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Stacey M. Trevathan-Tackett,[1] Jeffrey Kelleway,[1] Peter I. Macreadie,[1,2] John Beardall,[3] Peter Ralph,[1] and Alecia Bellgrove[4,5]

[1]*Plant Functional Biology and Climate Change Cluster, School of Environment, University of Technology Sydney, Ultimo, New South Wales 2007 Australia*
[2]*Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Burwood, Victoria 3125 Australia*
[3]*School of Biological Sciences, Monash University, Clayton, Victoria 3800 Australia*
[4]*Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Warrnambool, Victoria 3280 Australia*

Abstract. Many marine ecosystems have the capacity for long-term storage of organic carbon (C) in what are termed "blue carbon" systems. While blue carbon systems (saltmarsh, mangrove, and seagrass) are efficient at long-term sequestration of organic carbon (C), much of their sequestered C may originate from other (allochthonous) habitats. Macroalgae, due to their high rates of production, fragmentation, and ability to be transported, would also appear to be able to make a significant contribution as C donors to blue C habitats. In order to assess the stability of macroalgal tissues and their likely contribution to long-term pools of C, we applied thermogravimetric analysis (TGA) to 14 taxa of marine macroalgae and coastal vascular plants. We assessed the structural complexity of multiple lineages of plant and tissue types with differing cell wall structures and found that decomposition dynamics varied significantly according to differences in cell wall structure and composition among taxonomic groups and tissue function (photosynthetic vs. attachment). Vascular plant tissues generally exhibited greater stability with a greater proportion of mass loss at temperatures >300°C (peak mass loss ~320°C) than macroalgae (peak mass loss between 175–300°C), consistent with the lignocellulose matrix of vascular plants. Greater variation in thermogravimetric signatures within and among macroalgal taxa, relative to vascular plants, was also consistent with the diversity of cell wall structure and composition among groups. Significant degradation above 600°C for some macroalgae, as well as some belowground seagrass tissues, is likely due to the presence of taxon-specific compounds. The results of this study highlight the importance of the lignocellulose matrix to the stability of vascular plant sources and the potentially significant role of refractory, taxon-specific compounds (carbonates, long-chain lipids, alginates, xylans, and sulfated polysaccharides) from macroalgae and seagrasses for their long-term sedimentary C storage.

This study shows that marine macroalgae do contain refractory compounds and thus may be more valuable to long-term carbon sequestration than we previously have considered.

Key words: blue carbon; carbon sequestration; macroalgae; mangrove; plant cell wall; pyrolysis; saltmarsh; seagrass; thermogravimetry.

Introduction

With the threat of a changing climate, scientists are searching for options to combat and prevent the imminent consequences of rising CO_2 levels including global warming and ocean acidification. Recently, one such option, natural carbon (C) sequestration in coastal habitats, has become a hot topic of international research and policy. Coined "blue C" habitats, these aquatic systems capture atmospheric carbon dioxide (CO_2) and convert it into a more permanent form of fixed C that is stored primarily in live tissues and sediments (McLeod et al. 2011). Dominated by macrophytes (saltmarsh, mangrove, and seagrass), blue C systems are more efficient at long-term sequestration of organic C than are terrestrial systems (Laffoley and Grimsditch 2009, Nellemann and Corcoran 2009, McLeod et al. 2011).

While blue C habitats are highly productive (Laffoley and Grimsditch 2009), much of their C sink capacity is primarily attributed to their sediments (Duarte et al. 2005, Donato et al. 2011, Fourqurean et al. 2012), where both autochthonous C (produced within the ecosystem) and allochthonous (external C transported to the ecosystem) may accrete. Allochthonous sources can contribute significant proportions (e.g., up to 72%; Gacia et al. 2002) of this sediment C, and depending

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[5] Corresponding author.
E-mail: alecia.bellgrove@deakin.edu.au

on the surrounding environment, macrophyte contributors to organic C stocks, i.e., C donors, may include terrestrial plants, other coastal vascular plants (saltmarsh plants, mangroves, seagrasses), macroalgae, and microalgae (Fig. 1a–e). The least well-studied of these potential C donors is macroalgae (Hill et al., *in press*). Macroalgae have exceptionally high biomass production, with a global extent of 2–6.8 million km^2 and primary production of 0.19–0.64 Pg C/yr, exceeding that of all other coastal blue C habitats combined (Duarte et al. 2013). Most macroalgal species spend most of their life history attached to hard benthic substrata (Lüning 1990) where they are unable to accumulate belowground C, thus limiting their capacity to act as C sinks that accumulate and maintain long-term C in their own right. However, once macroalgae become dislodged, they undergo a period of transport in surface waters by wind and water movement, until eventually sinking, decaying, or becoming cast upon depositional areas, including land (Thiel 2003, Thiel and Gutow 2005, McKenzie and Bellgrove 2009, Macreadie et al. 2011), the deep sea (Wada et al. 2008, Dierssen et al. 2009), and coastal blue C habitats (Hyndes et al. 2012, Macreadie et al. 2012; Fig. 1e). Considering the exceptional production of macroalgae and their high rates of fragmentation (Thomsen and Wernberg 2005) and transport (Macaya et al. 2005, Fraser et al. 2011), macroalgae would appear able to make a significant contribution as C donors to blue C sediments (Hill et al., *in press*).

Once C is deposited into a blue C habitat, there are three possible fates of the C: (1) it may be broken down by microbes and assimilated into new biomass, (2) it may be respired by microbes back into CO_2, or (3) the particulate or dissolved organic C is in a form (or environment conditions are such) where it cannot be utilized by microbes and thus may contribute to the long-term C stock (Hill et al., *in press*). There is debate as to which factors most strongly influence plant decomposability, and therefore the ability to act as a long-term C storage. Large-scale models suggest that the environment or climate affect plant decomposition (Heimann and Reichstein 2008); however, finer-scale studies suggest that the microbial community and litter quality drive decay rates (Enríquez et al. 1993, Cleveland et al. 2014). Enriquez et al. (1993) reviewed terrestrial, freshwater, and marine plant decomposition studies and showed that 83% of the variation in decay rates was attributed to litter quality (nitrogen, phosphorus, and C), which has also been shown in a number of field and laboratory studies (Josselyn and Mathieson 1980, Kristensen 1994). Decomposition is not only influenced by nutrient content, but also by the structural complexity of the cell wall (Rice and Hanson 1984, Twilley et al. 1986, Kristensen 1990, Wada et al. 2008). For vascular plants, this complexity is a lignocellulose matrix that varies among blue C plants according to their requirements for structural rigidity. Subtidal seagrasses, for example, require little aerial support and must be pliable enough to avoid breakages from wave action (Klap et al. 2000), while mangrove tissues may support significant aboveground biomass. In contrast, macroalgae have various components, such as alginates, xylans, carrageenans, agars, and mannans, that give them their structural complexity, which can be as variable as their diverse lineages (van den Hoek et al. 1995, Domozych 2001). Because of this high variability in macromolecular structural complexity of the cell wall (i.e., its stability), there may also be critical differences in decomposition rates and thus, C donor potential, among the macroalgae and blue C plants.

Thermal methods aid the investigation of organic matter stability and may be used to detect patterns of organic matter decomposability (Persson et al. 1986, Plante et al. 2009). Thermogravimetric analysis (TGA) has been used to identify macromolecules in both terrestrial and algal biofuel studies (Yang et al. 2006, Wang et al. 2007, Ross et al. 2008, Carrier et al. 2011) where typical patterns of thermal curves are used to quantify molecular composition. TGA has also been used as a proxy for the biogeochemical stability of marine sediments (Capel et al. 2006); however, such sediment studies need to consider the influence of mineral matter on the thermal stability of a sample (Plante et al. 2009). For pure organic matter sources such as marine plant tissues, this proxy is more straightforward: TGA assesses the degradation of plant material under increasing temperature, providing quantitative and qualitative information on the biochemical components and their propensity for decomposition, based on the temperature ranges in which they degrade.

