Sediment anoxia limits microbial-driven seagrass carbon remineralization under warming conditions

This is the accepted manuscript.

© 2017, FEMS

This is a pre-copyedited, author-produced version of an article accepted for publication in *FEMS Microbiology Ecology* following peer review. The version of record:


is available online at: [http://www.dx.doi.org/10.1093/femsec/fix033](http://www.dx.doi.org/10.1093/femsec/fix033)

Downloaded from DRO:
[http://hdl.handle.net/10536/DRO/DU:30096928](http://hdl.handle.net/10536/DRO/DU:30096928)
Sediment anoxia limits microbial-driven seagrass carbon remineralization under warming conditions

Stacey M. Trevathan-Tackett, Justin R. Seymour, Daniel A. Nielsen, Peter I. Macreadie, Thomas C. Jeffries, Jonathan Sanderman, Jeff Baldock, Johanna M. Howes, Andrew D. L. Steven, Peter Ralph

1Climate Change Cluster, University of Technology Sydney, NSW, Australia.
2School of Life and Environmental Sciences, Centre for Integrative Ecology, Deakin University, Burwood, VIC, Australia.
3Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW, Australia.
4CSIRO Agriculture and Food, Glen Osmond, SA, Australia
5Woods Hole Research Center, Falmouth, MA, USA
6CSIRO Ocean and Atmosphere Flagship, Dutton Park, QLD, Australia

* Corresponding author: Climate Change Cluster, University of Technology Sydney, 15 Broadway, Sydney, 2007 Australia (Current Address: School of Life and Environmental Sciences, Centre for Integrative Ecology, Deakin University, Burwood, VIC 3215 Australia), s.trevathan.tackett@gmail.com, +61 0424483513

Keywords: bacterial remineralization, succession, oxygen availability, seagrass microbiology, temperature, carbon cycling
Abstract

Seagrass ecosystems are significant carbon sinks, and their resident microbial communities ultimately determine the quantity and quality of carbon sequestered. However, environmental perturbations have been predicted to affect microbial-driven seagrass decomposition and subsequent carbon sequestration. Utilizing techniques including 16S-rDNA sequencing, solid-state NMR and microsensor profiling, we tested the hypothesis that elevated seawater temperatures and eutrophication enhance the microbial decomposition of seagrass leaf detritus and rhizome/root tissues. Nutrient additions had a negligible effect on seagrass decomposition, indicating an absence of nutrient limitation. Elevated temperatures caused a 19% higher biomass loss for aerobically decaying leaf detritus, coinciding with changes in bacterial community structure and enhanced lignocellulose degradation. Although, community shifts and lignocellulose degradation were also observed for rhizome/root decomposition, anaerobic decay was unaffected by temperature. These observations suggest that oxygen availability constrains the stimulatory effects of temperature increases on bacterial carbon remineralization, possibly through differential temperature effects on bacterial functional groups, including putative aerobic heterotrophs (e.g. Erythrobacteraceae, Hyphomicrobiaceae) and sulfate-reducers (e.g. Desulfobacteraceae). Consequently, under elevated seawater temperatures, carbon accumulation rates may diminish due to higher remineralization rates at the sediment surface. Nonetheless, the anoxic conditions ubiquitous to seagrass sediments can provide a degree of carbon protection under warming seawater temperatures.
Introduction

Seagrass meadows fundamentally influence the biogeochemistry of coastal habitats due to their high productivity and rates of organic carbon (C) burial (Duarte and Chiscano 1999; Marbà et al. 2006; McLeod et al. 2011), and are consequently important within the context of global C sequestration (Duarte et al. 2010; McLeod et al. 2011). Within seagrass habitats, microbes play pivotal roles in several biogeochemical cycling processes, including nitrogen-fixation by leaf- and root-associated bacteria (Borowitzka et al. 2006), as well as nitrogen, sulfur and iron cycling within the rhizosphere and surrounding sediments (Duarte et al. 2005). Bacteria are also the primary decomposers of seagrass detritus in these habitats and thus drive the transformation and cycling of seagrass-derived C (Harrison 1989), which ultimately influence the rates and efficiency of C sequestration. While bacteria have been shown to oxidize 30-80% of seagrass-derived substrate back into carbon dioxide (CO₂) (Blum and Mills 1991; Pollard and Moriarty 1991), a significant proportion of the detritus escapes remineralization and is sequestered into the sediment (Burdige 2007).

