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Release of dissolved organic carbon from seagrass wrack and its implications for trophic connectivity

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ABSTRACT: The export of old leaves and stems (wrack) from seagrass meadows provides a mechanism for trophic connectivity among coastal ecosystems. As little of this wrack is consumed by mesograzers, leached dissolved organic carbon (DOC) may determine the importance of wrack as a trophic subsidy. However, few studies have examined the effect of seagrass type or age on the release of DOC or its bioavailability. We examined the amount and composition of DOC released from different wrack: *Posidonia sinuosa*, *Amphibolis antarctica* and the alga *Laurencia* sp. We then examined the effect of age on DOC leaching from *P. sinuosa* wrack. The bioavailability of the DOC was also assessed using a bacterial bioassay. The rate of DOC leaching from *P. sinuosa* leaves decreased exponentially with time. According to that exponential model, ~50 % of the total DOC release occurred in the first 14 d and it would require a further 2.94 yr to release the same amount again. Fresh algae *Laurencia* sp. leached the greatest amount of DOC in the first 16 h (6.7 g kg^{-1} fresh weight (FW) wrack), followed by fresh *P. sinuosa* leaves (1.7 g kg^{-1} FW), *A. antarctica* leaves (1.1 g kg^{-1}) and stems (0.6 g kg^{-1}), 4 wk old *P. sinuosa* (67 g kg^{-1}) and fine detritus (74 g kg^{-1}). In all cases, the composition of the DOC was similar and dominated by the hydrophilic component (in *P. sinuosa*, predominantly sugars and amino acids). Leachates from all fresh wrack supported bacterial growth over 24 h. Leachate from older wrack either failed to support bacterial growth or only supported it for a limited time. Given the exponential decay in DOC release rate, the interacting timescales of transport and leaching will affect the value of wrack as a vector for trophic subsidies.

KEY WORDS: Dissolved organic carbon · DOC · Seagrass · Wrack · Geographe Bay

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INTRODUCTION

Seagrass meadows are conspicuous and highly productive components of coastal ecosystems worldwide (Green & Short 2003). A portion of seagrass production is continually shed as old leaves, which can contribute significantly to wrack (detached macrophyte) accumulations in adjacent coastal habitats (Kirkman & Kendrick 1997, Mateo 2010). Export rates of detached leaves from meadows varies enormously, but can be as high as 100 % of leaf production (Cebrian & Duarte 2001, Mateo et al. 2006, Mateo 2010). Given that these older leaves also contain nutrients other than carbon, even after resorption before shedding (e.g. Prado et al. 2008), this wrack export represents a significant potential loss of nutrients from the habitat and a potential trophic subsidy to adjacent recipient habitats, particularly in oligotrophic environments where alternative sources of nutrient may be limited.

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Despite this potential, there is little published evidence of seagrass wrack being an important source of nutrients to adjacent habitats. Several studies concluded that seagrass wrack was unlikely to be a significant contributor to mesograzer production in recipient habitats, including unvegetated marine habitats (Hyndes & Lavery 2005), beach ecosystems (Ince et al. 2007), surf zones (Crawley et al. 2009) and within seagrass meadows (Smit et al. 2005, 2006). Wrack typically has a large proportion of macroalgae, and many mesograzers demonstrate a preference for this over seagrass detritus (Doropoulos et al. 2009), likely due to the lower C:N ratio of algae. This suggests that if the nutrients within seagrass wrack are to be recycled within meadows or provide a subsidy to adjacent systems, then mechanisms other than direct consumption of seagrass detritus must be important, with microbial pathways utilising dissolved organic carbon (DOC) being among the most likely.

Fluxes of DOC to overlying water are higher in seagrass meadows than adjacent unvegetated meadows (Barrón & Duarte 2009). Up to 50% of this DOC is consumed by bacteria (Ziegler & Benner 1999), which can be rapidly transferred to higher trophic levels through consumption by flagellates and ciliates (Robertson et al. 1982). The seagrass leaves themselves are a major contributor to this DOC flux. Through exudation or autolysis and leaching, seagrass leaves typically release 2 to 10% of net primary production (Moriarty & Pollard 1982, Barrón & Duarte 2009), and this leaf-derived DOC has been shown to support bacterial production (Brylinsky 1977, Kaldy et al. 2006).

As seagrass leaves degrade much more slowly (Klumpp & Vandervalk 1984, Moore & Fairweather 2006) than the rate at which they are transported by currents and storm-driven advection, DOC leaching may occur over extended periods of time and encompass a range of different habitats. This may provide a mechanism of cross-habitat trophic subsidy not dependent on the direct consumption of leaves. Thus, the loss of DOC from seagrass leaves is potentially a key contributor to the total DOC flux from seagrass ecosystems and cross-habitat trophic subsidies.

Almost all of the studies that have examined the loss of DOC from seagrass leaves have focused on living or fresh leaves (e.g. Brylinsky 1977, Wetzel & Penhale 1979, Robertson et al. 1982). Yet there is evidence that the age of detached leaves has a significant impact on DOC leakage and on the composition (and therefore bioavailability) of that DOC (Velimirov 1986, Maie et al. 2006), which may be crucial for the transfer of seagrass-derived nutrients to adjacent ecosystems. Furthermore, the amount and rate of DOC leaching, and the ability of microbes to utilise the leachate, varies among different vascular plants (Benner et al. 1986, Maie et al. 2006), suggesting that the export and bioavailability of seagrass-derived DOC may be species dependent. Among the seagrasses, interspecific differences could relate to the anatomy of the plants (e.g. membranous, leafy species such as *Posidonia* spp. versus heavily lignified species such as *Amphibolis* spp.) or the amounts and forms of soluble compounds within the tissues. These differences among species in DOC leaching and its apparent bioavailability led Maie et al. (2006) to call for more studies on the bioavailability of the DOC fractions that are released from macrophyte leaves. Furthermore, the potential significance of seagrass as a source of DOC to the coastal zone, coupled with the rapid decline in seagrass cover in recent decades (Green & Short 2003), prompted Barrón & Duarte (2009) to call for more studies on seagrasses to understand the export of DOC from these systems and its significance.