We applied TGA under N_2 (i.e., pyrolysis) to assess the thermochemical stability of organic and inorganic C content of marine macroalgae and vascular plants from coastal blue C habitats. The objective was to assess the structural complexity of multiple lineages of plant and tissue types with differing cell wall structures and compositions in order to identify their likely contribution to long-term stores of sedimentary C. Specifically, we hypothesized that thermogravimetric signatures (i.e., decomposition dynamics across all temperature intervals) will differ among different lineages of aquatic plants, consistent with differences in their cell wall composition and structure. Gross differences in cell walls between vascular plants and protists might affect decomposition and C storage potential, but also diversity in composition within lower taxonomic levels may mean some taxa have greater C storage potential than others.

Materials and Methods

Sample collection and preparation

Species were selected based on abundance/dominance and likely contribution to autochthonous and allochthonous inputs to blue C systems and their evolutionary lineage. Five phaeophycean algal species from two orders (*Durvillaea potatorum* (Labillardière) Areschoug,

FIG. 1. (a) A mosaic of succulent (*Sarcocornia quinqueflora*) and grass (*Sporobolus virginicus*) saltmarsh and mangrove (*Avicennia marina*; background) species; (b) dense *A. marina* leaf litter coverage among seedling growth on a mangrove forest floor; (c) *Halophila ovalis* and *Zostera muelleri*, diverse members of the seagrass order Alismatales; (d) southern Australian macroalgal diversity represented in a shallow rock pool with several *Cystophora* and *Sargassum* species, *Phyllospora comosa*, small *Macrocystis pyrifera* plants, *Zonaria* sp., *Ulva* sp., *Codium* spp., *Caulerpa* spp., and various red coralline species in the pool, fringed by a mixed intertidal assemblage dominated by *Hormosira banksii*, *Ulva* sp. and *Capreolia implexa*, with the bull kelp *Durvillaea potatorum* in the wave-exposed upper subtidal margin; (e) macroalgae (*Hormosira banksii*) deposited on seagrass (*Zostera* sp.; foreground) meadow and adjacent to mangrove forest. Photo credit: (a, b, and e) J. Kelleway, (c) P. I. Macreadie, and (d) D. Squire.

Hormosira banksii (Turner) Decaisne, *Phyllospora comosa* (Labillardière) C. Agardh, *Ecklonia radiata* (C. Agardh) J. Agardh, *Macrocystis pyrifera* (Linnaeus) C. Agardh), two rhodophytes from different orders (*Corallina officinalis* Linnaeus, *Plocamium angustum* (J. Agardh) J. D. Hooker and Harvey), one chlorophyte (*Caulerpa cactoides* (Turner) C. Agardh), the three seagrass species (*Amphibolis antarctica* (Labillardière) Sonder and Ascherson ex Ascherson, *Zostera muelleri* Irmisch ex Ascherson, *Zostera nigricaulis* (J. Kuo) S. W. L. Jacobs and D. H. Les) common in southern Australia, two common saltmarsh species (*Puccinellia* Parl., *Tecticornia arbuscula* (R. Br.) K.A. Sheph. and Paul G. Wilson) from different orders and the single mangrove species in the region (*Avicennia marina* (Forssk.) Vierh.) were sampled (Table 1). All samples were collected from southwestern Victoria, Australia in August/September 2013. Macroalgal and seagrass samples were collected from Pea Soup (38°23′ S, 142°13′ E) and The Passage (38°23′ S, 142°14′ E) in Port Fairy or from Warrnambool (38°25′ S, 142°29′ E); saltmarsh plants were collected adjacent to the Moyne estuary (38°22′ S, 142°14′ E), Port Fairy, and mangrove leaves were collected from the Barwon estuary (38°16′ S, 144°30′ E), Barwon Heads (Table 1). Three replicate samples of each species were haphazardly selected from different individuals or isolated patches (for seagrasses, saltmarsh plants, and stoloniferous macroalgae) to ensure independence. For the macroalgal species that have distinct tissues with different structural complexity, TG analyses were performed separately for blades and holdfasts/stipes; *Caulerpa* was separated into stolon/rhizoids and blades, while analyses for other species were conducted on the whole, largely undifferentiated, thalli (Table 1). Similarly, aboveground and belowground structures of saltmarsh plants and seagrasses

TABLE 1. List of macrofloral species, taxonomic order, collection location (see *Materials and methods: Sample collection and preparation*), and tissue types collected for thermogravimetric analyses, southwestern Victoria, Australia, August/September 2013.

Order and species	Location	Tissue type						
		1° photosynthetic tissue			Attachment organs			
		Blade	Thallus	Leaf	Holdfast	Stolon	Rhizome + root	Root
Macroalgae								
Caulerpales								
Caulerpa cactoides	Pea Soup	X				X		
Corallinales								
Corallina officinalis	Pea Soup		X					
Fucales								
Durvillaea potatorum	The Passage	X			X			
Hormosira banksii	Pea Soup		X					
Phyllospora comosa	Pea Soup	X			X			
Laminariales								
Ecklonia radiata	Pea Soup	X			X			
Macrocystis pyrifera	Warrnambool	X			X			
Plocamiales								
Plocamium angustum	Pea Soup		X					
Vascular plants								
Alismatales								
Amphibolis antarctica	Pea Soup			X			X	
Zostera muelleri	The Passage			X			X	
Zostera nigracaulis	Pea Soup			X			X	
Caryophyllales								
Tecticornia arbuscula	Moyne			X				X
Lamiales								
Avicennia marina	Barwon			X				
Poales								
Puccinellia sp.	Moyne			X				X

were separated. Only leaves were sampled for the mangrove. Tissues were washed in freshwater then dried at 50°C until weights were stable. Samples were ground to a consistent texture with a coffee grinder (model EM0405, Sunbeam, Botany New South Wales, Australia) before analysis.

Thermogravimetric analysis (TGA)

For TGA, a SDT Q600 (TA Instruments, New Castle, Delaware, USA) with a 0.1-μg balance sensitivity was used for all samples. An aliquot of ground sample (10 mg) was place in a platinum cup and heated under N_2 (gas flow, 100 mL/min) at 20°C/min for macroalgae samples (Wang et al. 2007) or at 10°C/min for vascular plant samples (Yang et al. 2006, Ncibi et al. 2009) to 800°C. Universal Analysis software (TA Instruments, New Castle, Delaware, USA) was used to aid the identification and quantification of mass loss within specific temperature intervals.

Definition of temperature intervals

Due to differences in major component definitions reported in the literature between macroalgae and vascular plant studies (see *Discussion*), it was deemed necessary to define appropriate temperature intervals that allow comparison of decomposition dynamics between the two groups. From analysis of all thermograms and rate-of-change derivatives produced in this study, four temperature intervals of distinct mass losses were common to all taxa, except *Corallina officinalis* and *Caulerpa cactoides*. The first temperature interval (TI_1: protein, soluble carbohydrates, hemicellulose) ranged from 160–180°C to 300°C for vascular plants and macroalgae, respectively, followed by TI_2 (cellulose, lipids, insoluble polysaccharides) from 300°C to 400°C. TI_3 (lignin and insoluble polysaccharide residues) extended from 400°C to 600°C before inorganics and residual organics decomposed from 600°C to 800°C (TI_{inorg}). The total range of mass loss (TI_{total}) was from 180°C to 800°C and 160°C to 800°C for macroalgae and vascular plants, respectively. As thermograms for *Corallina officinalis* and *Caulerpa cactoides* did not coincide with the four temperature intervals selected for analysis, these taxa were excluded from statistical analyses and are discussed separately.