On average, half of the C sequestered in seagrass meadows (20 - 101 Tg C year⁻¹ globally) originates from seagrass detritus (Duarte et al. 2013), with the remaining C from algal or terrestrial sources and captured within seagrass meadows (Kennedy et al. 2010). Seagrass meadows sequester ~40 times more C on an area basis than terrestrial ecosystems (McLeod et al. 2011), and are considered a ‘blue carbon’ (blue C) habitat for their globally significant role in natural, long-term sequestration of CO₂ (Laffoley and Grimsditch 2009). However, environmental perturbations, linked to climate change and coastal eutrophication, may influence the rates and amounts of C sequestered in seagrass meadows, making it difficult to predict the conditions for optimal sequestration or identify threats to the preservation of stored C.
In terrestrial environments, temperature and litter quality (i.e. nitrogen and lignocellulose content) influence aerobic microbial metabolism of plant litter, which subsequently controls organic C cycling and sequestration (Cleveland et al. 2014; Couteaux et al. 1995; Silver and Miya 2001). In general, higher temperatures enhance microbial metabolism and growth, resulting in higher CO$_2$ production, until temperatures increase beyond an optimum range or microbes become acclimated (Pendall et al. 2004; Pietikäinen et al. 2005; von Lützow and Kögel-Knabner 2009). In marine ecosystems, elevated temperatures have also been shown to enhance labile and refractory C remineralization in primarily anaerobic coastal and marine sediments, as well as the pelagic ocean (Bendtsen et al. 2015; Matsui et al. 2013; Weston and Joye 2005). As such, increases in marine microbial remineralization of organic C as a result of elevated seawater temperatures can be expected to drive future reductions in the efficiency of marine C burial and potentially lead to enhanced CO$_2$ release, i.e. shifting from a C sink to a C source. Therefore, given their large role in marine C sequestration, it is fundamentally important to resolve the potential effects of predicted seawater temperature increases on seagrass C remineralization.

Due to the relatively low nutrient (high C:N:P) and high lignocellulose content of seagrasses, bacterial metabolism of seagrass detritus is typically slower than that of other sources of marine detritus (e.g. macroalgae; Enríquez et al. 1993; Harrison 1989; Godshalk and Wetzel 1978; Rice and Tenore 1981). However slower, seagrass lignocellulose C is susceptible to microbial attack. Detritus-colonizing bacteria have been shown to utilize a diversity of enzymes to breakdown seagrass components, including cellulose (Harrison 1989; Wahbeh and Mahasneh 1985). Furthermore, seagrass habitats are often located in close proximity to the mouths of rivers, estuaries or lagoons where the input of external nutrient sources (i.e. runoff of inorganic nutrients or labile organic matter) can occur (Ralph et al. 2006). As a source of nutrients or organic matter, these allochthonous additions may boost
microbial remineralization of refractory C (lignocellulose), a process described as microbial priming (Cloern 2001; Guenet et al. 2010; Kuzyakov et al. 2000). The result of priming is not only a shift in sediment biogeochemistry, but also a reduction in the C sequestration and storage potential of seagrass meadows (López et al. 1998).

Global warming processes are projected to drive substantial increases in both global (Stocker et al. 2013) and local (Astles and Loveless 2012) seawater temperature and increases in eutrophication caused by terrestrial runoff into coastal habitats (Cloern 2001; Orth et al. 2006). Consequently, the future C sequestration capacity of seagrass meadows may be altered as a consequence of shifts in the activity and composition of microbial assemblages (Guenet et al. 2010; Pendall et al. 2004). Here, we conducted a three-month laboratory experiment on the temperate seagrass Zostera muelleri to examine the effect of inorganic nutrient additions and elevated seawater temperature on the microbial remineralization of seagrass C. Zostera muelleri was chosen as a model species due to its prevalence along the southern and eastern coasts of Australia, as well as being a part of the widely-studied and globally-distributed Zostera genus (Moore and Short 2006; Short et al. 2007). We achieved this through quantifying the changes in seagrass macromolecular chemistry, the seagrass-associated microbial communities and the sediment biogeochemistry. We hypothesized that elevated temperatures and nutrient additions to the sediments would enhance microbial remineralization of seagrass carbon and lead to enhanced loss of organic C. Our goal was to understand how microbial remineralization of seagrass C under different environmental conditions could alter C-cycling and sequestration processes in coastal ecosystems.

