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Contributions of degrading *Spartina alterniflora* lignocellulose to the dissolved organic carbon pool of a salt marsh

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ABSTRACT: As *Spartina alterniflora* lignocellulose degrades, it contributes soluble degradation products to the bulk dissolved organic carbon (DOC) pool in salt marsh environments. Experiments with radiolabeled *S. alterniflora* lignocelluloses show that during the initial 6 mo of decomposition, DOC accounts for 50 to 60% of the total degradation products (DOC plus CO_2) of the lignin fraction of lignocellulose; by contrast, only 20 to 30% of the polysaccharide portion of *S. alterniflora* lignocellulose accumulates as DOC during decomposition. The differences in net accumulation are most likely due to differential rates of microbial utilization of the soluble compounds derived from these 2 fractions and not to differential rates of formation. As a result, although lignin comprises only 7% of undegraded *S. alterniflora* lignocellulose, it may contribute as much as 30% of the carbon in lignocellulose-derived DOC. Soluble compounds derived from lignin show evidence of significant chemical modification, such that only a small fraction of lignin-derived carbon is present as recognizable lignin phenols. The long residence time of lignin-derived carbon in salt marsh DOC pools, relative to that for polysaccharide-derived carbon, demonstrates a mechanism by which lignin may serve as a source of aquatic humic substances and contribute to the bulk DOC pool of salt marshes in greater proportion than expected from the ratio of lignin : polysaccharides in undegraded plant material. Based on quantification of lignin oxidation products, we estimate that *S. alterniflora* lignocellulose, both the lignin and polysaccharide components, contributes 44% of the carbon in the bulk DOC pool of a Georgia salt marsh creek.

INTRODUCTION

Recognition of the quantitative importance of dissolved organic carbon (DOC) in aquatic environments has given rise to interest in its ecological significance within the microbial loop (Azam et al. 1983, Ducklow et al. 1986, Sherr et al. 1987). Because of the chemical complexity and the difficulty in separation and identification of the various components, approaches to the study of DOC oftentimes have focused on the concentration and dynamics of uncharacterized bulk material (Bott et al. 1984, Ford & Lock 1987). However, this approach is limited in that dynamics of minor components may be masked. An alternative approach involves identification and investigation of a single component of the DOC pool (Azam & Hodson 1977). The rationale for this approach lies either in determining the quantitative importance of the chosen component or in demonstrating that it can serve as a useful model compound for tracing the fate of a particular type of DOC (e.g. very labile compounds). Our approach in this investigation was a hybrid of these 2 methodologies, in that we studied a complex mixture of dissolved organic compounds which are derived from a single source, the lignocellulosic component of marine vascular plants. Although composed initially of insoluble structural polymers, lignocellulose is a potentially important source of DOC in aquatic ecosystems through degradation-mediated solubilization (Moran & Hodson 1989). Lignocellulose-derived DOC may be particularly important in shallow aquatic ecosystems with extensive vascular plant growth, such as the coastal salt marshes of the southeastern USA. However, its importance in this and other ecosystems has never been quantified.

Lignocellulose is a heteropolymer made up of polysaccharides (cellulose and hemicellulose; ca 90 to 95% of aquatic vascular plant lignocellulose) and lignin (ca 5 to 10%). These 2 chemical fractions are degraded by aquatic microorganisms at distinctly different rates.

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Experiments following either the formation of $^{14}CO_2$ from specifically-radiolabeled lignocelluloses degrading in laboratory microcosms (Maccubbin & Hodson 1980, Benner et al. 1984a, 1986) or the relative changes in remaining particulate material in both laboratory and field incubations (Hodson et al. 1984, Moran et al. 1989) have demonstrated that rates of degradation of the lignin moiety of lignocellulose are significantly lower than rates of polysaccharide degradation, with differences ranging from 2- to 10-fold. As a result of the differential rates of microbial decomposition, lignin may comprise up to 30 % or more of remaining particulate *Spartina alterniflora* detritus after 1 yr of decomposition (Moran et al. 1989).