In this study, we compared the amount, composition and bioavailability of DOC released from different types of seagrass wrack. We also examined the effect of wrack age on the amount of DOC released and on its bioavailability. There were 3 main objectives. (1) To determine the amount and functional composition of DOC released from different wrack materials. We tested the hypotheses that the amount and functional compsition of DOC released would vary among different types of wrack. (2) To examine whether the amount and composition of DOC released from *Posidonia sinuosa* wrack depends on wrack age. We tested the hypothesis that the amount of DOC released would diminish with age of wrack and that the composition would differ among wrack of different ages. (3) To assess the bioavailability of DOC released from wrack and whether this is affected by wrack age. We hypothesised that bacterial biomass would increase more rapidly when grown in leachate than blank solution, and more rapidly in leachate from fresh wrack than aged wrack.

MATERIALS AND METHODS

Study region

The study was conducted on wrack accumulations in Geographe Bay (Fig. 1), a 100 km wide north-facing embayment on the southwestern coast of

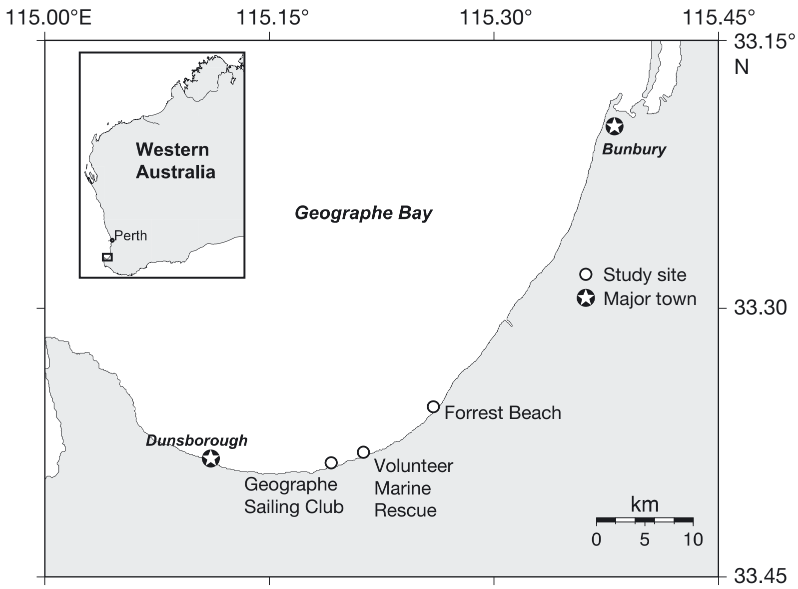

Fig. 1. Geographe Bay, Western Australia, showing the location of the 3 sites used to sample beach wrack composition

Australia. It is a relatively protected bay with extensive beds of seagrass *Posidonia sinuosa* Cambridge & Kuo, and *Amphibolis antarctica* (Labillardière) Sonder & Ascherson ex Ascherson. *Posidonia* and *Amphibolis* are the dominant meadow-forming genera of seagrasses in southwest Australia. *Posidonia* spp. produce aboveground shoots with 1 to 3 strap-like leaves that are periodically shed. *Amphibolis* spp. have heavily lignified and persistent stems on which clusters of short leaves are borne (Ducker et al. 1977). The stems are often heavily covered by epiphytes, particularly red (rhodophyte) macroalgae such as *Laurencia* sp. (Lavery & Vanderklift 2002). Extensive accumulations of detached seagrass leaves and stems are typical of the region, especially in winter (McMahon et al. 1997). The bay is exposed to the northwest winds that characterise the early phase of storms in the region. This exposure results in the transport of wrack throughout the bay, providing a high degree of connectivity among seagrass and other habitats in the region. The wrack is typically dominated by *P. sinuosa*, with significant amounts (up to 30% by weight) of *A. antarctica* at times and generally a low amount (but occasionally up to 15%) of red algae (K. McMahon et al. unpubl.). Geographe Bay is an oligotrophic water body and, as such, the decomposition of wrack in subtidal and beach habitats may be a vital source of recycled nutrients (Robertson & Lenanton 1984).

Release of DOC from different wrack material

The effect of wrack type on the amount and composition of DOC leachate was examined by comparing DOC leached from wrack commonly found in the region: the seagrasses *Posidonia sinuosa* (leaves) and *Amphibolis antarctica* (leaves and stems); the red algae *Laurencia* sp., which is common as both a free-living algae and epiphytic on seagrasses; and the fine particulate organic fraction (>0.1 to <1 mm) of beach-cast wrack that had been on the beach for at least 2 mo. Fresh samples of *P. sinuosa*, *A. antarctica* and *Laurencia* sp. were collected from meadows in 0.5 m depth. Beach-cast wrack was collected from Busselton Beach, Geographe Bay (33° 39.317′ S, 115° 16.812′ E) and then sieved to separate the fine fraction. Samples were stored at 4°C, for no more than 12 h, until the leachate was collected.

The effect of wrack age on DOC leaching was examined using one wrack type, *Posidonia sinuosa* leaves. We focused on *P. sinuosa* as this was the dominant component of the beach-cast wrack, typically accounting for >60% by biomass. Fresh samples of *P. sinuosa* were collected from a meadow at 0.5 m depth. The fresh material was transported immediately to the laboratory and stored in a cool room at 4°C, for a maximum of 2 d, until the leachate was collected. For 'aged' material, approximately 500 g of fresh *P. sinuosa* was placed in nylon mesh litterbags (mesh size <5 mm), which were then placed on the surface of wrack accumulations on the beach, held in place by pickets driven into the wrack accumulations and exposed to the ambient weather conditions. Replicate bags (n = 3) were removed after 1, 2 and 4 wk and returned to the laboratory for leachate experiments.

DOC leachate extraction

Each wrack type and age was incubated to extract DOC. All leaves of both seagrass species and the *Laurencia* thallus were lightly scraped with a razor blade to remove algal epiphytes. For each wrack type, 4 replicate samples of 100 to 150 g wet weight plant material were placed in 0.5 l of sterile, artificial seawater (ASW; Red Sea Salt™, salinity 35) in acid-washed 2 l glass beakers. The beakers were sealed with scientific-grade rubber stoppers and incubated for 16 h at 18°C on shaker trays with periodic, gentle agitation. Blanks were prepared as described above but with no wrack. After 16 h, the leachate was iltered through a series of Whatman GFF filter papers and then a 0.45 µm hydrophilic polypropylene membrane filter (Pall Life Sciences) and analysed for total DOC with a Shimadzu Total Organic Carbon Analyser 9000. Scraped leaves were used in the experiment to avoid DOC release from epiphytic algae. However, to test for any effect of scraping on DOC release, simultaneous incubations were performed on unscraped leaves with epiphytes gently removed by hand. Even with the removal of epiphytic algae and animals, a microbial biofilm is likely to remain on the leaf surface, which can reduce the flux of DOC to the surrounding waters through direct uptake (Maie et al. 2006). Consequently, the changes in DOC concentration of the incubating water are referred to as net DOC release.

