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Identifying plant cell wall remnants in detritus of a subtropical wetland with fluorescence labeling

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ABSTRACT

Sediment accretion in wetlands represents a significant carbon burial pathway. While litter studies can quantify the loss rates of plant leaf material, those studies do not provide insight into the specific cell wall polymers being retained or lost within the detrital matrix. The Everglades ecosystem has been dramatically altered due to anthropogenic eutrophication and hydrologic modifications. The results are changes in macrophyte species composition and sediment accretion- and loss- rates. To improve ecological conditions, active management strategies are re-establishing open water slough environments. A question remains about the persistence of new- and old- plant cell wall material in sediments because of active management. In this pilot project we utilized immuno-fluorescence labeling with lectins applied to plant leaf material and detrital flocculent collected from created open and control plots in the Everglades to observe the presence, absence, and overlap of specific cell wall polymers between macrophytes and detrital flocculent in increasingly recalcitrant materials that would most likely contribute to peat accumulation. The persistence and loss of specific polymers between treatment and control plots provided insight into the differing levels of recalcitrance amongst plant cell walls and their relative potential as a carbon sink. This study provides a novel method for testing for the presence and persistence of specific cell wall polymers in detritus to gain a better understanding of plant material persistence in wetland ecosystems.

1. Introduction

Wetlands provide long-term storage of carbon through sediment accretion. A significant component of sediment organic matter (OM) is contributed by above-ground plant cell wall biomass after death (Reddy and DeLaune 2008). Plant cell walls are broadly grouped as cellulose, hemicelluloses, pectins, and lignins which are a mixture of cross-linked compounds comprised of saccharides, aromatics, proteins, lipids, uronic acids, and ester sulfates (Srivastava et al. 2017). Sediment accretion or subsidence rates are directly related to the amount of contributed plant biomass and cell wall composition which will alter susceptibility to physical breakdown and microbial decomposition, as well as wetland physicochemical characteristics (e.g., anaerobic conditions) that influence microbial community composition and metabolic rates (Qualls and Richardson 2000; Kayranli et al. 2010).

The Florida Everglades, a massive wetland complex encompassing the greater portion of southern Florida, USA, has changed dramatically over the past century due to anthropogenic draining and cultural eutrophication which have altered OM and sediment subsidence and accretion rates (Qualls and Richardson 2000; Hagerthey et al. 2008). Where drained, peat loss has accelerated due to persistent aerobic conditions stimulating greater microbial activity, whereas flooding and excessive phosphorus (P) loading has driven replacement of the diverse periphyton-submerged aquatic vegetation-sawgrass (*Cladium jaimiacaense*) ridge-and-slough environment with monotypic stands of the emergent plant cattail (*Typha dominigensis*) (Hagerthey et al. 2008). Ridge-and-slough landscape sediment accretion rates were relatively slow due to alternating redox conditions and mixed inputs of labile and recalcitrant OM. Conversely, the dense monotypic cattail stands of P-impacted regions contribute significantly more above- and below-ground OM, resulting in higher biological oxygen demand and persistent anaerobic conditions leading to slower decomposition rates. Coupled with deposition of new detrital matter at rates exceeding decomposition, rapid sediment accretion has resulted in a loss of open water sloughs (Hagerthey et al. 2008; Reddy and DeLaune 2008).

To increase the functionality of the P-impacted regions, active

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Table 1
Immuno-fluorescence labeling results for plants and detritus. Strong detection (+), weak detection (–+), or absence (–) of each cell wall polymer (and associated fluorescence probe) given from the unfractionated sample, each soluble fraction, and applied to the remaining residue. The percent of the initial mass recovered after lyophilization from each soluble fraction provided. Abbreviations: AGP – arabinogalactan protein; EC – enriched control plot; EO – enriched open plot; HG – homogalacturonan; TC – transitional control plot; TO – transitional open plot;