The pool of soluble products formed during microbial decomposition of lignocellulose contains compounds derived from both the lignin and polysaccharide components of the lignocellulose matrix. Because of the heterogeneous nature of soluble lignocellulose degradation products, the potential exists for microorganisms to preferentially take up certain components of lignocellulose-derived DOC, while discriminating against others. If, as predicted from degradation dynamics of particulate lignocellulose, DOC derived from the lignin fraction of lignocellulose is more refractory to microbial decomposition than is polysaccharide-derived DOC, lignin will contribute more to the resident pool of dissolved compounds in aquatic environments than the initial lignin : polysaccharide ratio in undegraded plant material would suggest; conversely, polysaccharides will contribute relatively more to microbial secondary production, at least in the short term. Refractory lignin-derived compounds are possibly an important source of dissolved humic substances, largely uncharacterized compounds with long residence times that make up 50 % or more of the total DOC in aquatic environments (Thurman 1985).

In the studies presented here, we examine the relative contributions of lignin and polysaccharide to lignocellulose-derived DOC in a Georgia salt marsh and make a preliminary estimate of the quantitative importance of lignocellulose-derived DOC in the salt marsh system.

MATERIALS AND METHODS

The relative contributions of lignin and polysaccharide to the DOC pool of a Georgia (USA) salt marsh were examined by following the fate of radiolabeled DOC derived from the degradation of specifically-radiolabeled and uniformly-radiolabeled lignocelluloses by natural microbial assemblages. For specific labeling, cut stems of the salt marsh grass *Spartina alterniflora* were allowed to incorporate radiolabeled precursors for the biosynthesis of lignin (^{14}C-cinnamic acid) or polysaccharides (^{14}C-glucose). After several days, stems were harvested, dried at 55 °C, and ground to particles < 425 µm in size. This size range of particles is representative of small pieces of particulate lignocellulose fragmented from plant stems via physical and biological activity; such fragmentation is thought to be the fate of ca 30 % of the standing-dead *S. alterniflora* in coastal Georgia salt marshes (Newell et al. 1989). Plant particles were sequentially extracted in boiling ethanol, ethanol-benzene (1 : 2, vol : vol), and water for removal of non-lignocellulosic components. Chemical analyses of specifically-radiolabeled lignocelluloses (Benner et al. 1984a) indicated that they were substantially free of radiolabeled contaminants. Mineralization of [^{14}C-lignin]lignocelluloses has previously been demonstrated to track the decomposition of the lignin component of *S. alterniflora* lignocellulose, and likewise mineralization of [^{14}C-polysaccharide]lignocelluloses to track decomposition of the polysaccharide component (Hodson et al. 1984).

For preparation of uniformly labeled lignocellulose ([U-^{14}C]lignocellulose), live potted *Spartina alterniflora* plants were grown in mylar bags containing an atmosphere of $^{14}CO_2$. Labeling occurred at intervals over a 1 mo period, following which plants were cut, dried, ground, and extracted as described above. [U-^{14}C]lignocellulose preparations were checked for uniformity of labeling by comparing percentages of total lignocellulose, polysaccharides, and lignin in the plant material on a weight basis with the percentages of radioactivity in each component. The distribution of radioactivity among the components of the labeled plants was confirmed to be similar to the natural distribution by weight, i.e. the various components of lignocellulose had similar specific activities.

Degradation of labeled lignocelluloses was followed in small (125 ml) bottle microcosms containing 10 to 50 mg lignocellulose or in large (4 l) bottle microcosms containing 350 to 400 mg lignocellulose. Salt-marsh creek water or sediment-slurry inocula from salt marshes of Sapelo Island, Georgia, were added to 3 or 4 replicate incubations to give a final concentration of 1 mg ml^{-1} particulate lignocellulose. Microbial mineralization of the labeled moiety (lignin, polysaccharide, or lignocellulose) was quantified by flushing humidified air through the microcosms for 15 to 30 min (depending on the size of the microcosms) and trapping evolved $^{14}CO_2$ in a CO_2-absorbing scintillation medium followed by radioassay using liquid scintillation spectroscopy. Microcosms were aerated and $^{14}CO_2$ was collected at frequent intervals during the initial stages of decomposition (2 to 4 d) and at longer intervals in later stages of decomposition (up to 14 d). Dissolved degradation products (DO^{14}C) were quantified either at the end of the incubation period (termination sampling) or

simultaneously with quantification of $^{14}CO_2$ (interval sampling). Microcosm contents were acidified to pH 2.5 to 3.0 and filtered through a 0.2 µm pore-size filter; radiolabel was quantified in 1 ml subsamples of the filtrate.