The initial leaching experiments indicated high release rates of DOC from fresh seagrass wrack in the first 1 to 2 wk of aging. To obtain increased temporal resolution of early leaching rates, the experiment was repeated using *Posidonia sinuosa* leaves to determine the change in release rate over this initial period of high DOC leaching. Three replicate leaf samples (180 g wet weight) were incubated in 1.4 l of ASW in acid-washed 2 l glass beakers. A blank of ASW was incubated at the same time. Samples of the leachate were collected after 1, 3, 5, 7, 10 and 14 d of incubation, and the ASW replaced each time. The leachate was filtered and analysed as described above.

Characterisation of DOC

DOC composition was characterised by fractionation using open column chromatography following modified methods of Chow et al. (2004) and Cleveland et al. (2004). The leachate was fractionated into 3 components: (1) hydrophobic DOC (fulvic and humic acids), by retention on DAX-8 resin; (2) transphilic DOC, by retention on XAD-4 resin; and (3) hydrophilic DOC, the eluent passing through the DAX-8 and XAD-4 column (Thurman & Malcolm 1981). The hydrophilic fraction is composed predominantly of low molecular weight compounds, including carbohydrates and amino acids (Cleveland et al. 2004).

DAX-8 Superlite (Sigma-Aldrich) and XAD-4 Amberlite (Sigma Aldrich) resin columns were prepared in a similar manner, following the manufacturer's instructions. The resin was mixed with Milli-Q water to form a slurry and poured into a glass column (30 × 1.5 cm) fitted with a tap. The bed volume for the resin was approximately 20 ml. The column was conditioned by passing 200 ml Milli-Q water though the column dropwise, followed by 6 alternating washes of 0.1 M NaOH (40 ml) and 0.1 M HCl (40 ml), again eluted dropwise. The final wash was with 0.1 M HCl to leave the column acidified.

The DOC leachate (200 ml) was acidified with 35% HCl to pH 2 then passed through the DAX-8 resin (dropwise or at <3 ml min^{-1}). The first 10 ml of eluent was discarded (as it was simply displaced acid). When most of the leachate had been applied to the column, 0.1 M HCl (2 bed volumes or 40 ml) was applied to the top of the column. The acid was passed through the column dropwise to elute all but the hydrophobic fraction. The eluent (leachate and acid) was then passed through the XAD-4 column in a similar manner, in this instance the acid eluting the hydrophilic fraction. A sample of deionised (Milli-Q) water was passed through the columns and treated in an identical way as the leachates to act as an analytical blank.

The hydrophobic and transphilic fractions retained on the DAX-8 and XAD-4 resins, respectively, were eluted with 0.1 M NaOH. The volume of base added was typically 5 bed volumes (100 ml) or until the absorbance of the eluent at 254 nm was similar to the blank, indicating an absence of DOC.

The total volume of each fraction was recorded. All pre-filtered leachate samples and fractionated samples were acidified with 35% HCl to pH 2 and analysed for total DOC with a Shimadzu Total Organic Carbon Analyser 9000. The UV absorbance for each of the acidified samples was also recorded at 254 nm on a Shimadzu UV-1601 spectrophotometer.

Wrack composition on beaches

The DOC leaching studies were conducted on wrack collected in subtidal habitats and incubated in submerged conditions, typical of subtidal seagrass wrack. Initial results showed differences in the net release of DOC from wrack of different ages. As large amounts of wrack accumulate on beaches, we examined the age of beach-cast wrack to determine whether beach wrack was likely to have arrived while it was 'fresh' (and with higher net DOC release rates), and, therefore, whether the bulk of DOC leaching (and potential trophic subsidy) occurs in subtidal habitats or on beaches. The composition of beach wrack was determined at 3 sites on 5 occasions over the period of maximum wrack accumulation on beaches (May to October; K. McMahon et al. unpubl.). Samples were collected at Forrest Beach, Volunteer Marine Rescue and Geographe Sailing Club (Fig. 1) on 19 to 22 May, 9 to 11 June, 12 to 15 August, 22 to 25 September and 20 to 22 October 2008. At each site and time, 4 replicate wrack accumulations were sampled. About 0.001 m^3 of wrack was collected from the surface of the accumulation with a quadrat and from the sediment immediately below the accumulation with a corer (90 mm inside diameter × 10 cm deep). The wrack was rinsed to remove sand and sorted into categories based on its estimated age. Age was defined as either old (no green leaves) or new (green leaves or stem) on the basis of the colour of the leaves: pilot work showed that moist leaves above the surface of the sediment turned brown within 2 wk (*Posidonia sinuosa*) or 2 to 4 wk (*Amphibolis antarctica*) (C. E. Oldham et al. unpubl.).

Bioavailability of DOC

We used a bacterial bioassay to test the bioavailability of the filtered DOC leachate produced by *Posidonia sinuosa* leaves, *Amphibolis antarctica* leaves and stem, *Laurencia* sp. and fine particulate wrack during the 16 h incubations using the methods of Cleveland et al. (2007). The response of a bacterial inoculum to the different DOC leachates was observed as growth rate over a 24 h period.

Filtered DOC leachate (200 ml) was combined with a bacterial inoculum (2 ml) in acid-washed glass flasks, wrapped and capped in aluminum foil. A bacterial inoculum was created by combining 100 g of moist beach sediment, 100 g of moist wrack and 800 ml sterile ASW. This was left in the dark for 24 h at 18°C and then filtered through a Whatman 3 filter paper with the filtrate used as the bacterial inoculum. For each DOC leachate, 4 replicates and 4 blanks (200 ml ASW + 2 ml bacterial inoculum) were incubated at 25°C. Triplicate 1 ml sub-samples were taken after 0, 3, 18 and 24 h of incubation and fixed with 0.5% glutaraldehyde for 15 min in the dark (Marie et al. 1997), and then stored in liquid nitrogen until further processing. Heterotrophic bacterial cell counts were determined on a FACS Canto II flow cytometer. Samples were diluted with tris-ethylenediaminetetraacetic acid buffer (1:50 dilution) and stained with SYBR Green I for 15 min at 80°C. Acquisition was run for 2 min at a speed of 1 µl s^{-1}. Data were stored as FCS 2.0 files, and cell counts (cells ml^{-1}) were calculated using the CYTOWIN 4.3 software.