			Cell Wall Protein	Hemicellulose		Pectin			
Site Plant or Substrate	Fraction	% Recovered	AGP (LM2)	Xylan (LM11)	Xyloglucan (LM15)	(1,4)-β-D-galactan (LM5)	Arabinan (LM6)	HG (JIM5)	HG (JIM7)
EC Cattail	Unfractionated		–	+	+	+	–	–	+
	MeKOH	7.5	–	+	+	–	–	–	–
	DMSO	2.4	–+	+	+	+	–	–	–
	Hot water	1.5	–	+	+	+	–	+	–
	CDTA	5.5	–	+	–	–+	–	–	–
	Na_2CO_3	2.4	–	+	–+	–+	+	–	–
	1 M KOH	0.1	–	+	+	–+	–	–	–
	4 M KOH	66.8	–	+	+	+	–	–	–
	Residue	13.8	–	+	–	–	–	–	–
EC Flocculent	Unfractionated		–+	+	+	–	–+	–+	–
	MeKOH	27.3	–	–+	–	–	–	–	–
	DMSO	1.6	–	–+	–	–	–	–	–
	Hot water	3.1	–	+	–+	–	–	–	–
	CDTA	3.4	–	–+	–	–	–	–	–
	Na_2CO_3	2.9	–	–	–	–	–	–	–
	1 M KOH	3.8	–	–+	–	–	–	–	–
	4 M KOH	46.1	–	–	–	–	–	–	–
	Residue	11.9	–+	–	–	–	–	–	–
TC Sawgrass	Unfractionated		+	–+	+	+	+	+	+
	MeKOH	9.6	–	+	–	–	–	–	–
	DMSO	0.6	–	+	–+	–+	+	–+	–
	Hot water	2.0	–	+	–+	–+	+	–+	–
	CDTA	1.8	–	+	–+	–+	–	–	–+
	Na_2CO_3	2.5	–	+	–+	+	–	–	–
	1 M KOH	30.4	–	+	–	–	+	–	–
	4 M KOH	33.0	–	+	+	+	–	–	–
	Residue	20.2	+	+	–	–	–	–	–
TC flocculent	Unfractionated		–+	+	+	+	+	–	+
	MeKOH	25.1	–	+	+	–	–	–	–
	DMSO	6.1	–	–	–	–	–	–	–
	Hot water	2.7	–+	+	+	+	–	–	–
	CDTA	4.3	–	–+	–+	–	–	–	–+
	Na_2CO_3	1.8	–	–	–	–	–	–	–
	1 M KOH	2.1	–	+	–	–	–	–	–
	4 M KOH	37.2	–	+	+	–	–	–	–
	Residue	20.8	–	–+	–	–	–	–	–
EO Muskgrass	Unfractionated		–+	+	–	–+	–	+	+
	MeKOH	6.5	–	+	–	–	–	–	–
	DMSO	1.0	–	–	–	–	–	–	–
	Hot water	4.2	–	+	–	–	–	–	–
	CDTA	2.6	–	–	–	–	–	–+	–
	Na_2CO_3	1.5	–	+	–	–	–	–+	–
	1 M KOH	33.6	–	+	–	–	–	–	–
	4 M KOH	0.1	–	+	–+	–	–	–	–
	Residue	50.3	–	–+	–	–	–	–+	–+
EO flocculent	Unfractionated		–+	+	+	–+	–	–+	–+
	MeKOH	21.7	–	+	–	–	–	–	–
	DMSO	2.5	–	+	–	–	–	–	–
	Hot water	4.6	–	–+	–+	–	–	–	–
	CDTA	1.0	–	+	–	–	–	–	–
	Na_2CO_3	3.0	–	–	–	–	–	–	–
	1 M KOH	7.9	–	–+	–	–	–	–	–
	4 M KOH	27.2	–	–+	+	–	–	–	–
	Residue	32.2	–	–	–	–	–	–	–
TO flocculent	Unfractionated		+	+	+	+	–+	–	+
	MeKOH	31.8	–	+	+	–	–	–	–
	DMSO	5.2	–	–	–	–	–+	–	–
	Hot water	4.2	–	+	–+	–	–	+	–

(*continued on next page*)

Table 1 (*continued*)

Site Plant or Substrate	Fraction	% Recovered	Cell Wall Protein	Hemicellulose		Pectin			
			AGP (LM2)	Xylan (LM11)	Xyloglucan (LM15)	(1,4)-β-D-galactan (LM5)	Arabinan (LM6)	HG (JIM5)	HG (JIM7)
	CDTA	3.4	–	–+	–+	–	–	–	–+
	Na_2CO_3	1.5	–	–	–	–	–	–	–
	1 M KOH	2.7	–	+	–	–	–	–	–
	4 M KOH	12.2	–	+	+	–	–	–	–
	Residue	38.8	–+	–+	–	–	–	–	–

management efforts have utilized herbicide and fire to remove the cattail stands, thereby creating an open water environment (Tian et al. 2010; Sklar et al. 2012). The newly created "sloughs" support submergent aquatic vegetation, notably the macroalgae muskgrass (*Chara* spp.) and periphyton. Wind mixing coupled with submerged primary producers has re-established aerobic conditions in the water column and surface detritus, resulting in a bacterial community shift (Bellinger et al. 2012). It was hypothesized that the altered physicochemical conditions, microbial communities, and substrate quality would stimulate faster decomposition of new organic matter and remnant macrophyte detritus relative to control plots. In this pilot study we utilized immuno-chemical fluorescence labeling techniques to provide a novel look at the presence and/or absence of specific cell wall polymers within the detrital floccculent from extant and previous macrophyte species. While there is an abundance of litter bag studies evaluating changes in plant mass decomposition (e.g., Qualls and Richardson 2000; Chimney and Pietro 2006), there are few studies documenting persistence or loss of specific cell wall polymers within the detrital matrix. Lectin labeling provides a rapid and less analytically intensive method relative to typical biochemical analyses (e.g., mass spectrometry, nuclear magnetic resonance) required to identify cell wall polymers.