For interval-sampled microcosms, contents were readjusted to the original pH with NaOH and reinoculated with fresh salt-marsh water for the next sampling interval. Detailed descriptions of sampling procedures are given in Benner et al. (1984a) and Moran et al. (1989). Total degradation, expressed as percent of original particulate labeled moiety (lignin, polysaccharide, or lignocellulose), is calculated as the sum of mineralization plus soluble degradation products.

Water from a tidal creek and dissolved degradation products from laboratory incubations of unlabeled *Spartina alterniflora* lignocellulose were analyzed for content of lignin-derived phenols. In August, 1988, 40 l of water were collected from the Duplin River, a large tidal creek draining the Sapelo Island salt marshes, on 2 consecutive days at slack high tide (Fig. 1). Humic substances were isolated from the filtered (Whatman GF/F filters), acidified water samples by passage through an Amberlite XAD-8 resin (Aiken 1985). Humic substances were eluted from the resin with 0.1 N NaOH and the eluent desalted by passage through a column of Bio-Rad AP MG-50 ion exchange resin. Freeze-dried humic substances were reacted with cupric oxide for 3 h at 170 °C to produce a suite of 11 characteristic lignin-derived phenols. These phenols fall within 4 families: vanillyl phenols (vanillin, acetovanillone, and vanillic acid), syringyl phenols (syringaldehyde, acetosyringone, and syringic acid), *p*-hydroxyl phenols (*p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone, and *p*-hydroxybenzoic acid), and cinnamyl phenols (*p*-coumaric acid and ferulic acid). Oxidation products were extracted with ether, converted to trimethylsilyl derivatives, and analyzed for the 11 phenols via capillary gas chromatography (Hedges & Ertel 1982).

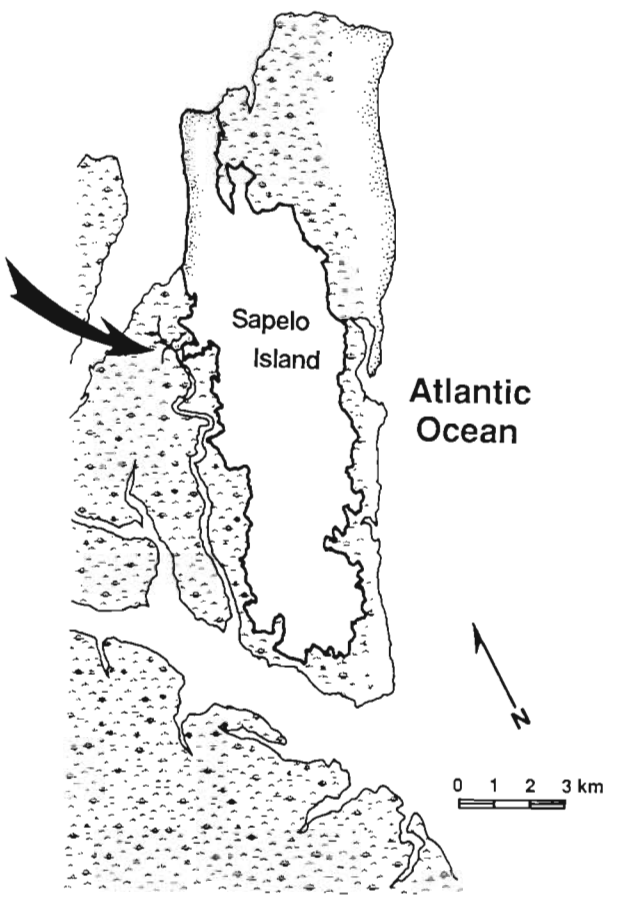

Fig. 1. Sample collection site (arrow) in the Duplin River at Sapelo Island, Georgia, USA

