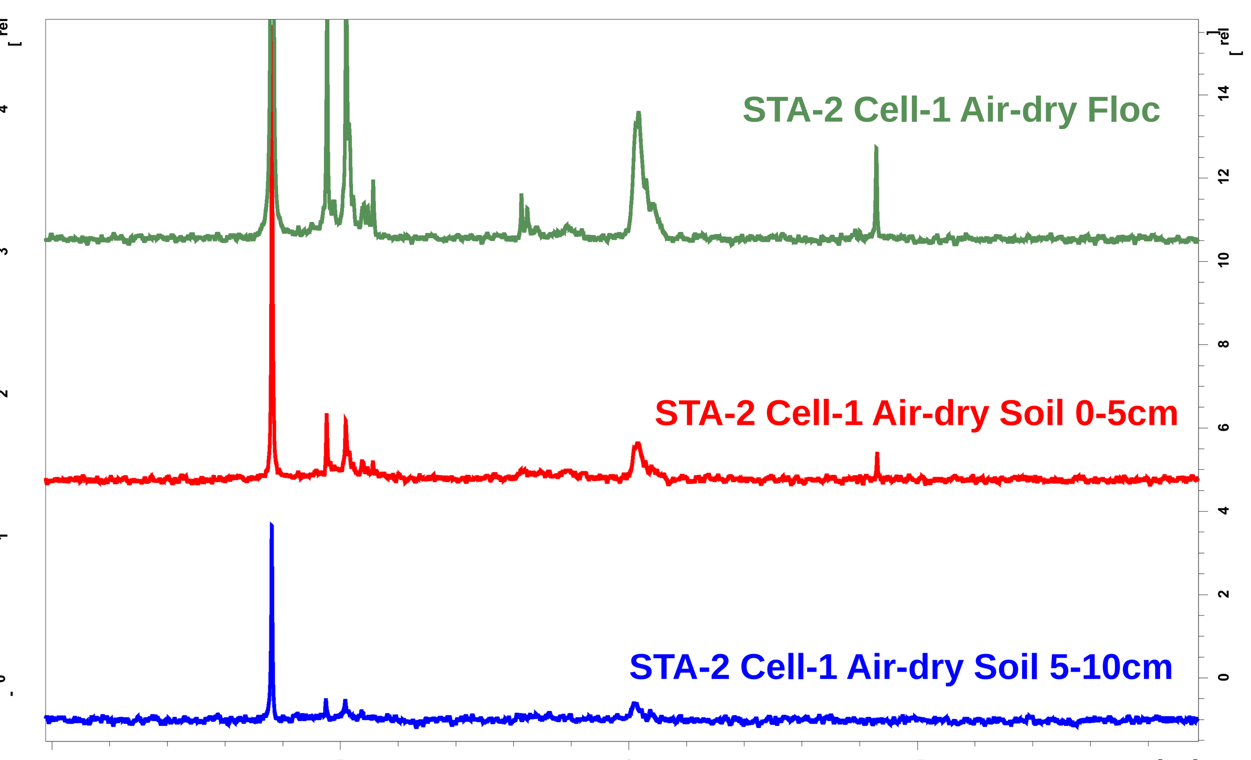
Parameters/ Unit	WCA-2A U-3 Floc	WCA-2A U-3 Soil (0-5 cm)	STA-2 Cell-1 Floc	STA-2 Cell-1 Soil (0-5 cm)	STA-2 Cell-1 Soil (5-10 cm)
LOI (%)	59 ± 2	53 ± 0	74 ± 1	82 ± 1	86 ± 1
TP (mg kg^{-1})	161 ± 8	246 ± 27	2186± 58	946 ± 24	316 ± 13
HCl-P$_i$ (mg kg^{-1})	32	62 ±2	518 ± 25	238 ± 15	38.7 ± 4

Table 1. Characterization of floc and soil samples collected from U-3 site of WCA-2A.