**Materials and Methods**

Laboratory experimental design
We conducted a seagrass decomposition experiment using leaf detritus, rhizome/root biomass and sediments collected from a *Zostera muelleri* Irmisch ex Ascherson meadow in Fagans Bay (33.4306 S, 151.3210 E), located on the east coast of Australia. Leaf detritus was collected as fresh wrack along the shoreline. Live plants were collected for rhizome/root material and subsequently cleaned of sediments and attached animals using a combination rinsing in seawater and removal by hand. All seagrass material was stored frozen prior to the start of the experiment to help inhibit the microbial degradation of the seagrass material.

Fagans Bay sediments were characterized by high organic matter content (OM ≥ 9% by loss on ignition) and 2-4% organic C and 0.4% total N (elemental analysis). Particle size analysis by laser diffraction using ultrasonication and Malvern Mastersizer 2000 showed the top 10 cm contained 50% in the clay and silt fraction (≤ 62.5 μm), ~ 40% as fine to medium sand (≤ 500 μm) and ~ 10% as coarse sand (≤ 2 mm). Prior to the experiment, coarse particulates were removed from the sediments with a 3.0 mm sieve and stored in aerated seawater to maintain moisture and redox levels. To ensure that all treatments received the same initial seawater, 1000 L of ≤ 5 μm filtered seawater at a salinity of ~25 (Rose Bay and Jervis Bay, NSW sources) were mixed overnight prior to experimental setup to ensure full homogenization of microbial communities.

For the decomposition experiment, *Z. muelleri* biomass was added to 1.0 mm nylon mesh litter bags (whole leaf detritus ~ 12 g DW in 15 cm x 15 cm bag; 2-4 cm segmented rhizome/roots ~6 g dry weight (DW) in 12 cm x 12 cm bag; nylon mesh from Miami Aquaculture, Inc., Florida, USA). To enable microsensor profiling through the litter bags, a 6 mm diameter hole was punched through all bags prior to filling with seagrass material. Although litter bags do not allow for natural movement of litter and can prevent macrofaunal influence on decay, they enable precise tracking of seagrass biomass loss and have therefore been used extensively in decomposition studies (Harrison 1989). To replicate natural decomposition
conditions, rhizome/root litter bags were buried under 1 cm of sediment near the natural rhizosphere, and leaf litter bags were kept on top of the sediment. Each incubation container had an area of 225 cm$^2$ with a total sediment depth of 3.5-4.0 cm. Water pumps (600 L hr$^{-1}$) and air stones were used to maintain oxygenated, circulating water in 80 L tanks (100 cm x 50 cm x 17.8 cm).

The temperature and inorganic nutrient treatments were designed orthogonally with four replicates (Fig. S1), with control or ‘ambient’ treatments represented by samples not subject to elevated temperature or nutrient addition. Temperatures were chosen based on current average annual temperatures at Fagans Bay, with an annual mean ambient temperature of 23°C and an approximate mean maximum summer temperature of 30°C (data unpublished). The elevated temperature was chosen based on a recent assessment that predicted current annual high water temperatures in shallow-water estuaries in New South Wales will increase in duration under multiple climate change scenarios (Astles and Loveless 2012). The ambient temperature treatment (23.5°C ± 2°C) was controlled with the test room’s automatic thermostat, while the high temperature treatment (30°C ± 1°C) was maintained with aquatic heaters (Aqua One, NSW Australia). The nutrient treatments were applied by mixing controlled-release fertilizer pellets into the sediments (1 g per L of Grow! Controlled-Release Fertiliser Pellets; 16.1% N as ammonium, nitrate and urea N; 1.2% P as water and citrate soluble P; NSW, Australia), following methods previously used to simulate nutrient enrichment and fertilization within coastal sediments (López et al. 1998, Leoni et al. 2008).