For laboratory studies, *Spartina alterniflora* lignocellulose was incubated at concentrations of 10 to 15 mg ml^{-1} in continuously bubbled (to provide aeration) 1 l flasks containing artificial seawater and inorganic nutrients. This concentration of lignocellulose is higher than that used in incubations with radiolabeled lignocelluloses, but was necessary in order to obtain sufficient material for chemical analyses. After 2 or 7 wk of decomposition, DOC was harvested by filtration (Whatman GF/F followed by Rainin 0.2 µm pore-size filters). Humic substances were isolated on an XAD-8 resin and analyzed for content of lignin-derived phenols as described above.

For undegraded particulate *Spartina alterniflora* lignocellulose, the quantity and signature of lignin-derived phenols was determined by direct oxidation of the ground material with cupric oxide. The lignin content of particulate *S. alterniflora* lignocellulose was also determined gravimetrically, by the detergent fiber method of Goering & Van Soest (1970). Ground material was successively extracted with a neutral detergent solution, acid detergent solution, and 72 % sulfuric acid.

RESULTS AND DISCUSSION

In 9 short-term decomposition studies (incubation periods ranging from 10 to 23 d) in which *Spartina alterniflora* [^{14}C-lignin]lignocelluloses and [^{14}C-polysaccharide]lignocelluloses were degraded under identical conditions, DO^{14}C accumulation at the end of the decomposition period accounted for 13 to 71 % of the total degradation products (Table 1A). In all 9 experiments, despite differences in inoculum with regard to source (water or sediment) or season of the year, the percent of degradation products recovered as DOC

Table 1. Net accumulation of dissolved organic carbon ($DO^{14}C$) derived from degrading *Spartina alterniflora* [^{14}C-lignin]lignocelluloses and [^{14}C-polysaccharide]lignocelluloses (A), and [U-^{14}C]lignocelluloses (B), expressed as percent of total degradation products ($DO^{14}C \times 100/^{14}CO_2 + DO^{14}C$)

	Incubation length (d)	Accumulation of $DO^{14}C$ Lignin-labeled	Polysaccharide-labeled	Source
A	10	14.6	13.5	This paper
	10	68.7	57.4	This paper
	10	57.1	33.1	This paper
	8	50.7	48.3	This paper
	10	67.2	23.6	Benner et al. (1986)
	10	57.5	15.9	Benner et al. (1986)
	10	71.0	30.1	Benner et al. (1986)
	10	60.5	21.9	Benner et al. (1986)
	23	39.4	12.8	Benner et al. (1984c)
	Mean ± SE	54.1 ± 5.9	28.5 ± 5.2	
		(Uniformly-labeled)		
B	7	16.3		This paper
	7	14.3		This paper
	7	11.8		This paper
	14	13.5		This paper
	14	11.7		This paper
	29	16.1		This paper
	29	11.0		This paper
	Mean ± SE	13.5 ± 0.8		

was higher for degradation of lignin than for polysaccharide (paired t-test, $p < 0.002$), averaging 54.1 % of the products of lignin decomposition but only 28.5 % of the products of polysaccharide decomposition. Explanations for the difference in DOC accumulation for carbon derived from the 2 lignocellulose moieties include the possibilities that: (1) lignin-derived DOC is formed at a higher rate than is polysaccharide-derived DOC during microbial decomposition of lignocellulose; or (2) lignin-derived DOC is formed at equal or lower rates than is polysaccharide-derived DOC, but subsequent microbial utilization of the lignin-derived compounds occurs more slowly. As *S. alterniflora* lignocellulose degrades, the lignin content of the particulate material gradually increases, accounting for up to 30 % of the remaining material following 1 yr of decomposition (Hodson et al. 1984, Moran et al. 1989). Furthermore, rates of lignin degradation (mineralization plus solubilization) have been directly demonstrated to be lower than rates of polysaccharide degradation (Benner et al. 1984a, 1986). Thus the best available evidence suggests that decomposition of lignin, and therefore the formation of lignin-derived DOC, proceeds at a lower rate than degradation of the polysaccharide component. Observed differences in the accumulation of lignocellulose-derived DOC are therefore most likely attributable to differential rates of microbial utilization of the soluble products of lignocellulose degradation and not to differential rates of formation.