Statistical analysis

A 1-way ANOVA was used to test for differences in the total amount of DOC released among different types of wrack, with Wrack type as fixed factor. A 2-way ANOVA was then used to test for differences among wrack types and DOC fraction on the total amount of DOC released, with Wrack type and DOC fraction treated as fixed factors. A 1-way ANOVA was used to test for significant effects of Wrack age on the total DOC released, with Age as fixed factor. A 2-way ANOVA was then used to test for effects of wrack age and form of DOC on the amount of DOC released, with Age and DOC fraction as fixed factors. A repeated measures ANOVA was used to test for significant effects of scraping on the release of DOC from leaves over time, with Scraping a fixed factor.

The assumption of homogeneity of variances was tested using Cochran's test. When variances were heterogeneous, data were ln- or arcsine-transformed for proportions and percentage values. Where significant main effects were detected, post hoc comparisons (Tukey's) were conducted to determine the sources of significant variation.

RESULTS

Amount and composition of DOC

The net DOC leaching from wrack over 16 h differed significantly among wrack types, with fresh algae *Laurencia* sp. leaching about 4 times the DOC released by fresh *Posidonia sinuosa* leaves, 6 times that released by *Amphibolis antarctica* leaves, more than 11 times that released by *A. antarctica* stems and more than 90 times that released by the fine fraction of natural wrack accumulations (Table 1). The recovery of DOC after fractionation into hydrophobic, transphilic and hydrophilic fractions was high, ranging from 78 to 94% (Table 1). For all wrack types, the hydrophilic fraction dominated the total DOC (37 to 68%), followed by the hydrophobic fraction (17 to 31%) and the transphilic fraction (4 to 11%). Nonetheless, there were subtle, but significant, differences in the percentage contribution that the hydrophobic and hydrophilic components, but not the transphilic component, made to the total DOC

Table 1. Dissolved organic carbon (DOC) composition in leachates derived from different wrack material (mean ± SD). Within each class of DOC (totals, hydrophobic, transphilic and hydrophilic), shared superscript letters indicate no significant differences among wrack types (α = 0.05). *Laurencia* = *Laurencia* sp.; *Posidonia* = *P. sinuosa*; *Amphibolis* = *A. antarctica*; fine fraction = 0.1 to 1.0 mm size class of natural wrack accumulations. FW: fresh weight wrack; n = 4 in all cases. % values indicate the proportion of the total DOC in the various DOC fractions

Wrack type	DOC released in 16 h (mg kg^{-1} FW) Total	Hydrophobic	Transphilic	Hydrophilic	DOC recovery
Fresh *Laurencia*	6749 ± 278[a]	1998 ± 64 30%[a]	675 ± 95 10%[a]	3554 ± 213 53%[a]	93%
Fresh *Posidonia* leaves	1724 ± 76[b]	298 ± 14 17%[b]	138 ± 6 8%[b]	1173 ± 58 68%[b]	93%
Fresh *Amphibolis* leaves	1102 ± 24[c]	284 ± 26 26%[b]	48 ± 4 4%[b,c]	676 ± 21 61%[c]	91%
Fresh *Amphibolis* stems	588 ± 31[d]	180 ± 13 31%[c]	58 ± 4 10%[b,c]	312 ± 29 53%[d]	94%
Fine fraction	74 ± 1[e]	22 ± 3 30%[d]	8 ± 5 11%[c]	27 ± 1 37%[e]	78%

(Table 1); this was reflected in a significant interaction between wrack type and DOC fraction (2-way ANOVA Wrack type × DOC fraction, df = 8,59, $p < 0.001$). The proportion of DOC present as hydrophilic (and presumably the most bioavailable) DOC was highest in fresh *P. sinuosa* leaves (68%) followed by *Laurencia* and *Amphibolis* tissues (53 to 61%) and least in the fine fraction of beach-cast wrack (37%).

Influence of aging of *Posidonia sinuosa* wrack on DOC leaching

The net DOC leaching from *Posidonia sinuosa* leaves declined with increasing age of the wrack (Table 2). Fresh and 1 wk old leaves released similar amounts of DOC (>1400 mg kg^{-1}) over 16 h, at least 10 times the amount released after 2 wk of aging and 20 times that released after 4 wk. The composition of the leachate released by leaves of different ages varied subtly and not systematically (Table 2), with a significant interactive effect of Wrack age and DOC fraction (2-way ANOVA, df = 6,35, $p < 0.001$; see Table 2 for post-hoc pairwise comparisons). However, in all cases the leachate was dominated by the hydrophilic fraction (48 to 67%), followed by the hydrophobic (16 to 27%) and the transphilic (11 to 17%) fractions.

Table 2. Dissolved organic carbon (DOC) composition of leachate from *Posidonia sinuosa* leaves of different ages (mean ± SD). % values indicate the proportion of the total DOC in the various DOC fractions. ANOVA revealed a significant interaction of Age and DOC fraction ($p < 0.001$). Shared superscript letters indicate no significant difference in mass of DOC released (Tukey's test, $p > 0.05$) among treatments within each class of DOC. FW: fresh weight wrack. n = 4 in all cases

Age	DOC release over 16 h (mg kg^{-1} FW) Total	Hydrophobic	Transphilic	Hydrophilic	DOC recovery
Fresh	1419 ± 93[a]	223 ± 18 16%[a]	181 ± 17 13%[a]	855 ± 45 60%[a]	89%
1 wk	1627 ± 192[a]	417 ± 13 26%[b,c]	277 ± 32 17%[a]	783 ± 120 48%[b]	91%
2 wk	133 ± 17[b]	36 ± 4 27%[b]	19 ± 4 14%[a]	67 ± 7 50%[b]	91%
4 wk	67 ± 2[c]	12 ± 1 18%[c]	7.2 ± 0.2 11%[a]	45 ± 1 67%[a]	96%

DOC release during the first 14 d

The rate of net DOC released from fresh *Posidonia sinuosa* leaves during the first 14 d was affected by scraping (Fig. 2), with a significant Time × Scraping interaction ($p < 0.05$). For scraped leaves, the leaching rate (A_t) was described by a single-stage exponential decay with a half-life of 1.8 d: $A_t = 752\ e^{(-0.317t)}$. For unscraped leaves, the net release rate of DOC was describe by a 2-stage model, with an increasing rate of DOC release for the first 5 d, after which the leaching rate was described by an exponential decay with a half-life of 1.65 d: $A_t = 1610\ e^{(-0.385t)}$, which approached the decay curve for the scraped leaves.