Chemical analyses

Microsensor profiling of sediments

Oxygen, hydrogen sulfide (H$_2$S) and pH microprofiles were measured for leaf detritus and rhizome/root samples and unamended control sediments using electrochemical
microsensors and a motorized setup (Unisense, A/S, Aarhus, Denmark). The profiles were measured within the control sediments (no seagrass) and in the ambient temperature and no added nutrient treatments in the litter bags only due to the substantial time required for each profile to be conducted. The profiling provided information of the oxic or anoxic conditions within the litter bags and how the addition and decomposition of seagrass within sediments influenced microbial metabolism. Oxygen (O$_2$) and hydrogen sulfide (H$_2$S) profiles were measured using Clark-type electrochemical O$_2$ (tip size 100 µm, 0.5% stirring sensitivity, 6 s response time) and H$_2$S (tip size 100 µm, 2% stirring sensitivity, < 10 s response time) microsensors. pH was measured using a pH minisensor (tip size 500 µm, < 10 s response time) with an external reference (Kühl and Jørgensen 1992; Revsbech 1989) (Unisense A/S, Aarhus, Denmark). Each microsensor profile was performed in triplicate except for the leaf H$_2$S profiles due to high risk of breakage of the sensor tip through the litter bag.

For litter bag samples, the profiles were measured through the 6 mm holes in the center of the litter bags, while profiles in control sediments were measured in the center of the sample container. For leaf samples sitting atop the sediment, all three profiles were taken at 1 mm intervals beginning at 3 mm above the litter bag surface and ending at 35 mm (~5-7 mm below the sediment surface). For control sediment and rhizome/root samples, oxygen profiles were taken at 100 µm intervals starting at 500 µm above the sediment surface to an anoxic depth. Data for H$_2$S and pH profiles for rhizome/root and sediment samples were taken at every 1 mm beginning at 3 mm above the sediment surface to 25 mm depth to ensure a complete profile through the bag. Oxygen profiles were adjusted so that the surface of the sediment (0 mm) was directly below the linear section of the profile, representing the diffusion boundary layer (Jørgensen and Revsbech 1985). Triplicate profiles were averaged to obtain a mean profile for each analyte for each sample. The mean profiles were then averaged for the true replicate samples (n=3, control sediment, leaf and rhizome/root).
Total sulfide ($S_{tot}^{2-}$) and average sulfate reduction rates (mmol m$^{-2}$ d$^{-1}$) were calculated based on the retrieved H$_2$S profiles. Total sulfides ($S_{tot}^{2-} = H_2S + HS^- + S^{2-}$) were calculated as per manufacturers guidelines as

$$S_{tot}^{2-} = [H_2S] \times (1 + k_1/H_3O^+),$$

where

$$H_3O^+ = 10^{\text{pH}},$$

$$k_1 = 10^{\text{pk1}},$$

$$\text{pk1} = -98.08 + (5765.4/T) + (15.04555*lnT) + (-0.157*S0.5) + 0.0135*S,$$

and $T$= temperature in Kelvin and $S$= salinity in % (Unisense A/S H2S Manual). Diffusive flux for $S_{tot}^{2-}$ was calculated using Fick’s First Law:

$$\text{flux} = -D \times \text{porosity} \times \Delta C/\Delta T,$$

where $D$ is the diffusion coefficient, porosity = 0.8 following the coastal silt sediment estimates in Bahr et al., (2001), $\Delta C$ is the change in concentration over the linear region of the profile (nmol cm$^{-3}$), and $\Delta T$ is change in distance over the linear section (cm). The diffusion coefficient is calculated based on the temperature and salinity dependent diffusivity of O$_2$.

$D_{oxygen}$, obtained from unisense.com, where

$$D_{H2S} = D_{oxygen} \times 0.7573$$

Average sulfate reduction rates (mmol m$^{-2}$ d$^{-1}$) were calculated per sampling event for each sample type, and were estimated as the sum of the upward and downward sulfide flux for rhizome samples (Kühl and Jørgensen 1992).