Results of 7 additional studies with uniformly-labeled *Spartina alterniflora* lignocellulose, which track the degradation of the entire lignocellulose matrix rather than specific components of lignocellulose, show that accumulation of $DO^{14}C$ accounts for an average of 13.5 % of the degradation products after 1 to 4 wk of decomposition (Table 1B). As *S. alterniflora* lignocellulose is 93 % polysaccharide by weight, uniformly-labeled lignocellulose preparations are dominated by polysaccharides and measurements of the degradation of the overall complex largely reflect polysaccharide decomposition dynamics. As expected, DOC accumulations from degrading [U-^{14}C]lignocellulose were more similar to the lower values found for the polysaccharide moiety than for the higher values characteristic of the lignin moiety.

In previous studies, we have tracked the degradation kinetics of lignocelluloses derived from other woody and non-woody vascular plants in a variety of marine and freshwater environments. Results from these studies can be used to compare accumulation of DOC derived from the lignin and polysaccharide moieties of lignocellulose degrading under identical conditions. As was found for *Spartina alterniflora*, degradation of lignocelluloses from the sedge *Carex walteriana* (Okefenokee Swamp, Georgia; Benner et al. 1984b, Benner et al. 1986, Moran & Hodson unpubl.), the rush *Juncus romerianus* (Georgia salt marsh; Benner et al. 1984b), red mangrove *Rhizophora mangle* (Bahama Islands; Benner & Hodson 1985), and cypress *Taxodium ascendens* (Corkscrew Swamp, Florida; Benner et al. 1986) in the presence of natural microbial populations under both aerobic and anaerobic conditions resulted in significantly greater accumulation of lignin-derived DOC (averaging 50.0 ± 3.6 % of total lignin degradation products for all vascular plant species including *S. alterniflora*; n = 35) than polysaccharide-derived DOC (30.6 ± 3.3 %; n = 35). The difference in percent accumulation of soluble compounds between the 2 components of lignocellulose was significant (paired t-test, $p < 0.001$).

Short-term studies are subject to criticism because they span the degradation of only a small percentage of the original lignocellulose. Although lignocellulose preparations were checked for appropriate distribution of label with respect to weight distributions of the lignocellulose components, small amounts of contaminants may be present in the preparations. If present, such contaminants would affect the accumulation of $DO^{14}C$ more dramatically for short-term incubations;

for example labile (readily mineralized) contaminants might skew the ratio of DOC:CO_2 in the initial stages of decomposition. However, we can expect that contaminants would have an ever-decreasing effect on apparent lignocellulose-derived DOC accumulation as decomposition time is increased and larger proportions of the particulate material are degraded. In long-term studies of *Spartina alterniflora* lignocellulose degradation, uniformly-labeled and lignin-labeled *S. alterniflora* lignocelluloses were degraded for 8 or 24 wk, with aeration and collection of $^{14}CO_2$ and $DO^{14}C$ every 2 to 14 d. $DO^{14}C$ accounted for 68.1 (± 1.6) and 56.8 % (± 2.4 %) of the total degradation products of lignin decomposition after 8 and 24 wk degradation, respectively (Fig. 2). Significantly lower percentages [20.8 (± 0.4) and 19.1 % (± 0.6 %)] were recovered as $DO^{14}C$ from the degradation of whole lignocellulose (t-test, $p < 0.001$ for both decomposition periods). Because over 30 % of the original weight of particulate lignocellulose had been lost after 24 wk of decomposition, minor labeled contaminants could not be responsible for the large differences observed in DOC accumulation during decomposition of the lignin portion of lignocellulose vs whole (largely polysaccharide) lignocellulose.