Statistical analyses

Multifactorial permutational analysis of variance (PERMANOVA) was used to investigate differences in the size of the four temperature intervals (TI_1, TI_2, TI_3, and TI_{inorg} normalized to total mass loss) between taxa. Preliminary two-factor analyses comparing either orders (seven levels) or species (nine levels) and tissues (two levels, photosynthetic [leaf, blade] and attachment [root, rhizome, holdfast]) identified that there were significant differences among tissue types (pseudo-$F_{1,44} = 11.61$, P

[perm] = 0.001) and orders (pseudo-$F_{4,44} = 56.14$, P [perm] = 0.001), but differences between tissue categories were not consistent among species (species × tissue interaction, pseudo-$F_{8,36} = 3.64$, P [perm] = 0.001). Therefore, three-factor PERMANOVAs examined differences in decomposition dynamics for photosynthetic structures and attachment structures independently comparing kingdom (two levels, Protista [macroalgae] and Plantae [vascular plants]), orders nested within kingdom (macroalgae, Fucales, Laminariales, and Plocamiales; vascular plants, Alistimales, Lamiales, Caryophyllales, and Poales) and species nested within order (nine levels; Table 1). Pairwise comparisons of orders within each kingdom were also undertaken, as were pairwise comparison of species within orders. Monte Carlo-approximated P values (P(MC)) were used to interpret comparisons with low numbers of unique permutations (i.e., <100). Permutational analysis of multivariate dispersions (PERMDISP) was used to identify if differences were due in part to heterogeneity in dispersion. Similarity percentage (SIMPER) analysis was used to identify the contributions of each temperature interval to observed differences among kingdoms and orders. Multidimensional scaling (MDS) plots provide spatial representations of taxon similarity in which each sample is defined by its proximity to the most closely related samples. Goodness of fit of MDS plots was determined with a stress value <0.2 considered acceptable (Clarke 1993). Analyses were based on untransformed data and Euclidean distance resemblance matrices calculated by PRIMER, version 6 for Windows (PRIMER-E). Analyses were also performed using PRIMER, version 6 with PERMANOVA+ add on.

Results

There were significant differences in the decomposition of organic matter under pyrolysis between macroalgae and vascular plants of both photosynthetic tissues (pseudo-$F_{1,24} = 129.30$, P [perm] = 0.001; Fig. 2) and attachment tissues (pseudo-$F_{1,18} = 112.79$, P [perm] = 0.001; Fig. 3; Appendix A: Fig. A1). For the attachment tissues, these differences were due at least in part to heterogeneity in dispersion of Euclidean distances among temperature intervals between kingdoms (PERMDISP, $F_{1,25} = 17.06$, P [perm] = 0.002; Fig. 3). Similarly, the differences between macroalgae and vascular plants were reflected in the mean thermogravimetric signatures and the rates of change for both tissue types (Figs. 4 and 5). Vascular plant tissues exhibited a greater proportion of mass loss than did macroalgal tissues at temperatures >300°C, with a peak rate of change at approximately 320°C (Figs. 4d and 5d, Table 2). This is consistent with the SIMPER results, which indicated that 50% and 30% of the differences in the thermogravimetric signatures were due to differences in TI_2 for photosynthetic and attachment tissues, respectively. In contrast, macroalgae showed a peak mass loss between 175–300°C (Figs. 4c and 5c, Table 2), again consistent with similarity percentage contributions of 37–39% for TI_1 for both tissue groups. The remaining contribution to differences in signatures between macroalgae and vascular plants was of inorganics, accounting for 9% of the difference for photosynthetic tissues, but 26% for attachment tissues. This is illustrated by peaks at ~750°C for some macroalgal taxa (Figs. 4c and 5c, Table 2) with variations in size of the peak between taxa (*Corallina officinalis* > *Plocamium angustum* > *Hormosira banksii*). Consistent with the diversity of cell wall composition amongst groups, there was greater variation in thermogravimetric signatures within and among macroalgal taxa relative to vascular plants (Figs. 4 and 5, Table 2).

Two algal species were unique whereby they did not follow the consistent thermogram patterns exhibited by other algal taxa (Figs. 4a and 5a). Firstly, *Corallina* is the only calcifying alga analyzed in this study, which is clearly indicated by the high relative mass loss (74%) above 600°C, compared to other taxa (3–25%; Table 2). Organic matter content was approximately a quarter of the total mass compared to the other thallus tissues (13% vs. 46%), with the majority of organic mass loss between 250–380°C (Fig. 4c, Table 2). Secondly, both blade and stolon tissues from *Caulerpa* show high variability within replicates (Figs. 4a and 5a). At 800°C, mass loss ranged from 20–74% for the blades (Fig. 4a) and 17–65% for the stolon (Fig. 5a). When additional samples of *Caulerpa* were analyzed, the variability remained (data not shown).

At the next taxonomic levels studied, there were significant differences in decomposition between macroalgal and vascular plant orders for both photosynthetic and attachment tissues (orders within kingdoms, P [perm] < 0.001; Figs. 2 and 3) and between species within orders (P [perm] < 0.001). Ordinal differences were due, at least in part, to differences in dispersion (PERMDISP, $F_{6,29} = 6.47$, P [perm] = 0.003, and $F_{4,22} = 5.31$, P [perm] = 0.011, respectively) driven by greater dispersion in Laminariales than Plocamiales for photosynthetic tissues (Fig. 2) and Alistamales compared to Poales for attachment tissues (Fig. 3; pairwise PERMDISP, P [perm] < 0.05).

For photosynthetic tissues, the decomposition dynamics for laminarian and fucoid macroalgae did not differ, but both differed significantly from those of Plocamiales (represented by *Plocamium angustum*; pairwise tests, P [perm] < 0.05; Fig. 2). This was primarily due to differences in mass loss in TI_1 (SIMPER, 55% and 61% for Plocamiales vs. Laminariales and Fucales, respectively), where the laminarian and fucoid algae had greater rates of mass change than *P. angustum* (Fig. 4c, Table 2), and TI_{inorg}, where rates of mass change for *P. angustum* exceeded those of the brown algae (SIMPER, 37% and 34% for Plocamiales vs. Laminariales and Fucales, respectively; Figs. 2 and 4, Table 2). All vascular plant orders showed significant differences (pairwise tests, P(MC) < 0.05), with differences in TI_2