For leaf and control sediment samples, only the upward flux was captured in the profiles, and thus these measurements represent minimum estimates. As sulphide may interact with iron to form an iron-sulfide precipitate, which would alter the total flux of free sulphide in the sediment, these measurements represent estimates of sulfate reduction rates only. However, as all sediments were identical at the onset of the experiment, such unquantifiable interactions were assumed to be similar across incubations.
Seagrass chemistry

Destructive sampling of the litter bags occurred at days 2, 7, 14, 28 and 84 (n = 4, total N = 160). After sampling for bacterial analyses (see below), the remaining bulk litter was washed of sediments and weighed before and after oven drying at 60°C. Initial and final dry masses of the seagrass were used to calculate the proportion of dry mass remaining. Decay rates were calculated by fitting the proportion of dry mass remaining to single-component

\[ \frac{W_t}{W_0} = e^{-kt} \]

and double-component

\[ \frac{W_t}{W_0} = \alpha e^{-k_1 t} + (\alpha - 1)e^{-k_2 t} \]

exponential decay models (tolerance = 0.001, 1000 iterations, Dynamic Fit Wizard, Sigma-Plot, Systat Software, Inc., San Jose, CA, USA).

The dried seagrass samples were mixed before grinding a representative subsample into a fine powder (Pulvisette 7, Fritsch, Germany) for elemental and biochemical analyses. Elemental analysis, diffuse reflectance Fourier-transform mid-infrared (FTIR) spectroscopy and solid-state $^{13}$C nuclear magnetic resonance (NMR) spectroscopy were performed to investigate how seagrass organic components changed with decay from day 0 and during early (day 7) and later (day 84) stages of decay (n = 3). A LECO elemental analyser was used to quantify the bulk carbon (C) and nitrogen (N) content of the seagrass tissue (LECO TruSpec, MI, USA). FTIR spectra were attained with the instrumentation described in Baldock et al. (2013a). In total, 60 scans were acquired and averaged to produce a reflectance spectrum for each individual sample, and the Omnic software (Version 8.0; Thermo Fisher Scientific Inc.) was used to convert the acquired reflectance spectra into absorbance spectra (log-transform of the inverse of reflectance). For FTIR data processing, second derivatives
were generated for the spectra using the Savitsky-Golay function with 15 smoothing points, before removing scattering effects using the Standard Normal Variate (SNV) correction algorithm. The spectra were then split into two groups: a calibration set consisting of about two thirds of the data, and a validation set made up of the remaining third. These were then used to build PCA models in order to reveal groupings within the data that would be otherwise hidden within the complex spectra.

Solid-state $^{13}$C-Cross-Polarisation Magic-Angle Spinning (CPMAS) nuclear magnetic resonance (NMR) spectra were acquired for one representative sample of each tissue type per treatment for each day, selected by applying a Kennard-Stone algorithm to the FTIR spectra acquired for all samples (Baldock et al. 2013b). All NMR spectra were acquired according to Baldock et al. (2013a). The acquired total signal intensity was divided into a series of chemical shift regions: amide/carboxyl/ketone (215–165 ppm), O-aromatic (165–145 ppm), aromatic (145–110 ppm), di-O-alkyl (110–95 ppm), O-alkyl (95–60 ppm), N-alkyl/methoxy (60–45 ppm), and alkyl (45 to 10 ppm). The loss of C-containing compounds over the decomposition process was assessed based on changes in the distribution of NMR signal intensity (Smernik and Oades 2000a; Smernik and Oades 2000b). First, the differences between the initial and final spectra were calculated based on C observed by the NMR. These ‘loss spectra’ were then normalized to the biomass loss and the elemental C loss experienced between the initial and final samples during decomposition. The signal intensity associated with the resulting loss spectra was integrated across the spectral regions, and then entered in a molecular mixing model for terrestrial soils to predict molecular composition (Baldock et al. 2004). The original terrestrial protein component of the mixing model was altered to be reflective of the amino acid composition of seagrasses using data from *Posidonia australis*, *Thalassia testudinum*, *Halodule wrightii* and *Syringodium filiforme* (Torbatinejad et al. 2007; Zieman et al. 1984). Preliminary tests indicated that the 5-component molecular mixing
model including contributions from carbohydrates, lignin, protein, lipids and carbonyls had the best fit between measured and predicted composition for both tissue types (root mean squared error ≤ 1.31), and therefore was used for all samples.