We conducted another long-term study of *Spartina alterniflora* lignocellulose degradation in salt-marsh microcosms which differed in that the soluble degradation products were collected only at the termination of the experiment, rather than at intervals during decomposition. Accumulation of soluble degradation products again accounted for a greater percentage of total decomposition products for lignin than for whole lignocellulose, averaging 25.5 (± 3.2) and 20 % (± 2.4 %) of the products of lignin degradation after 2 and 7 wk of decomposition, respectively, but only 8 (± 0.6) and 5 % (± 0.9 %) of the products of overall lignocellulose degradation (Fig. 3; t-test, $p < 0.01$ for both decomposition periods). As $DO^{14}C$ was not removed from the microcosms during the course of these experiments, greater opportunity existed for microbial uptake and mineralization of the soluble compounds. As a result, smaller percentages of dissolved compounds were collected from these termination-sampled microcosms than from the interval-sampled microcosms (compare Week 8, Fig. 2 to Week 7, Fig. 3). Microbial utilization of DOC formed from degradation of refractory particulate lignocellulose has also been reported previously (Moran & Hodson 1989) and indicates that lignocellulose-derived soluble compounds may be important substrates for DOC-based microbial food webs, particularly in aquatic ecosystems with substantial production or input of vascular plant material.

Lignin-derived DOC formed during degradation of *Spartina alterniflora* lignocellulose may remain relatively intact, and therefore consist largely of recognizable lignin oligomers or monomers, or it may be significantly degraded, such that many compounds are no longer identifiable by standard chemical analyses as being derived from polymeric lignin. Although the unique chemical signal of lignin may be extinguished by modification during decomposition, carbon derived from lignin will still be identifiable with radiotracer methods. We investigated the extent of modification of lignin-derived DOC by comparing the lignin phenol approach (Hedges & Ertel 1982) with radiotracer studies. Characteristic lignin-derived phenols were quantified in undegraded *S. alterniflora* lignocellulose and in the DOC accumulating during lignocellulose degradation. For undegraded particulate lignocellu-

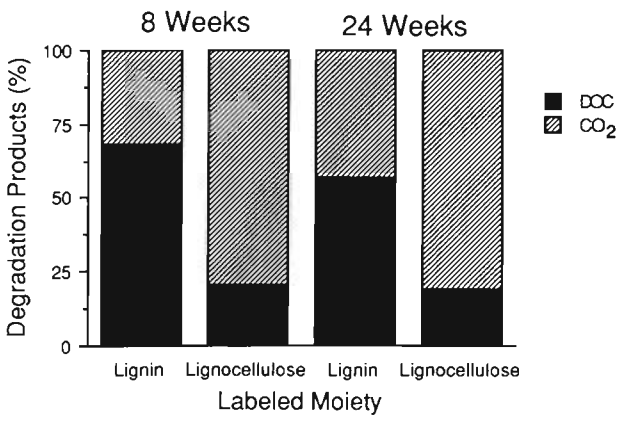

Fig. 2. Accumulation of dissolved organic carbon ($DO^{14}C$) and formation of $^{14}CO_2$, expressed as percent of total degradation products ($DO^{14}C$ plus $^{14}CO_2$), collected from degrading *Spartina alterniflora* [^{14}C-lignin]lignocelluloses and [U-^{14}C]lignocelluloses during an 8 and 24 wk study (n = 3)