Despite the differences in initial net DOC release rates, the total mass (mean ± SD) of DOC released (M_{DOC}) from scraped and unscraped leaves was similar over the initial high release period (first 5 d: 1650 ± 122 vs. 1600 ± 77.0 mg kg^{-1}) and then the full 14 d (1920 ± 131 vs. 1740 ± 80.0 mg kg^{-1}) of the experiment, indicating that the effect of scraping the leaves was minimal in terms of quantity of DOC leached.

The accumulated mass released over the 14 d of incubation approached 2000 mg (Fig. 3), with the rate of release dramatically slowing by Day 14. Assuming that the mechanism of

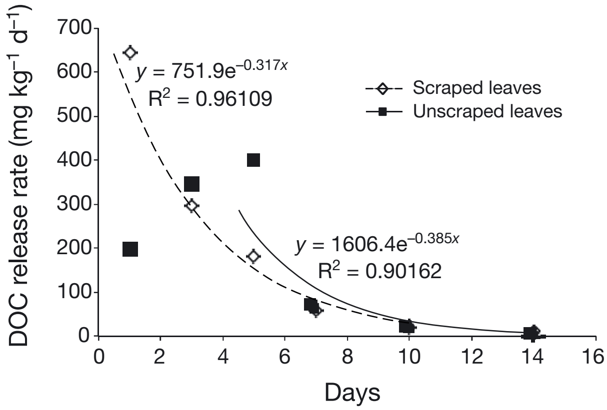

Fig. 2. Net dissolved organic carbon (DOC) release rates from scraped and unscraped *Posidonia sinuosa* leaves during 14 d incubations. The regression for unscraped leaves is for Days 5 to 14

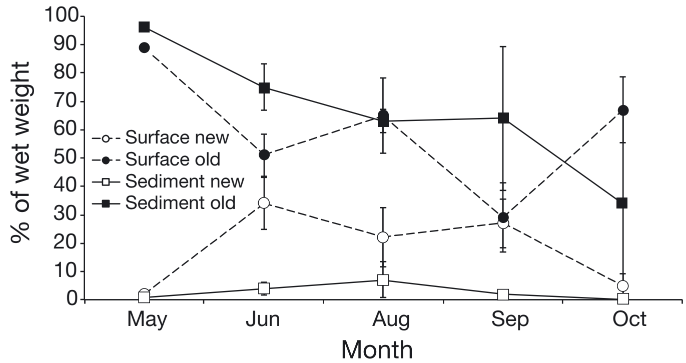

Fig. 4. Composition (new versus old) of wrack on beaches of Geographe Bay, Western Australia, from May to October 2008. All values are mean ± SD, n = 4

DOC release remained constant over time, the curve fit to the full dataset (scraped and unscraped leaves, $M_{DOC} = 716 + 470\ \ln(t)$) predicts that a further 1100 days (3 yr) would be required to release the next 2000 mg.

Composition of wrack on beaches

The wrack accumulating on Geographe Bay beaches was typically dominated by old material (Fig. 4). In May, the period just before the first autumn/winter storms, the wrack on the sand surface and that within the underlying beach sediments was dominated by old material (generally >90%). During the winter storm period (June to September) the proportion of fresh wrack increased in both zones, reaching 25 to 30% at the surface of accumulations, but was always less than 10% in the sediment layer below accumulations. By October (spring), the proportion of fresh wrack had declined in all accumulations, approaching 5% in the surface layer and negligible in the sediment layer.

Bioavailability of DOC leachates

For leachates from all types of fresh wrack material, there were significant exponential increases in bacterial abundance over time following inoculation (Fig. 5 & Table 3; in all cases $p < 0.001$). The fine fraction from beach-cast wrack also showed a significant increase in bacterial abundance over time ($p < 0.05$),

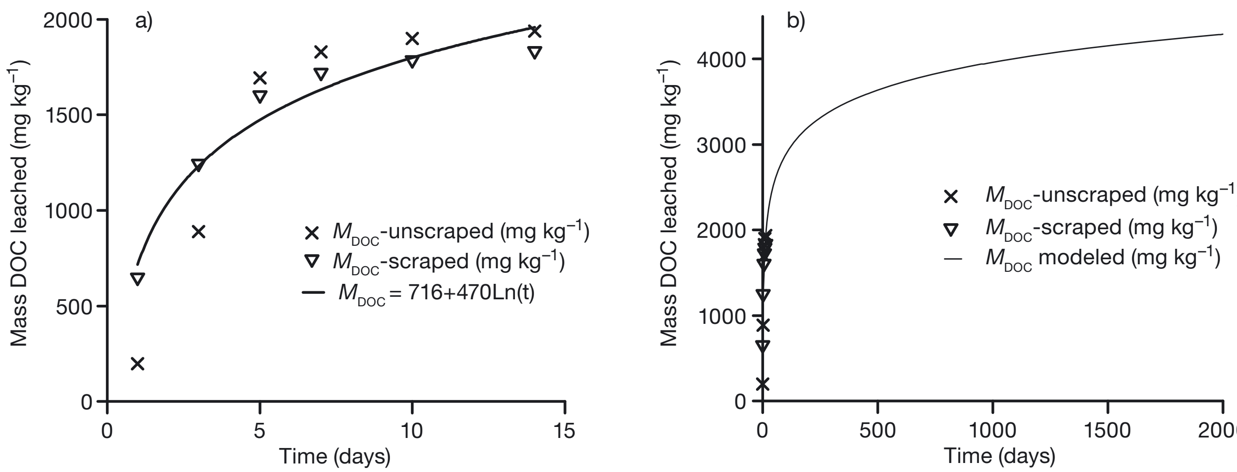

Fig. 3. Cumulative net mass of dissolved organic carbon (M_{DOC}) released over time: a) measured release over the first 14 d; b) released over 2000 d modelled from the curve fit to the measured data (a)