Microbial community analyses

Samples for microbial abundance and community characterization were subsampled immediately after litter bags were removed from the incubation. Abundance analyses were performed for days 0, 7, 28 and 84 (n = 4), and community characterization were performed for days 0, 7 and 84 (n = 3). Seagrass litter was sampled by cutting 1-2 cm sections from the bulk sample, and sediments (a reference for bacterial communities on the seagrass) were sampled from the top 1 cm of sediment as a microbial community reference using a 3 mL cut-off syringe. Seagrass litter for bacterial enumeration using flow cytometry were sampled in the same manner then fixed in 0.01% glutaraldehyde. All bacterial samples were snap frozen in liquid N₂ and stored at -80°C before processing. Quantification of bacteria cells by flow cytometry followed Trevathan-Tackett et al. (2014).

Genomic DNA was extracted from the seagrass and sediment samples using the PowerSoil DNA Isolation Kit (MoBio Technologies, CA, USA). Extracted DNA was amplified using the V4 variable regions of 16S rDNA gene with the bacterial primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3’) and 806R (5′GGACTACVSGGGTATCTAAT-3’) (Caporaso et al. 2011) prior to sequencing using the Illumina MiSeq platform following the manufacturer’s guidelines (www.mrdnalab.com, TX, USA). Sequence data was analyzed using the QIIME package (Caporaso et al. 2010). Following de-multiplexing, barcodes were removed and sequences shorter than 200 bp, containing ambiguous base calls (Ns) and bases with a quality score < Q20 were removed. Paired ends were joined using fastq-join (Aronesty 2011), and operational taxonomic units (OTUs) were defined de novo as sequences sharing
97% similarity using UCLUST (Edgar 2010). Representative sequences of each OTU were compared against the Greengenes database (DeSantis et al. 2006) using BLAST (E< 0.001) (Altschul et al. 1997) to assign taxonomy. Chimeras were removed using ChimeraSlayer (Haas et al. 2011). To ensure even sampling depth, the samples were rarefied to 3795 sequences, which represented the minimum count per sample and 100% of the sampling effort. Weighted UNIFRAC was performed to take into account community differences associated with different lineages in a phylogenetic tree (Lozupone and Knight 2005). QIIME was also used to calculate the alpha diversity indices Chao1 and Simpson’s evenness on de novo-picked genus-level taxa (Caporaso et al. 2010). Raw sequences can be found on the ENA database under project PRJEB18754. An additional set of OTUs were defined ‘closed-reference’ against Greengenes (97%) and analyzed using the PICRUSt package to predict bacterial functional capacity from the 16S rDNA profiles (Langille et al. 2013). Before rarefication at 2706 sequences, OTUs were normalized by copy number, and functions were predicted for Level 2 and 3 KEGG pathways.

Statistical analysis

A 2-way Analysis of Covariance (ANCOVA) was used to analyze the effects of temperature and nutrient addition (independent variables) on biomass loss, elemental content, bacterial abundance and bacterial alpha diversity (dependent variables) with time as a covariate. A Sidak test was used for post-hoc analysis. A 1-way ANOVA was used to analyze the differences in sulfate reduction rates over the course of the experiment. As initial seagrass samples did not receive temperature nor nutrient manipulation, they were considered a separate treatment. All main and interaction effects with \( \alpha < 0.05 \) were considered statistically significant. Samples were log-transformed to achieve homogeneity of variance where required. ANCOVA and ANOVA analyses were performed using SPSS (v19, IBM).
Multivariate statistics were used to statistically analyze the weighted UNIFRAC bacterial taxa using Primer (version 6.1.11, Primer-E; Clarke and Gorley, 2006). Bacterial OTU data were square-root transformed prior to creating a Bray-Curtis similarity resemblance matrix. Data were visualised using multidimensional scaling (MDS). Analysis of Similarities (ANOSIM) was applied to determine if differences between treatments were significantly different from a randomly permuted distribution. Similarity percentage analysis (SIMPER), with 90% cut off for low contributions, was used to identify the bacterial taxa driving the shift between the clusters observed in the MDS plots. The predicted PICRUST metagenome functions were analyzed using the Statistical Analysis of Metagenomic Profiles (STAMP) program (Parks et al. 2014). All STAMP analyses were performed with a two-sided Welch’s t-test and Bonferroni correction to minimize Type I errors.