Sample pre-treatment	P conc. (mg kg^{-1}) NaOH-TP	Ortho P	Monoesters	Diesters
Fresh	42.2	40.2	1.9	ND
Freeze-dry	57.1	30.5	20.8	5.7
Air-dry at 35°C	64.1	36.3	18.3	8.6
Oven-dry at 70°C	98.9	51.8	19.4	27.7
Oven-dry at 110°C	94.5	33.6	41.3	18.9

Figure 4. Organic P forms of Soil samples from U-3 site of WCA-2A with sample pre-treatment variables



Samples	P conc. (mg kg^{-1}) NaOH-TP	Ortho P	Monoesters	Diesters	Pyro P
Floc	1091	489	325	245	32
Soil 0-5cm	481	183	137	152	8.7
Soil 5-10cm	194	109	53	32	ND

Figure 5. NMR spectra and Organic P concentration (mg kg^{-1}) of air-dry floc and soil samples from STA-2 Cell-1.

Sample Treatment	Solid-Solution ratio	Floc NaOH-TP (mg kg^{-1})	Floc Extraction Efficiency (%)	Soil (0-5 cm) NaOH-TP (mg kg^{-1})	Soil (0-5 cm) Extraction Efficiency (%)
Fresh	1:20	24.8 ± 1.2	15.4	42.2 ± 2.9	17.2
Freeze-dry	1:10	NA	NA	54.2	22.0
	1:20	44.9 ± 1.0	27.9	57.1 ± 4.1	23.2
	1:40	NA	NA	52.0	21.1
Air-dry at 35°C	1:10	25.4	15.8	36.2	14.7
	1:20	48.2 ± 4.8	29.9	64.1 ± 6.9	26.1
	1:40	26.1	16.2	74.2	30.2
Oven-dry at 70°C	1:10	40.0	24.8	77.8	31.6
	1:20	63.3 ± 8.5	39.3	98.9 ± 2.2	40.2
	1:40	72.4	45.0	93.4	38.0
Oven-dry at 110°C	1:20	NA	NA	94.5 ± 2.4	38.4

Table 2. Influence of sample pre-treatment variables and solid to solution (NaOH-EDTA) ratios on extraction of P from floc and soil samples from WCA-2A U-3 (slough). Data without standard deviation refers to the composited samples with one replicate.

Sample Treatment	Floc NaOH-TP (mg kg^{-1})	Floc Extraction Efficiency (%)	Soil 0-5 cm NaOH-TP (mg kg^{-1})	Soil 0-5 cm Extraction Efficiency (%)	Soil 5-10 cm NaOH-TP (mg kg^{-1})	Soil 5-10 cm Extraction Efficiency (%)
Air-dry	1091 ± 56	50	481± 26	51	194 ± 4	61
Freeze-dry	963 ± 71	44	461 ± 34	49	207 ± 15	66

Table 3. Influence of sample pre-treatment variables on extraction of P from floc and soil samples from STA-2 Cell-1 (EAV).

DISCUSSION AND CONCLUSIONS

- **Oven drying of soils at 70ºC improved both organic P extraction efficiency (NaOH-EDTA) and NMR spectra, though it is likely that relative proportion of P forms were altered.**
- **Sample pretreatments such as air-drying and freeze-drying had minimal effect on delineation of organic P functional groups.**
- **Air-drying of samples at 35ºC appeared to be a suitable option for reducing sample heterogeneity.**
- **Field moist, fresh samples exhibited low P extraction efficiency and resulted in unreliable NMR spectra.**
- **Soil to solution (NaOH-EDTA) ratios of 1:20 and 1:40 provided reliable spectra (Step A Figure 2).**
- **20mL of NaOH-EDTA extract plus 1mL of methylene-diphosphonic acid (MDP) solution were optimal prior to freeze drying to create the lyophilized powder (Step B Figure 2).**
- **For low P soils, concentrating the solutions before loading into NMR tubes improved the overall NMR spectra (Step C Figure 2).**
- **Most of the P functional groups were present in soil samples from Ordway Preserve sites and STA-2, while only monoesters and low levels of diesters were recorded in soil samples from WCA-2A due to low P concentration in the soil.**

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