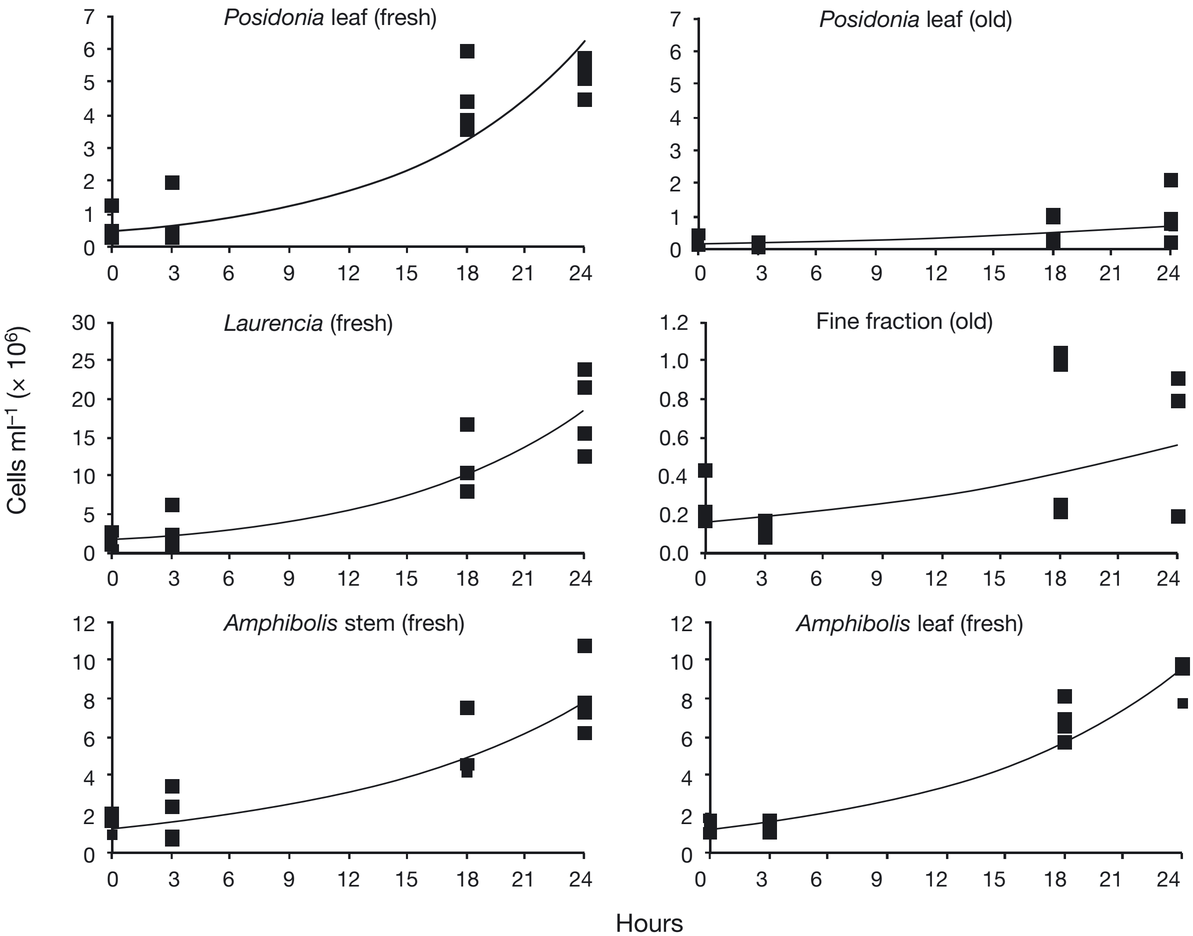

Fig. 5. Abundance of heterotrophic bacterial cells following addition of an inoculum to dissolved organic carbon leachates from different wrack types. *Posidonia* = *P. sinuosa*; *Laurencia* = *Laurencia* sp.; *Amphibolis* = *A. antarctica*; fine fraction = 0.1 to 1.0 mm size class of natural wrack accumulations

Table 3. Exponential curve fits describing the change in abundance of bacterial cell abundance over 24 h in leachates from different types of wrack following bacterial inoculation. $^*p \leq 0.05$, $^{***}p \leq 0.001$. In all cases, x = time in hours; nsd = no significant difference; wrack types as in Table 1

Wrack type	Correlation	r	p
Posidonia leaf (fresh)	$4.54 \times 10^5\ e^{0.109x}$	0.95	***
Laurencia (fresh)	$1.65 \times 10^6\ e^{0.101x}$	0.90	***
Amphibolis leaf (fresh)	$1.26 \times 10^6\ e^{0.076x}$	0.89	***
Amphibolis stem (fresh)	$1.23 \times 10^6\ e^{0.086x}$	0.97	***
Posidonia (old)	$3.92 \times 10^4\ e^{0.129x}$	0.34	nsd
Fine fraction	$7.64 \times 10^4\ e^{0.084x}$	0.51	*
Blank (fresh *Posidonia* and *Laurencia*)	$9.58 \times 10^4\ e^{-0.011x}$	−0.83	***

though the rate of increase was much smaller. In leachate from 1 mo old *Posidonia sinuosa* leaves, there was no significant increase in bacterial abundance over time. In all cases, when the number of bacteria in the blank incubations was plotted against time, the slope was not significantly different to zero, indicating little or no bacterial growth, except for the fresh *P. sinuosa* and *Laurencia* sp. leachate incubations, where there was a significant exponential decay in bacterial abundance.

The age of *Posidonia sinuosa* wrack affected the ability of the leachate to support bacterial growth. For leachate from fresh *P. sinuosa* leaf material, the linear increase in bacterial abundance over a 24 h period had an average slope of 2.04×10^5 cells h^{-1}. For the leachate from 4 wk old *P. sinuosa* leaves, bacterial abundance increased for the first 18 h, though the slope over this period was less than half that in the leachate from fresh leaves (9.20×10^4 cells h^{-1}), and declined thereafter.