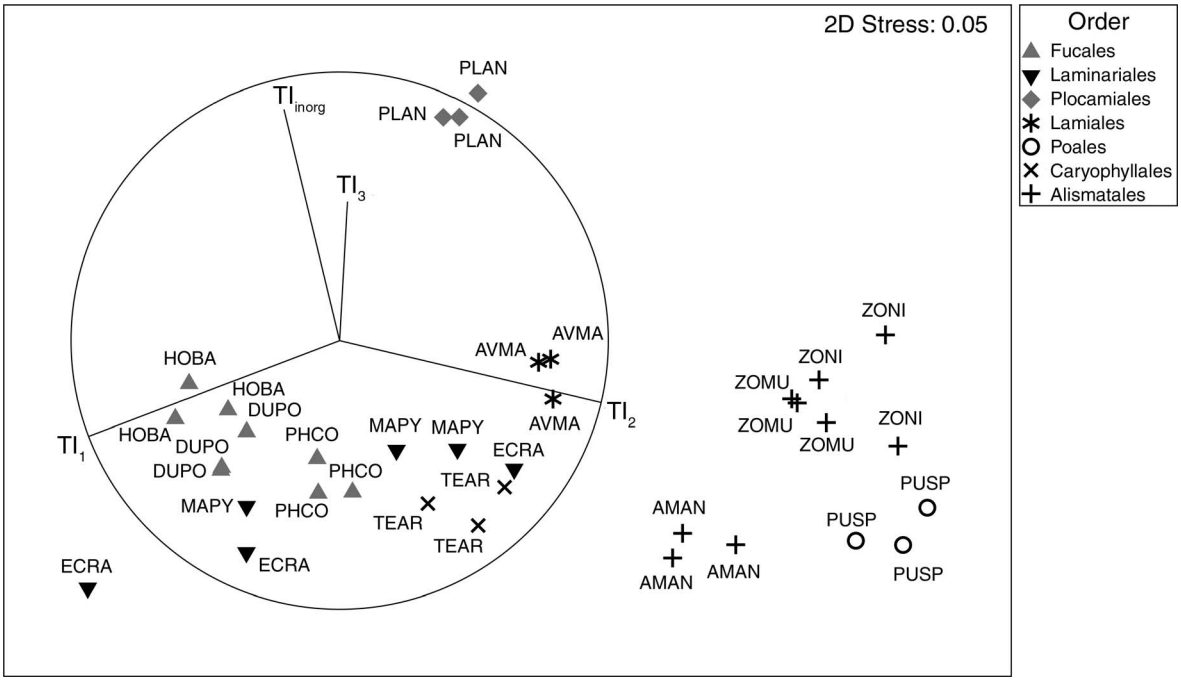

FIG. 2. Multidimensional scaling (MDS) plot based on Euclidean distance matrix of photosynthetic structure decomposition. Macroalgae (solid shapes) and vascular plant (open shapes) samples are grouped by order. *Caulerpa* and *Corallina* were excluded from analysis. Abbreviations are AMAN, *Amphibolis antarctica*; AVMA, *Avicennia marina*; DUPO, *Durvillaea potatorum*; ECRA, *Ecklonia radiata*; HOBA, *Hormosira banksii*; MAPY, *Macrocystis pyrifera*; PHCO, *Phyllospora comosa*; PLAN, *Plocamium angustum*; PUSP, *Puccinellia* sp.; TEAR, *Tecticornia arbuscula*; ZOMU, *Zostera muelleri*; and ZONI, *Zostera nigracaulis*. Vectors are temperature intervals for mass loss, TI_1, where TI_1 is the relative mass loss for organic matter 180–300°C for algae and 160–300°C for vascular plants; TI_2 is the relative mass loss for organic matter 300–400°C; TI_3 is the relative mass loss for organic matter 400–600°C; and TI_{inorg} is the relative mass loss for inorganics 600–800°C. Stress value is a type of goodness of fit calculated during the formation of MDS plots in PRIMER. Values of <0.2 are considered acceptable.

(highest in the monocot orders Poales and Alistamales) and TI_1 (highest in the Caryophyllales; SIMPER, 44% and 37%, respectively) driving the separation (Table 2). TI_3 and TI_{inorg} had smaller contributions to the separation of orders (SIMPER, 13% and 5%, respectively; Fig. 2, Table 2). The leaf samples of the mangrove *A. marina* (Lamiales) drove the differences in TI_3 with a distinctly higher rate of mass loss in the temperature range 400–500°C (Fig. 4d). While there was no significant difference in decomposition patterns between the laminarian *E. radiata* and *M. pyrifera* (P(MC) = 0.509) for within-order comparisons, all fucoid species differed significantly, as did seagrass species (P(MC) < 0.05; Appendix A: Table A1). Among the fucoid species, *P. comosa* had higher mass loss under TI_2 than both *D. potatorum* and *H. banksii* (SIMPER, 63% and 61%, respectively), while *H. banksii* had a higher relative loss under TI_{inorg} (SIMPER, 53%) and higher loss under T_2 (SIMPER, 35%) than *D. potatorum* (Appendix A: Table A1). For the seagrasses, *A. antarctica* had higher mass loss under TI_1 than both *Z. muelleri* and *Z. nigracaulis* (SIMPER, 64% and 71%, respectively), while differences between *Zostera* species were mostly due to TI_{inorg} (SIMPER, 30%) and TI_2 (SIMPER, 28%; Appendix A: Table A1).

For attachment tissues, laminarian and fucoid macroalgae had significantly different decomposition dynamics (P [perm] = 0.005) with within-order differences for fucoids (*Durvillaea potatorum* ≠ *Phyllospora comosa*; P(MC) = 0.002) but not laminarians (*Ecklonia radiata* = *Macrocystis pyrifera*; Fig. 4; Appendix A: Table A1). Macroalgal ordinal differences were primarily due to marginally lower rates of mass change in TI_1 and lower rates in inorganics for laminarians relative to fucoids (SIMPER, 40% and 37%, respectively; Table 2). Similarly, the decomposition dynamics of attachment tissues of vascular plants differed significantly among orders (P [perm] < 0.01 or P(MC) < 0.01 where appropriate), with TI_1 (higher in Carophyllales) and TI_2 (higher in Poales and Alistamales) each contributing 37% and 31% (SIMPER), respectively, to the separation (Table 2). The Alismatales was the only vascular plant order with a significant rate of change above 600°C (inorganics; Fig. 5d, Table 2). For the Alistamales, the two *Zostera* species had similar decomposition dynamics (P(MC) > 0.05), but differed to that of *Amphibolis antarctica* (P(MC) < 0.05; Fig. 3), primarily due to higher rates of mass change for TI_2 (SIMPER, 50% and 27% for *Z. mulleri* and *Z. nigracaulis* vs. *A. antarctica*, respectively) and lower inorganics (SIMPER, 46% and

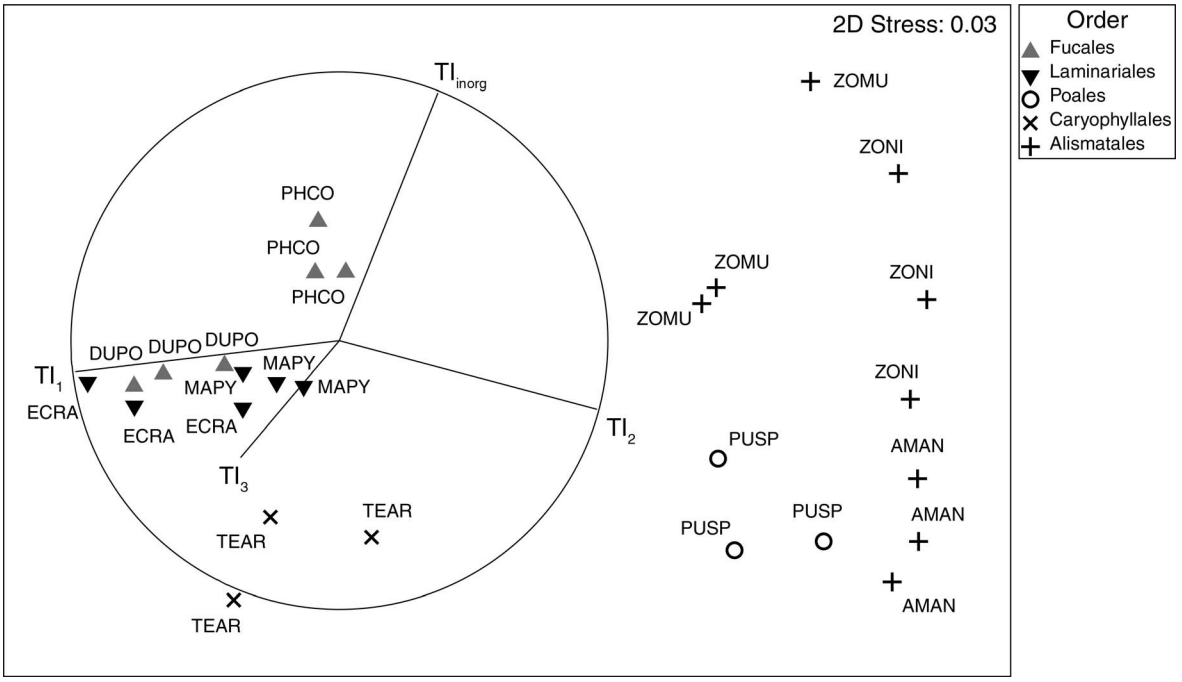

FIG. 3. Multidimensional scaling (MDS) plot based on Euclidean distance matrix of attachment structure decomposition. Macroalgae (solid shapes) and vascular plant (open shapes) samples are grouped by order. See Fig. 2 for definitions of the abbreviations.

53% for *Z. mulleri* and *Z. nigracaulis* vs. *A. antarctica*, respectively) in *A. antarctica* relative to *Zostera* spp. (Appendix A: Table A1). There was greater variability within *Z. nigracaulis* attachment tissues than the other seagrass species (Fig. 4; Appendix A: Table A1).