**Results and Discussion**

No effect of nutrients

Both seagrass macromolecular chemistry and the abundance and composition of the associated microbial communities changed significantly throughout the three month study and were primarily influenced by tissue type, time and temperature (ANCOVA and ANOSIM Statistics Supplementary Tables S1 and S2). However, the effects of added nutrients on both seagrass decay and microbial community dynamics were minimal. Specifically, the addition of nutrient pellets increased nutrient concentrations in the porewater by 1.3-1.5-fold for NH₄ and 1.8-4.4-fold for PO₄ after one week. The nutrient addition sediments maintained higher porewater nutrient levels throughout the experiment, except for PO₄ in the sediments of leaf detritus and rhizome/root samples. These PO₄ concentrations declined to control levels for the 23°C treatment by day 84. Despite the increases in sediment porewater nutrient
concentrations, the effects of elevated nutrients were limited to an increase in rhizome/root %N (pairwise tests, p = 0.022) and a slight increase in rhizome/root-associated bacterial abundance (pairwise tests, p = 0.014).

In contrast to our hypothesis that nutrient inputs will increase microbial remineralization of seagrass, the addition of inorganic nutrients to the sediment did not influence seagrass decay. Mixed conclusions about the influence of exogenous nutrients on seagrass decomposition have been derived from previous studies. In a temperate *Posidonia oceanica* meadow, nutrient addition led to an increase in sediment bacterial activity and a reduction in sediment organic matter content (López et al. 1998). López et al. (1998) suggested a direct relationship between inorganic nutrient availability and C metabolism by bacteria. However, another study that tracked sediment C and N cycling during 33 days of *Zostera marina* decomposition showed that bacteria utilized N from the surrounding sediments, rather than seagrass-derived N, while breaking down seagrass C-based molecules (Pedersen et al. 1999). The unamended sediments in this study had a comparatively low C:N ratio of 7.52 ± 0.48, however not abnormal for terrigenous sediments rich in clays and silts (Holmer et al. 2004; Kennedy et al. 2010). It is likely that the bacteria had sufficient access to N in the sediments or porewater to supplement the breakdown of seagrass C in this short-term experiment (Kenworthy and Thayer 1984). This scenario would explain the lack of response among most of the seagrass biochemical and microbial variables after nutrient addition for both leaf and rhizome/root decomposition. As such, our results are supported by the hypothesis for apparent or negative priming effect, which states that under non-nutrient-limiting conditions, the addition of nutrients will have little impact on the magnitude of microbial priming and microbial metabolism (Guenet et al. 2010; Kuzyakov et al. 2000). Since nutrient addition had minimal effect on seagrass decomposition, we henceforth primarily focus on the influence of tissue type and temperature on seagrass decay.
Sediment chemistry

Microsensor profiles of the control treatments (23°C, no nutrients added) illustrated that leaf detritus decomposition occurred under oxic conditions above the sediment surface, where oxygen levels were consistently higher than 50 µM (Fig. 1E). Conversely, rhizome/root samples were buried in highly reduced, anoxic sediments with oxygen penetrating no deeper than 2 mm and never reaching the seagrass samples (Figs. 1C, D). The maximum total sulfide concentrations always occurred within the buried rhizome/root litter bag and peaked at day 6 (Fig. 1D). Estimated sulfate reduction rates in rhizome/root samples, calculated from the slopes of the sulfide profiles, reached a maximum rate of 50 mmol m⁻² d⁻¹ during the first week of decomposition followed by a decrease to ~22 mmol m⁻² d⁻¹ in the final weeks. This rapid peak in sulfide followed by a consistent decline thereafter is likely due to a combination of processes: a) the rapid use of labile substrate leaving behind ‘non-competitive’ substrates that sulfate reducers are unable to utilize (Capone and Kiene 1988), and b) a finite source of sulfate within the mesocosm sediment, limiting electron donor availability and allowing other anaerobic metabolic pathways to be used (Arndt et al. 2013).