DISCUSSION

Effect of wrack type on DOC release

Total net DOC released varied among types of wrack (algae > *Posidonia* leaves > *Amphibolis* leaves > *Amphibolis* stems > fine fraction). This is consistent with studies that found release rates were higher from algae than seagrasses (e.g. Brylinsky 1977). Algae have less structural carbon, and therefore more storage carbon, per unit biomass, which would account for this difference. Within the different types of seagrass wrack there were also differences in release rates of DOC. Fresh *P. sinuosa* leaves had the largest release rate of DOC, followed by *A. antarctica* leaves and then stems. The stems of *Amphibolis antarctica* are vertical rhizomes and serve as a major storage organ for carbohydrates. In the closely related species *A. griffithii*, soluble sugars account for 15 to 20% dry weight (DW), and starches account for 2 to 3% DW of the rhizome (Lavery et al. 2009). On this basis, we might expect higher fluxes of soluble carbohydrate compounds from the stems than the leaves. However, the lower net release rate for stems may reflect higher levels of other soluble compounds in the leaves, especially proteins associated with photosynthesis, and stronger barriers to diffusion, as stems are highly lignified and contain large amounts of vascular tissue. This may also explain the differences among leaves, as *P. sinuosa* leaves and those of *A. griffithii* typically have similar levels of soluble sugars and starches (*P. sinuosa* = 2 to 4% DW soluble sugars and 5 to 10% starches (Collier et al. 2009); *A. griffithii* = 5 to 15% soluble sugars and 2 to 3% starch (Lavery et al. 2009)).

The relatively low release rates from aged *Posidonia sinuosa* leaves (4 wk old) and the fine particulate fraction of wrack (at least 2 mo old) reflected the effect of aging on DOC release and the significant loss of DOC that occurred in the first few days of leaching. However, despite the low rate of bacterial growth on leachate from the 4 wk old *P. sinuosa* leaves, the initial growth over 18 h confirmed that the DOC leachate was bioavailable. The decline in bacterial biomass after 18 h indicated that it was more likely a function of the mass of DOC in the leachate than the composition that affected bacterial growth.

The composition of the DOC leached from different species of wrack was similar. This may partly reflect the level of resolution in our chemical characterisation of the leachates. The high percentage recovery of DOC following fractionation gives confidence that we have not underestimated a significant portion of the DOC. Maie et al. (2006) found differences in concentrations of sugars and phenols in leachate from a range of aquatic plants. However, the plants they studied covered a wide phylogenetic range, from algal periphyton to freshwater macrophytes, mangroves and seagrasses. In comparison, our wrack was all derived from seagrasses, with the exception of the alga *Laurencia* sp.

The similarity in leachate quality from all wrack types, including that of aged wrack, indicates that the quality of DOC that seagrass wrack contributes to recipient habitats is likely to be similar, irrespective of the type or age of the wrack, though the mass contributed will decline rapidly with age. It was surprising that the hydrophilic portion (which contains sugars, amino acids, small molecular weight fatty acids and other compounds likely to be more labile) continued to form a significant proportion (more than 50%) of the DOC leached from aged *Posidonia sinuosa* and the fine fraction. It is not clear whether, in the older wrack, the low molecular weight component is derived directly from the wrack, or is contained in exudates from bacteria growing on the wrack or in suspension. In any case, this makes little difference in terms of the potential benefit of the input to recipient ecosystems. If it is derived from exudates of bacteria growing on the wrack, then it is possible that this input of readily bioavailable DOC could persist for months, though at a very slow rate.

Mass and timescale of DOC release

The release rate of DOC declined rapidly with age of *Posidonia sinuosa* wrack. About 50% (2000 mg kg^{-1} FW wrack) was released in the first 14 d. Assuming that the mechanism of DOC release remains constant over the decay period of the wrack, it would take in the order of thousands of days to release the next 2000 mg kg^{-1} FW wrack. This assumption may not be the case but, nonetheless, it is clear that the rate of DOC release will fall dramatically after the first days. The *P. sinuosa* leaves used in our studies contained about 33% carbon DW or 11% FW (using a DW:FW ratio of 0.34; P. S. Lavery unpubl. data), so the total mass of carbon released over 14 d was about 1.8% of the leaf carbon, and if we project out to 2000 d, 3.6% of the leaf carbon. These values are similar to the total fixed carbon lost through DOC excretion reported for *P. oceanica* (Velimirov 1986), but much less than the 48% estimated by Kirkman & Reid

(1979) for *P. australis*, which has very similar leaf structure to *P. sinuosa* (Cambridge & Kuo 1982). However, it is likely that the value estimated by Kirkman & Reid (1979) severely overestimated the leaching of DOC from leaves. They used leaves with necrotic tissue and with a full complement of epiphytes, which would have contributed to the DOC leakage, and they measured the rates over 2 h, which is likely to produce much higher estimates of loss than that would occur for aged wrack tissue, as shown by our results.

Temporal variation in DOC release rates have been reported for other seagrasses. Velimirov (1986) found that the young, green portions of *Posidonia oceanica* leaves released negligible amounts of DOC, but high loss was observed for older, brown leaves, while the rate of DOC leaching from *Thalassia testudinum* leaves declined exponentially with age, with 84% of the DOC leached in the first 2 wk (Maie et al. 2006). In our case, the initial rate of DOC release was enhanced by scraping leaves to remove epiphytes. This could be due to the removal of epiphytes or damage to the leaf surface. Epiphytic organisms reduce the release of DOC from seagrass leaves to surrounding water (Wetzel & Penhale 1979, Velimirov 1986), presumably through assimilation of the DOC. Scraping is also likely to disrupt the tough cuticle and thick epidermal leaves of *P. sinuosa* (Cambridge & Kuo 1982), enhancing diffusive losses of cellular DOC. However, this effect was limited mainly to Day 1, with the total mass of DOC released over the first 5 d (the period of highest initial release rates) similar in both types of leaf, providing confidence that scraping had little effect on the total amount of DOC released beyond the first day. Despite the potential for scraping to introduce an experimental artefact, it may be representative of the condition of naturally shed leaves, which will have damage to the leaf surface, particularly the necrotic upper part of the leaves, through the action of grazers and abrasion by sediments as they are transported in bedload and suspended transport.

The bacterial growth in the assays demonstrates the bioavailability of the leachate DOC released from fresh seagrass and algae, with exponential increases in abundance indicating bacterial growth. Bacteria grown on seagrass DOC leachate can rapidly be converted into bacterial aggregates that are consumed by ciliates and flagellates at a much faster rate than the residual particulate organic carbon (Robertson et al. 1982). We did not enumerate the bacteria on the surface of the wrack, but these typically are much more abundant than bacteria in suspension.