We collected three plant species and detrital flocculent samples from paired plots in a northern Everglades water conservation area (WCA) in July of 2010 (detailed descriptions of sites in Sklar et al. 2012). Briefly, open water plots ("O"; 250 m × 250 m) were created in highly enriched ("E"; water P > 50 μg/L) and transitional ("T"; P 20 – 50 μg/L) areas of WCA-2A. Control ("C") plots in the "E" region were dominated by cattail, whereas in "TC" plots cattail and sawgrass were co-dominant. The created EO and TO plots were dominated by muskgrass with periphyton also present. Detrital flocculent from all plots was represented by the pourable fraction above the consolidated peat sediments (Bellinger et al. 2012). Within a plot, three floc sub-samples were collected and composited to capture spatial heterogeneity in plant, algal, and detrital biomass. Plant and floc material were lyophilized to determine initial dry weight (DW; g). Presence of specific cell wall components in macrophyte and detrital flocculent samples were screened by adding a lectin to the intact sample ("unfractionated"), and then to fractions isolated during sequential chemical extractions meant to solubilize distinctive cell wall polymers (Fry 1988). Freeze-dried material was sequentially extracted with hot (90 °C) methanolic KOH (MeKOH), followed by dimethyl sulfoxide (DMSO); hot water (>100 °C; HW); (1,2-cclohexylendedi nitrile)-tetraacetic acid (CDTA); Na_2CO_3; 1 M KOH; and lastly 4 M KOH with the remaining pellet collected (Fry 1988). Fractions were split to determine final DW after dialysis against dH_20 to remove salts, and for immunofluorescence labeling according to the methods of Knox et al. (1990). The suite of monoclonal antibodies applied targeted: pectins (1,4)-β-D-galactan (LM5), arabinan (LM6), and esterified homogalacturonan (HG; Jim5 and 7); the hemicelluloses xylan/arabinoxylan (LM11) and xyloglucan (LM15); and, the cell wall protein arabinogalactan protein (AGP; LM2). Antibodies were developed by Plant Probes (Paul Knox Cell Wall Laboratory, University of Leeds, England). Intensity of labeling within each fraction was qualitatively noted (i.e., "no", "weak", and "strong" fluorescence).

The total amount of material chemically extracted from the initial plant or detrital sample varied between 10 and 50 %, and among extractants, MeKOH and the 4 M KOH typically extracted the most material (Table 1). The residual material was generally not labeled by the lectins applied suggesting it was comprised of recalcitrant lignins and cellulose common in macrophytes. From the cattail, one polymer (HG-JIM7) was detected only in the unfractionated material and was not observed in any fraction or residue. Conversely, three other polymers (AGP, arabinan, and HG-JIM5) were identified in the chemical extracts of cattail but not the unfractionated material. It could be that cell wall configuration prevented successful labeling of the parent material. All the lectins applied to sawgrass labeled a polymer in an extracted fraction as well as unfractionated material.

There were only a few examples where the polymers present in the native plant material were absent in the flocculent (Table 1). For example, β-D-glacatan and Jim7-labeled HG polymers were absent from the EC flocculent material and fractions despite being present in both cattail and sawgrass, suggesting those polymers were effectively degraded within the anaerobic detritus. Conversely, presence of HG-JM5 in the EC flocculent and only sawgrass suggests that esterified HG persisted in the enriched plots past the transitional period where sawgrass was completely replaced by cattail. Finally, the strong presence of arabinan in floc from the TC plots but not EC plots suggests that that polymer was effectively degraded prior to cattail-dominance.

The unfractionated muskgrass plants were labeled for most of the same cell wall polymers as cattail and sawgrass except for arabinan and xyloglucan (Table 1). The labeling patterns of muskgrass observed here matched those of previous studies, confirming the ubiquitous presence of complex cell wall polymers in the Charophycean algae (Domozych et al. 2010). However, the absence of xyloglucan from the muskgrass sampled here is surprising as that cell wall polymer has been previously identified in muskgrass species (Domozych et al. 2009). Among the polymers identified from the flocculent, the persistence of HGs in open plots corroborates the potential for long-term burial of P through complexing with calcium carbonate precipitated on cell wall HGs during photosynthesis (Siong and Asaeda 2006). Absence of arabinan in muskgrass and only a weak labeling of flocculent material from the EO and TO sites suggests that after creation of the open plots the arabinans from emergent macrophyte cell walls were effectively decomposed by the new bacterial and fungal community (Bellinger et al. 2012). Conversely, xyloglucan observed in the EO/TO flocculent had persisted after the managed removal of cattail and/or sawgrass years prior to sampling.

The sequential extraction protocol applied to the flocculent material isolated several cell wall polymers that were also observed from intact plant material providing insight into the longevity of those polymers in the detrital matrix. The abundance of OM in the residue fraction represents material that can contribute to peat accumulation and is likely comprised of cellulose and lignins. Conventional thought regarding decomposition of cell wall polymers is that polysaccharides, aside from cellulose, are rapidly degraded by bacteria and fungi (Marchand et al. 2005; Reddy and DeLaune 2008). However, our results suggests that decomposition rates for a variety of cell wall components will vary depending on environmental conditions. While this was a qualitative approach to tracking cell wall accumulation and loss within a detrital

layer, these techniques could be coupled with additional sampling of deeper peat sediments and quantitative measures to measure how alterations to wetland ecosystems are impacting carbon cycling dynamics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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