DISCUSSION

Cell wall composition defines OM stability

We have recorded significant variability in the thermal stability of organic (and inorganic) matter among different lineages of aquatic plants and among different tissues within taxa. This variability is largely consistent with differences in cell wall composition and structure. The primary differences in the cell wall composition of marine macroalgae and vascular plants lie in a general lack of lignin and smaller proportion of cellulose in macroalgae (1–8% dry mass) than vascular plants (average 30% dry mass), and abundant matrix components (primarily polyanionic over neutral polysaccharides) in macroalgae relative to vascular plants (Kloareg and Quatrano 1988, Domozych 2011). Macroalgae also have abundant long-chain fatty acids (Schmid et al. 2014, Schmid and Stengel 2015; Skrzypczyk et al., *unpublished manuscript*). At the Phylum level, our comparison of macroalgae to vascular plants shows distinct differences in overall decomposition dynamics under increasing temperature and the differences are particularly defined by the regions that correspond to cellulose (TI_2) and soluble and insoluble polysaccharides (TI_1 and TI_2, respectively; Yang et al. 2006). In most cases, the macroalgal samples began to decompose at lower temperatures than the vascular plants, possibly due to catalysis by abundant inorganic salts in macroalgae (Wang et al. 2006) and/or lower thermal stability of polyanions vs. neutral polysaccharides (Villetti et al. 2002). However, some macroalgae had highly refractory compounds, such as calcium carbonate and sulfated polysaccharides. Our results are generally consistent with thermogravimetric characterizations made elsewhere for components of macroalgae (Wang et al. 2007, Anastasakis et al. 2011, Li et al. 2011) and vascular plants (Yang et al. 2006), but highlight the significant variability within each of these broad taxonomic groupings and also between attachment and photosynthetic tissues within taxa.

Variability in vascular plants

The vascular flora of blue C habitats contains remarkable diversity in physical structure between and within habitat types. Our results, along with previous biochemical studies, have shown this diversity to extend to chemical composition, with implications for long-term C sequestration in these habitats.

Hemicelluloses are polysaccharides that contribute to strengthening of plant cell walls by interaction with cellulose and lignin. Importantly, hemicellulose type varies predictably according to plant type, with xyloglucans dominating in primary walls of dicots and conifers, while glucuronoarabinoxylans dominate in commelinid monocots, which include grasses (Scheller

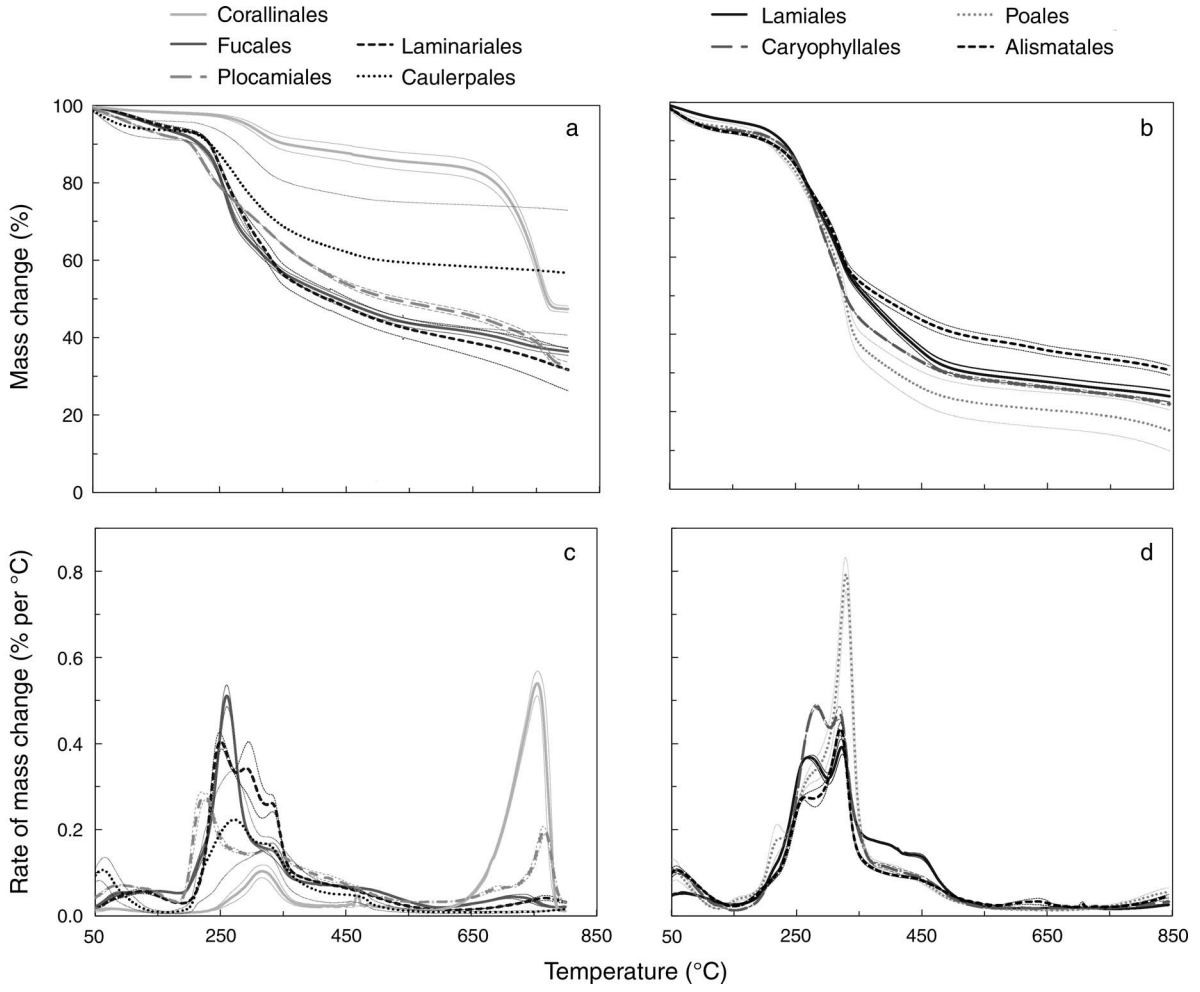

FIG. 4. Mean thermogravimetric plots for photosynthetic tissues of (a) macroalgal orders and (b) vascular plant orders with (c, d) corresponding derived rates of mass change. Each heavy line type with its associated pair of thin lines represents the mean ± SE for that order.

and Ulvskov 2010). This division along plant types is apparent in TI_1 (operationally defined to encompass hemicellulose), where all dicot samples exhibit a pronounced peak relative to monocots (grass and seagrasses). It is not known however, if this difference is due to (1) the dicots in our study having higher overall hemicellulose contents than grasses, (2) the two types of hemicellulose differing in their relative stability (for example, glucuronoarabinoxylans may have been degraded in TI_2 rather than TI_1), or (3) hemicellulose–lignin interactions increasing the stability of dicots.