Relative contribution of wrack to DOC in Geographe Bay

Although it was beyond the scope of this study to produce a full DOC budget for Geographe Bay, sufficient information is available to compare the potential contribution of wrack with some other sources of DOC (Table 1) to the study area. Oldham et al. (2010) estimated a total annual wrack production of 16 900 t DW of *Posidonia sinuosa* leaf wrack and 15 700 t of *Amphibolis antarctica* wrack from this region (i.e. an area of 60 km^2 with an average depth of 5 m — the area of seagrass coverage to the 10 m depth contour). At the initial DOC release rates recorded in our study, this mass of wrack would contribute 191 kg of DOC to the study region in 1 d. Actively growing phytoplankton can leak between 0.0005 and 0.055 pmol DOC $cell^{-1}$ d^{-1} (Biddanda & Benner 1997). Coastal waters to the north of Geographe Bay typically have about 91 000 cells l^{-1} of phytoplankton (Hanson et al. 2006). Assuming a similar cell count in Geographe Bay, phytoplankton in the study area would, at most, contribute 0.19 to 21 kg DOC d^{-1}, between 1 and 3 orders of magnitude less than seagrass wrack.

In contrast, living seagrass represents a very large DOC source relative to wrack. Velimirov (1986) compared the DOC release from healthy, living and scenescent seagrass leaves; healthy leaves released 0.2% of that released by scenescent leaves. However, the biomass of living meadow is much higher than that of wrack. Assuming a release rate comparable to that given by Velimirov (1986) for *Posidonia oceanica* (0.006 mg DOC g^{-1} h^{-1}) and using the biomass reported by McMahon et al. (1997) for *P. sinuosa* in Geographe Bay (115 to 470 g dw m^{-2}), live seagrass in the study area would provide 990 to 4000 kg DOC d^{-1} to the study area, 10 to 40 times that of wrack. Of course, this is a fixed source of DOC, compared with the more mobile nature of wrack, which permits interhabitat connectivity.

Surface beach sands can be a significant source of DOC to the water column in high-energy environments, with a net flux of 4 to 22 mmol DOC m^{-2} d^{-1} (Heymans & McLachlan 1996, D'Andrea et al. 2002, Avery et al. 2012). Geographe Bay has an ~0.5 m diurnal tide range and a mean beach slope of ~0.06 m m^{-1} (Oldham et al. 2010). Using the lower slope estimate, over the 30 km stretch of beach an area of 2.5×10^5 m^{-2} of beach face would be inundated each day, providing an estimated flux of DOC in the order of 12 to 66 kg DOC d^{-1}. While significant, this is a smaller source than wrack, and is likely to be most significant to the surf zone adjacent to the beach, unlike wrack, which can be transported and gradually release DOC over a wider area.

Implications for trophic connectivity

The exponential-decay model of DOC release from *Posidonia sinuosa* leaves indicates that there will be significant temporal variation in the release of DOC from leaves shed by plants, with significant implications for trophic connectivity. Wrack is constantly being produced in seagrass meadows, but in the case of *Posidonia sinuosa* and *Amphibolis antarctica* leaves, significant water velocities, in excess of 0.15 m s^{-1}, are required to suspend wrack, allowing it to be transported away from the meadow (C. E. Oldham et al. unpubl.). Typically, this results in wrack accumulating in offshore meadows during quiescent periods (spring through to early autumn in our study site) and leaves being transported to beaches and other habitats during storm events (K. McMahon et al. unpubl.), typically in autumn and winter. Leaves shed in spring and summer may, therefore, slowly degrade within the meadow for several weeks or months, with the majority of DOC released within the meadow itself. Adjacent habitats will only receive wrack in a high DOC-leaching phase under 2 scenarios: (1) during unusual storm events that are sufficiently energetic to dislodge and transport living material and when fresh wrack may constitute a significant portion of the total; and (2) during normal autumn/winter storms, when it will only constitute a small proportion of the total wrack exported (i.e. that shed in the previous 2 wk).

The above suggests that in our system and outside of storm events, when the timescale of leaching is typically much faster than the timescale of transport, wrack may be of limited value in supporting trophic subsidies. However, we have noted relatively high DOC concentrations in porewaters beneath wrack accumulations. While fresh wrack was never the dominant component of wrack accumulations, it frequently accounted for 25 to 30% of the mass during winter (when storm conditions dominate). During this time, beach accumulations can persist for several weeks, reaching biomasses of 4 kg m^{-2} under natural conditions but as much as 19 kg m^{-2} in areas affected by coastal structures (K. McMahon unpubl.). Assuming 4 kg m^{-2} of wrack with 30% fresh material, ~4.1 g of DOC m^{-2} would be released over 2 wk, which our data shows is capable of supporting bacterial growth. Thus, while seagrass wrack may have relatively little value as a source of trophic connectivity during periods of quiescent hydrodynamics, it may still be important during periods of higher-energy and faster transport, leading to the formation of biogeochemical hot moments (sensu McClain et al. 2003). Furthermore, under quiescent hydrodynamics, seagrass detritus may contribute, even if at slow DOC release rates, to the sedimentary organic carbon pool of offshore habitats, including oligotrophic unvegetated habitats, as suggested by Ziegler & Benner (1999). This could also apply to beaches and other recipient habitats if the wrack is buried and therefore can persist in these habitats for sufficiently long periods to allow an accumulation of DOC. This demonstrates a complex interaction of timescales of transport (or residence times) and timescales of leaching that must be taken into account when considering the potential for trophic subsidies.

CONCLUSIONS

We conclude that *Posidonia sinuosa* and *Amphibolis antarctica* seagrass wrack leaches bioavailable DOC. We also conclude that, for *P. sinuosa*, there is an initial rapid release of DOC within the first days to weeks followed by an extended period of low release rates. As similar time courses of DOC release have been shown for other seagrasses, such as *P. oceanica* (Velimirov 1986) and *Thalassia testudinum* (Maie et al. 2006), it is likely that wrack from many species of seagrass will demonstrate similar patterns of DOC release. Despite differences in the rate of DOC release from different types and ages of wrack, the composition was similar and it was bioavailable even when released from old wrack, though the amount released would limit bacterial growth. Given the known consumption of bacterial aggregates by higher levels of the food web, the leaching of DOC is one means of recycling the nutrients in seagrass detritus. The interaction of the timescales of transport and the timescale of leaching will be critical in determining the value of wrack as a vector for trophic subsidies. When fresh wrack is released during periods of rapid hydrodynamic transport, it has the potential to release most of its DOC into recipient habitats. However, during quiescent periods, the rapid leaching will result in most of the DOC being recycled within the seagrass meadow. Further work is required to determine the importance of bacterial growth on the surface of wrack and in suspension as a sink for seagrass DOC, and the efficiency of its subsequent incorporation into the food web of recipient ecosystems.

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