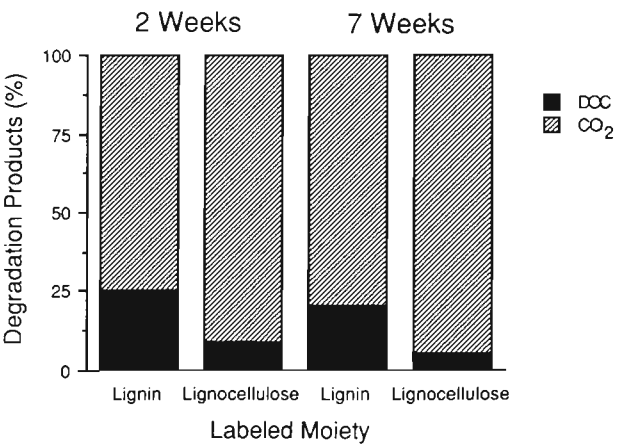

Fig. 3. Accumulation of dissolved organic carbon ($DO^{14}C$) and formation of $^{14}CO_2$, expressed as percent of total degradation products ($DO^{14}C$ plus $^{14}CO_2$), collected from degrading *Spartina alterniflora* [^{14}C-lignin]lignocelluloses and [U-^{14}C]lignocelluloses after a 2 and 7 wk study (n = 3)

lose, lignin-derived phenols (sum of 11 oxidation products) accounted for 5.6 % of lignocellulose dry weight, matching well the gravimetric determination of 7.1 % lignin and indicating that the lignin phenol approach is reasonably quantitative for microbially unaltered *S. alterniflora* lignin (Table 2). In DOC formed from *S. alterniflora* lignocellulose degrading for 2 and 7 wk in the presence of natural microbial assemblages, recognizable lignin-derived phenols accounted for less than 1 % of the carbon. This may be an underestimate of total lignin-derived phenols in the DOC pool, as the concentration step used during analysis excludes any lignin-derived phenols in the non-humic fraction of the DOC. Nonetheless, even if equivalent amounts of lignin-derived material were present in both the humic and non-humic fractions of *S. alterniflora* lignocellulose-derived DOC, the total amount of lignin phenols would still be only a few percent of the dissolved carbon pool. In contrast, the termination-sampled radiotracer study of lignocellulose degradation, which tracks lignin-derived carbon regardless of the extent of chemical modification, indicated that lignin contributed as much as 30 % of the carbon in *S. alterniflora* lignocellulose-derived DOC (Table 2). Assuming that the differences in particulate lignocellulose concentrations in labeled vs unlabeled incubations had only minor effects on the chemical composition of DOC formed during aerobic decomposition, the bulk of lignin-derived DOC is apparently significantly chemically modified, either during or subsequent to its formation, such that it no longer gives characteristic lignin phenols upon oxidation.

The lignin signature of vascular plant material provides additional compositional information through examination of the quantitative relationships among the 11 phenols. As expected for non-woody angiosperm lignin (Ertel et al. 1984), lignin oxidation products from undegraded *Spartina alterniflora* lignocellulose have a ratio of syringyl:vanillyl phenols (S:V) of 0.70 to 0.85 (n = 4) and a ratio of cinnamyl:vanillyl phenols (C:V) of 0.28 to 0.37 (n = 4). In contrast, dissolved degradation products of *S. alterniflora* lignocellulose have a lignin signature very distinct from that of undegraded particulate lignocellulose, with an S:V ratio of 0 to 0.25 (n = 8) and a C:V ratio of 0.04 to 0.08 (n = 8). Thus these laboratory incubations, in which the *S. alterniflora* lignocellulose was the only possible source of DOC, directly demonstrate the divergence of lignin phenol signatures of dissolved degradation products from those of the undegraded source plant. This is potentially due to preferential solubilization of vanillyl phenols (or those compounds oxidized by cupric oxide to vanillyl phenols) during microbial degradation of lignocellulose, or preferential microbial utilization and mineralization of cinnamyl and syringyl phenols from the pool of dissolved lignin-derived compounds, leading to the relative overrepresentation of vanillyl phenols in the remaining DOC. Significantly lower S:V and C:V ratios for lignin-derived phenols relative to those in the presumed source plants have been found previously for aquatic humic substances in Amazon River DOC (Ertel et al. 1986). Furthermore, the preferential loss of both syringyl and cinnamyl phenols has also been detected in the remaining particulate phase of degrading vascular plant material (Hedges et al. 1988a, S. Y Newell pers. comm.). These findings show that the use of lignin signatures to determine the source type of vascular plant-derived material in bulk DOC and particulate organic carbon (POC) can be complicated by radical changes in phenolic ratios of degradation products relative to that of undegraded plant material.

