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 31P 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 31P-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

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 uneroded 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.

Sample pre-treatment

<table>
<thead>
<tr>
<th>Sample pre-treatment</th>
<th>Solid Extraction</th>
<th>Floc</th>
<th>Soil (0-5 cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>NaOH-T</td>
<td>Ortho P</td>
<td>Monoesters</td>
</tr>
<tr>
<td>42.2</td>
<td>40.2</td>
<td>1.9</td>
<td>ND</td>
</tr>
<tr>
<td>Freez-dry</td>
<td>57.1</td>
<td>30.5</td>
<td>20.8</td>
</tr>
<tr>
<td>46.1</td>
<td>36.3</td>
<td>18.3</td>
<td>8.6</td>
</tr>
<tr>
<td>Oven-dry at 70°C</td>
<td>98.9</td>
<td>51.8</td>
<td>19.4</td>
</tr>
<tr>
<td>Oven-dry at 110°C</td>
<td>94.5</td>
<td>36.3</td>
<td>41.3</td>
</tr>
</tbody>
</table>

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

DISCUSSION AND CONCLUSIONS

- • Oven drying of soils at 70°C 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 35°C 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.