Due to its complex structure and aromatic bonds, lignin generally exhibits higher resistance to microbial degradation than most other plant constituents, including cellulose (Derenne and Largeau 2001). This has important implications for the C-sequestration potential of autochthonous plant biomass and litter as an allochthonous donor. Despite its abundance in vascular plants, overall lignin content varies greatly among terrestrial angiosperms, from grasses having 5–10% to hardwood trees with more than 40% (Novaes et al. 2010 and references therein). Variability in mass loss between 400–600°C is consistent with expected lignin contents across the structural variety of vascular plants studied, mangrove tree (Lamiales) > succulent shrub (Caryophllales) > saltmarsh grass (Poales) > seagrasses (Alismatales) (Figs. 4 and 5). This suggests that plant structure and composition may be an important determinant of lignin concentrations in coastal soils, as has been seen in blue C habitats where increased lignin storage rates occurred with a shift in vegetation from saltmarsh grass to *Avicennia germinans* mangrove (Bianchi et al. 2013). Although small, the increased content of lignin in saltmarsh grass relative to the seagrasses would also be expected based on the differing structural rigidity requirements of emergent (saltmarsh) relative to submerged (seagrass) vegetation. While the seagrass species used in this study are relatively lignin-deficient compared to the other vascular plants, more robust seagrass morphotypes, such as *Posidonia* or *Thalassodendron*,

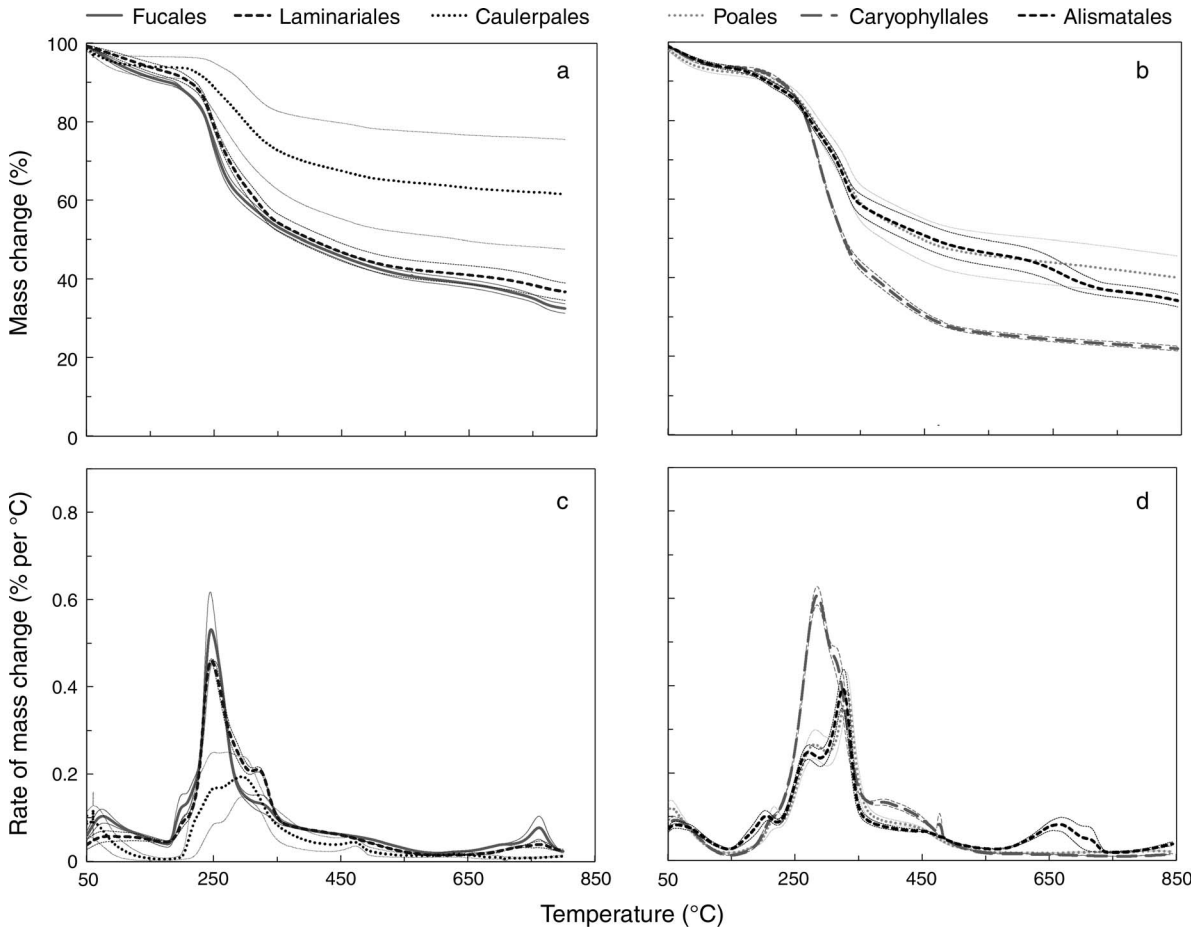

FIG. 5. Mean thermogravimetric plots for attachment tissues of (a) macroalgal orders and (b) vascular plant orders with (c, d) corresponding derived rates of mass change. Each heavy line type with its associated pair of thin lines represents the mean ± SE for that order.

have been shown to have similar lignin content to that of the saltmarsh grass *Spartina* (Klap et al. 2000). Therefore, such seagrasses could be greater contributors of refractory C in blue C habitats than others.

Significant thermal degradation above 600°C for the vascular plants only occurred in the seagrasses, namely in the rhizome/root tissue (Figs. 4d and 5d). The addition of inorganic compounds like salts to the organic matter can lower their decomposition temperature in thermogravimetric analysis (Wang et al. 2006), yet there is little information on the thermal properties of these inorganics above 600°C. It is possible that this characteristic inorganic peak is due to sulfated residues. Šimkovic et al. (2011) showed that sulfation of xylan had sulfur incorporated into the residual C leading to a peak of ~790°C under pyrolysis. Sulfation of polysaccharides is a common adaptation for marine plants, normally absent in terrestrial and freshwater plants, to aid in ionic balance (Kloareg and Quatrano 1988) and allelochemical interactions (McMillan et al. 1980; see also *Discussion: Variability in macroalgae*). Seagrasses may produce sulfated compounds as biochemical defenses (flavone glycosides and sulfated phenolic acids; McMillan et al. 1980, Jensen et al. 1998) or as adaptation to increased nutrient absorptions or to maintain ion balance (Aquino et al. 2005). In addition to production of S-containing compounds, seagrasses may accumulate high concentrations of sulfur in their tissues, particularly in their belowground tissues, due to high concentrations of sulfide from the anoxic, sulfate-rich pore waters (Holmer et al. 2006, Frederiksen et al. 2008). Not only are the roots and rhizome the site of exposure, but the natural storage function of the rhizomes may contribute to its greater S concentrations (Frederiksen et al. 2008).

Variability in macroalgae

There was significant variability in the thermogravimetric signatures among macroalgal orders, which reflects the diversity of cell wall constituents among lineages. While all macroalgal cell walls are composed of a fibrillar skeletal component and a matrix component,

TABLE 2. Thermogravimetric intervals for algae and vascular plant tissue groups.

Order	Tissue	Total mass loss† (%)	Relative mass loss (%)‡			
			TI_1	TI_2	TI_3	TI_{inorg}
Macroalgae						
Fucales	photosynthetic	56.41 (0.91)	51.92 (0.55)	19.63 (1.19)	17.56 (0.30)	10.90 (0.81)
	attachment	57.47 (1.74)	52.20 (1.40)	19.21 (0.84)	15.96 (0.99)	12.64 (1.48)
Laminariales	photosynthetic	56.30 (2.39)	50.76 (3.39)	24.55 (2.28)	15.45 (0.63)	9.24 (0.65)
	attachment	55.79 (0.95)	55.11 (1.34)	20.28 (1.11)	15.59 (0.32)	9.01 (0.41)
Plocamiales	photosynthetic	58.69 (1.06)	33.02 (0.68)	22.27 (0.25)	19.70 (1.17)	25.01 (0.41)
Overall average	photosynthetic	56.75 (0.91)	48.38 (2.00)	21.71 (1.07)	17.21 (0.46)	12.70 (1.42)
	attachment	56.63 (0.98)	53.66 (1.02)	19.74 (0.68)	15.78 (0.50)	10.83 (0.91)
Vascular plants						
Alismatales	photosynthetic	59.48 (1.30)	30.71 (1.85)	43.03 (0.73)	16.96 (0.97)	9.30 (0.84)
	attachment	58.39 (1.72)	32.22 (1.18)	38.92 (1.91)	13.75 (0.55)	15.11 (2.10)
Caryophyllales	photosynthetic	69.12 (0.45)	44.99 (1.17)	30.34 (0.70)	19.00 (0.89)	5.66 (0.59)
	attachment	70.83 (0.85)	51.68 (1.37)	24.42 (2.11)	20.42 (2.40)	3.49 (0.04)
Lamiales	photosynthetic	69.83 (1.24)	38.72 (0.26)	30.13 (0.47)	25.93 (0.54)	5.21 (0.29)
Poales	photosynthetic	74.32 (3.80)	29.56 (1.15)	50.15 (0.66)	15.10 (0.59)	5.19 (0.28)
	attachment	50.97 (4.84)	35.94 (1.08)	38.31 (2.29)	18.24 (1.72)	7.50 (1.01)
Overall average	photosynthetic	65.28 (1.69)	34.23 (1.67)	39.95 (1.82)	18.49 (0.99)	7.33 (0.64)
	attachment	59.39 (2.15)	36.85 (2.15)	35.90 (1.97)	15.98 (0.96)	11.27 (1.80)
Exceptional macroalgae§						
Caulerpales	photosynthetic	36.62 (13.72)		80.03 (2.52)	13.14 (0.80)	6.82 (1.73)
	attachment	32.14 (11.22)		76.77 (3.10)	15.50 (2.79)	7.75 (0.34)
Corallinales	photosynthetic	50.65 (0.72)		18.15 (3.15)	8.20 (0.74)	73.66 (3.88)