Table 2. Percent lignin-derived carbon in undegraded lignocellulose and dissolved organic carbon (DOC) derived from degrading lignocellulose. For the radiolabel method, the amount of DOC derived from *Spartina alterniflora* lignin and lignocellulose was calculated by dividing the dpm ml^{-1} in 0.2 µm filtrates by the specific activity of the lignin (in [^{14}C-lignin]lignocellulose) or lignocellulose (in [U-^{14}C]lignocellulose), respectively. Radioactivity in the DOC fraction after 2 wk degradation averaged 116 dpm ml^{-1} for [^{14}C-lignin]lignocellulose and 1364 dpm ml^{-1} for [U-^{14}C]lignocellulose, and after 7 wk averaged 183 dpm ml^{-1} for [^{14}C-lignin]lignocellulose and 1865 dpm ml^{-1} for [U-^{14}C]lignocellulose. Specific activities of *S. alterniflora* lignocelluloses are 31 670 dpm mg^{-1} for the lignin portion of lignin-labeled lignocellulose and 91 603 dpm mg^{-1} for uniformly-labeled lignocellulose. Percent lignin-derived carbon in DOC after 2 or 7 wk lignocellulose degradation was then calculated as: mg lignin-derived C × 100/mg lignocellulose-derived C

Method	Percent lignin in:		
	Undegraded lignocellulose	Lignocellulose-derived DOC (2 wk)	Lignocellulose-derived DOC (7 wk)
Lignin phenol	5.6 ± 0.38[a]	0.59 ± 0.08[b]	0.99 ± 0.28[b]
Gravimetric	7.1 ± 0.15[c]	n/a[d]	n/a[d]
Radiolabel	n/a[d]	24.0 ± 1.7[a]	29.4 ± 5.3[a]

[a] n = 3; [b] n = 4; [c] n = 5; [d] not applicable

In creek water draining the Sapelo Island salt marshes, characteristic lignin-derived phenols in the humic carbon fraction accounted for 0.35 % of the total DOC (mean of 2 samples). If, as chemical analysis of laboratory incubations indicates, recently-formed DOC from *Spartina alterniflora* lignocellulose contains 0.79 % recognizable lignin-derived phenols (mean of 2- and 7-wk laboratory studies; Table 2), then we can calculate lignocellulose contributions to natural DOC assuming simple dilution: [(0.35 × 100)/0.79 = 44]. Thus, we estimate that ca 44 % of the bulk DOC in Duplin River water is derived from *S. alterniflora* lignocellulose. This estimate is preliminary, however, in that 2 of the 11 lignin phenols (*p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) are not unambiguous lignin markers (Hedges et al. 1988b) and thus, in Duplin River water, may derive from non-vascular plant sources. Furthermore, the estimate involves relatively few samples, assumes that laboratory-degraded lignocellulose produces DOC with similar lignin content and phenolic signature as naturally-degraded lignocellulose, and does not consider the heterogeneities imposed by tidal and seasonal fluctuations in the salt marsh.

Although lignin accounts for only 7 % of the weight of *Spartina alterniflora* lignocellulose (polysaccharide accounts for the remaining 93 %), the lower rates of microbial uptake and mineralization of soluble lignin-derived compounds result in relative overrepresentation of lignin in lignocellulose-derived DOC; radiotracer studies presented here suggest that lignin may be the source of up to 30 % of the dissolved products accumulating during the decomposition of *S. alterniflora* lignocellulose. However, as comparisons of chemical and radiotracer analyses reveal, this pool of lignin-derived compounds is apparently substantially altered from intact lignin with regard to the amount and type of characteristic phenols. Because of its long residence time, lignin-derived DOC is a potentially important source of aquatic humic substances in the salt marsh and adjacent waters, whereas polysaccharide-derived DOC may be more rapidly incorporated into marine microbial food webs. Preliminary investigations suggest that *S. alterniflora* lignocellulose is the source of a significant fraction of the bulk DOC in a Georgia salt marsh creek, although the DOC is in a highly modified chemical state relative to the particulate source material.

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