Notes: Values represent mean (±SE) percentage total dry mass loss and relative percentage of mass loss among different temperature intervals, TI.

† Total mass loss at 180–800°C for algae and at 160–800°C for vascular plants.

‡ Temperature intervals for relative mass loss are as follows: for organic matter, TI_1, 180–300°C for algae and 160–300°C for vascular plants; TI_2, 300–400°C; TI_3, 400–600°C; for inorganic matter, 600–800°C.

§ For the exceptional orders, temperature intervals for relative mass loss are as follows: for organic matter, TI_A (in the TI_2 column) 180–410°C and TI_B (in the TI_3 column), 410–600°C; for inorganic matter, 600–800°C.

the composition of these components varies among lineages. The skeletal polysaccharides consist of long homopolymeric chains that aggregate into highly crystalline associations, yet the matrix components rarely consist of long homopolymeric chains (Kloareg and Quatrano 1988). The cell wall composition of the taxa tested in this study influenced the rates of decomposition for given temperature ranges, with implications for C sequestration potential.

Brown algal cell walls are composed of crystalline cellulose microfibrils in layers, parallel to the cell surface, but without defined orientation in each layer, surrounded by a polysaccharide matrix that may constitute 10–45% dry mass of the thallus (Kloareg and Quatrano 1988, Domozych 2011). The polysaccharide matrix is primarily composed of alginate (linear chains of β-1,4-d-mannuronic acid and α-1,4-l-guluronic acid), but sulfated polysaccharides (fucoidans, ascophyllans), laminarin, mannitol, proteins, and phenolics can also be abundant (Kloareg and Quatrano 1988, Chiovitti et al. 2001). The matrix components both embed and bond to the cellulose microfibrils resulting in structurally complex cell walls (Kloareg and Quatrano 1988, Domozych 2011) that are both strong and flexible enough to withstand wave forces (Denny and Gaylord 2002, Martone 2007, Guenther and Martone 2014). Composition of the polysaccharide matrix has been shown to vary between species, as well as seasonally and between tissues within species (Stewart et al. 1961, Lie et al. 1990, Westermeier et al. 2012).

Thermogravimetric analysis of commercial products of alginic acid and fucoidan showed peak mass loss in TI_1 range at 225°C and 202°C, respectively; while mannitol and laminarin peaked in TI_2 at ~340°C (Anastasakis et al. 2011). Laminarin demonstrated a second peak in TI_3 at 540°C as did fucoidan at 710°C in the inorganics range (Anastasakis et al. 2011). Among the brown algae from this study *Macrocystis pyrifera* (from Chile) and *Durvillaea potatorum* have been shown to have the highest alginate content with 47% and 35% dry mass, respectively, while *Hormosira banksii* and *Ecklonia radiata* averaged ~25% dry mass alginic acid (Stewart et al. 1961, Lie et al. 1990, Westermeier et al. 2012). The significant multivariate dispersion of the laminarian photosynthetic tissues relative to those of fucoids and Plocamiales is driven by a large difference in TI_1 between *M. pyrifera* and *E. radiata*, attributable to the differences in quantities of alginates in the blades of these species. As holdfast tissues contain less alginates for greater rigidity (Westermeier et al. 2012), the signatures in TI_1 of attachment tissues of the laminarian species were not similarly disparate. The peak in inorganics at ~710°C for fucoid algae is consistent with relatively high levels of the xylogalactofucan fucoidan,

particularly for the intertidal *H. banksii*, where it is thought to confer desiccation protection (Mabeau and Kloareg 1987, Lie et al. 1990, Anastasakis et al. 2011).

The skeletal framework of red algal cell walls in the Florideophyceae is also based on cellulose microfibrils that are less crystalline and less abundant (1–8% dry mass) than those of vascular plants (Craigie 1990) and arranged parallel to the cell surface with an entangled network of microfibrils in each layer, as in phaeophycean cell walls (Kloareg and Quatrano 1988). However, the matrix polysaccharides of rhodophytes are very different to those of phaeophytes, consisting primarily of linear sulfated galactans. In the Plocamiales, the matrix is largely agarose, which consists of regular repetition of β-d-galactosyl (1,4)-3, 6-anhydro-α-l-galactose (Kloareg and Quatrano 1988). *Plocamium angustum* (representing the Plocamiales in this study) exhibited peaks in the rate of mass change in TI_1 at ~230°C and TI_2 at ~340°C, consistent with pyrolysis of the congeneric *P. telefiariae* (Li et al. 2011) and attributable to agar in the cell wall matrix. These peaks were smaller than those of fucoid and laminarian algae from the same regions, however, suggesting that the brown algae had significantly more alginate and storage polysaccharides than the agarose content in this red alga. *P. angustum* also had high inorganic content with peak mass loss at >600°C exceeding that of the brown algae and likely attributable to the sulfation of agarose in this red algae.

The Corallinales are a unique group within the florideophycean red algae in that their cell walls are highly calcified. The cellulose framework is similar to that described for Plocamiales, but the polysaccharide matrix is significantly different to that of other red algae. The coralline algal matrix is composed of nonconventional sulfated xylogalactans (which have high xylose content) and alginic acids (similar to those of brown algae; Bilan and Usov 2001). The cell wall is heavily impregnated with $CaCO_3$ (in the form of calcite) in intergenicula segments with smaller, noncalcified geniculla that allow flexibility and reduce breakage by wave forces (van den Hoek et al. 1995). The thermograms of *C. officinalis* reflected this heavy calcification with 74% of total mass loss occurring at 600–800°C, unlike any of the other species tested. *C. officinalis* was more thermally stable than the other macroalgal taxa tested, with degradation beginning after ~250°C and first peaking at ~340°C, most likely attributable to decomposition of the cellulose skeletal framework and the xylose-rich matrix polysaccharides (Bilan and Usov 2001, Shen et al. 2010).

Chlorophyte algae have the greatest diversity of cell wall skeletal biochemistry with differences at multiple levels of the taxonomic hierarchy and between life phases within species (Kloareg and Quatrano 1988, Domozych et al. 2012). Unlike most other macroalgae, the Caulerpales do not contain cellulose. Instead the cell wall skeleton is very crystalline, built from tri-helical chains of β-1,3-xylans (with six xylose residues per helix turn) that can account for >50% dry mass of the cell walls (Kloareg and Quatrano 1988, Yamagaki et al. 1996). These microfibrils are arranged parallel to the cell surface as per the cellulose microfibrils of phaeophycean and florideophycean algae described earlier (Kloareg and Quatrano 1988). The cell wall matrix is largely β-1,3-glucans (Kloareg and Quatrano 1988). Another characteristic that sets *Caulerpa* apart from the other macroalgae tested is that it is siphonous, meaning the whole plant is a single cell with no cross walls (van den Hoek et al. 1995). The wall surrounds the branches and the interior is a large water- and ion-filled vacuole, and the cytoplasm lies between the vacuole and the cell wall (van den Hoek et al. 1995, Estevez et al. 2009). The rapid mass loss of *Caulerpa cactoides* at low temperatures (<150°C) is most likely due to dehydration of this large aqueous vacuole. The broad peak in average mass loss between 180–410°C for both blades and stolons of *C. cactoides* is consistent with an abundance of the insoluble β-1,3-xylan (Yamagaki et al. 1996, Shen et al. 2010, Ciancia et al. 2012) and possibly also novel secondary metabolites, including caulerpin (with a unique structure) and caulerpicin (which is a mixture of the hydroxyl amides N-acylspingosines 2 and N-acylsphinganines 3). The latter has been reported to be 0.15% dry mass in *C. cactoides* and the former 0.2% dry mass in other *Caulerpa* spp. (Vidal et al. 1984). Because of the siphonous structure of *Caulerpa*, it is likely that with differences in degree of branching between both blades and stolons, there may be significant differences in the amount of cell wall material for a given wet mass of tissue. Similarly there may be differences in the quantity and composition of secondary metabolites between individual plants in response to grazing and/or other environmental influences (Meyer and Paul 1992), both of which may explain some of the between-replicate variability observed for this species.

In addition to cell wall compounds, macroalgae also contain significant lipid reserves that may contribute to their ability to sequester carbon. Like the cell wall composition, the lipid biochemistry varies significantly among species; but long-chain fatty acids with at least 18 C molecules can account for as much as 70% of the total fatty acids for some of the laminarian and fucoid species in our study (Skrzypczyk et al., *unpublished manuscript*). The lipids may be more refractory than some of the macroalgal polysaccharides with thermal degradation typically beginning after 300°C (TI_2; Kapusniak and Siemion 2007). Thus, some of the differences in thermal stability observed within and between macroalgal orders (associated with TI_2) may be due to species-specific differences in long-chain fatty acids.

Macroalgae as blue C donors

While the role of ecosystem properties such as hydrology, temperature, and microbial community and function has been increasingly recognized in recent years, litter quality remains one of the most important,

yet poorly understood, determinants of the fate of C (Couteaux et al. 1995, Cleveland et al. 2014). Based on the labile or refractory characteristics of the organic C shown in this study, organic matter stability can give us insight in to how these plants would be processed in the detrital cycle and thus their potential to contribute to blue C sequestration.

Compared to aquatic vascular plants, macroalgae have a higher litter quality (lower C:N ratio) and lack lignocellulose tissue, making them more nutritious and energetically favorable for consumption (Enríquez et al. 1993), and thus a greater potential source of C for consumers in blue C habitats. The results in this study are in agreement with these consumption patterns as the two refractory decomposition stages associated with cellulose and lignin (TI_2 and TI_3, respectively) had higher mass loss for the vascular plants, while the organic matter for macroalgae were dominated by the labile TI_1. Not surprisingly, mangrove leaves appear the greatest contributor of refractory lignin (26% mass) among the studied taxa, and though not studied here, its woody biomass is likely to contribute an even higher proportion. While seagrasses and saltmarsh grass may contribute high cellulose ($\geq$40% mass) C stocks, the presence of even low concentrations of lignin among these taxa may support the retention of holocellulose (hemicellulose + cellulose), as seen in long-term decay studies (Wilson et al. 1986). Furthermore, residual lignin or phenolics occurring in the presence of N-containing compounds (from microbial by-products or plant residues) can promote geopolymerisation of humic substances, which are highly stable and practically permanent forms of C in sediments (Wilson et al. 1986, Harrison 1989, Klap et al. 2000).

A lack of lignin does not mean all macroalgae taxa are of low C sequestration value. Macroalgae may be rich in other unique metabolites or compounds that thermally decompose at high temperatures, which is characteristic of greater recalcitrance. With the exception of *Caulerpa*, all macroalgae taxa in the present study experienced mass loss >9% within the inorganics temperature range, with exceptionally high proportions for the calcifying *Corallina* (74%), as well as *Plocamium* (25%).

While blue C policy and funding mechanisms currently exclude inorganic C (as carbonate formation releases net CO_2), the long-term storage of inorganic C, which may otherwise end up as atmospheric CO_2, remains an important C pool. Carbonate deposition in coastal waters globally is around 0.17 Pg C/y, with the calcareous *Halimeda* alone estimated to contribute 0.4 Gt $CaCO_3$/y (0.06 Gt C/y) to carbonate accumulation (Hillis 1997). There are vast amounts of algal carbonates locked up as maerl (600 000 Mg/yr, wet mass in France alone), which are harvested commercially (Zemke-White and Ohno 1999), though growth rates of rhodolith-forming species are low (Nelson 2009).

Additionally, the sulfation of polysaccharides by both macroalgal and vascular plant taxa appears to increase organic matter stability, and we propose that it may make a contribution to long-term C stocks. This is particularly likely in anaerobic environments where decomposition via sulfate reduction had been recorded to cause additional incorporation of sulfur into decaying seagrass organic matter (Boon and Haverkamp 1982). However, more research is needed to further understand stability of organic matter after sulfation and to determine if this process does indeed lead to a degree of protection from microbial degradation.

Although both macroalgae and vascular plants have the biochemical foundation to contribute refractory or recalcitrant C to blue C stocks, another important factor is the fate of marine plant detritus, i.e., where is marine plant detritus ultimately buried and how quickly does this burial process occur? In general, plant detritus that undergoes rapid burial will have a high C sequestration rate, whereas detritus that undergoes slow burial has a greater chance of being exposed to microbial attack under oxic conditions, resulting in a lower C sequestration rate (Canfield 1989, 1994). Sassi et al. (1988) found that macroalgal decomposition over 25 days under aerobic vs. anaerobic conditions were 35% and 24%, respectively. Therefore, if the macroalgae detritus can be advected to a rapidly sedimenting region where it can be incorporated into an anaerobic sediment, this will slow down the breakdown and maintain the C within the sediment longer.

Burial of seagrass, saltmarsh, and mangrove plant material occurs with relatively high efficiency because they have the ability to transfer their own detritus directly into the sediments in which they grow, and because they are highly effective at trapping particles from the water column, their detritus is rapidly buried through accretion (Agawin and Duarte 2002, Gonneea et al. 2004). On the other hand, most macroalgae grow on hard substrates and therefore lack the ability to transfer their detritus belowground. This limits the capacity for macroalgae to act as long-term C sinks in their own right. However, they can make important contributions to global C sequestration by transferring their C (in the form of plant biomass, POC, or DOC) to receiver habitats for subsequent burial (Wada et al. 2008, Dierssen et al. 2009, Hyndes et al. 2012, Macreadie et al. 2012).

In tropical estuaries and bays, it is estimated that approximately half of the macroalgal biomass produced is exported to other habitats, whereas in the temperate open coast, most of the biomass is decomposed by detritivores, with a lesser amount being exported, and only small fractions being consumed or accumulated as detritus (Hyndes et al. 2013). Data on the uptake of macroalgal detritus within sediments is rare, but the limited information suggests that it can be taken up with relatively high efficiency (Hardison et al. 2010).

While the capacity of coastal vascular plants to act as biological sinks of carbon dioxide is well known, findings in the present study show that cell wall structure

and composition of macroalgae are central to their long-term carbon storage potential. Stability varies predictably according to cell wall differences and lipid chemistry among kingdoms, orders, within-order, and according to tissue function. While differences were most apparent in temperature ranges associated with hemicellulose, cellulose, and lignin, the presence of refractory compounds, including carbonates, long-chain lipids, alginates, xylans, and sulfated polysaccharides, will increase the C sequestration potential of otherwise labile source materials. Where these chemical characteristics are combined with an optimal preserving environment (retention in coastal habitat, quick burial, and anaerobic decomposition), macroalgae, like coastal vascular plants, could significantly contribute to long-term carbon storage.

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Supplemental Material

Ecological Archives

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