Microbial remineralization of seagrass through time

Comparison between substrates

Typical plant decomposition occurs in three stages. The first is the passive leaching of soluble compounds that benefits fast-growing r-strategist microbes, or copiotrophs, capable of rapidly decomposing labile C (Berg and McClaugthery 2008; Fierer et al. 2007). Subsequently, active microbial decomposition of the plant biomass typically occurs at a slower rate, which is finally followed by the very slow rate of microbial breakdown of refractory compounds, or the refractory stage. These latter two stages are dominated by
slower-growing \textit{K}-strategists, or oligotrophs, that can breakdown more refractory compounds under low-nutrient conditions, including hemicelluloses, cellulose and lignin (Fierer et al. 2007; Sinsabaugh et al. 2002).

In this study, each tissue type underwent relatively distinct decomposition patterns during the three-month incubation (Fig. 2). In general FTIR and elemental analyses showed that the leaf detritus contained higher N, protein (as amide content) and sucrose than rhizome/root tissues (Figs. 3 and S3). Rhizome/root tissues had higher carbohydrate content, as glucose and cellulose, than the leaf detritus throughout the decomposition process (Fig. 3). A single exponential decay model described leaf detritus decomposition better than the double-component model (Adj. $R^2 > 0.95$ vs. Adj. $R^2 = 0.80$-$0.86$). Conversely, the single exponential decay rate model fit poorly for the rhizome/root samples due to the large initial biomass in the first two days (~50\% DW; Adj. $R^2 = 0.03$-$0.13$). The double-component decay model had a better fit than the single-component model for rhizome/root tissues, though still weak, and predicted high k-values for both components (Fig. 2). The decay rates for both tissue types were within the ranges previously reported for seagrass detritus ($k = 0.001$-$0.110$ d$^{-1}$; Enríquez et al. 1993; Harrison 1989), although, leaf detritus decay was slower than previously shown for fresh \textit{Zostera} over similar time periods ($k = 0.011$-$0.019$ d$^{-1}$; Nicastro et al. 2012; Bourguès et al. 1996). This is potentially a result of using dead leaf detritus for the starting material in this study. Dead seagrass leaves typically have less soluble components and organic matter than green leaves due to the leaching process having already occurred (Harrison 1989). When the rapid biomass loss from the leaching phase is not a component of decay rates, the resulting decay constant k-values are lower than fresh biomass k-values (Harrison and Mann 1975).

The initial (day 0) bacterial community composition did not significantly differ between the leaf detritus and rhizome/root litter ($p = 0.10$), likely as a result of washing and
freezing preparations prior to the start of the experiment. However, during the first week of decomposition, pairwise analyses of the OTUs indicated that significantly different community signatures formed between leaf detritus and rhizome/root samples (p = 0.010; Fig. 4). During the same time-frame, the number of bacterial cells colonizing the seagrass rapidly increased in abundance by 4-5-fold and 6-7-fold in the leaf detritus and rhizome/root samples, respectively (Fig. S5). There was also a 2-fold increase in bacterial community evenness in the first week for both tissue types and a 2-fold increase in richness for the rhizome/root samples (Fig. S6). Subsequent to the first week, the bacterial communities became increasingly distinct between the leaf detritus and rhizome/root substrates, with both communities also significantly shifting in composition between day 7 and day 84 of decomposition (p = 0.004; Fig. 4).

SIMPER analyses revealed which taxa contributed to the dissimilarity between the microbial assemblages (Table 2, Figs. 5 and S7). Sediment bacterial communities remained relatively stable during the course of the experiment, with δ- and γ-proteobacteria making up ~40% of the population (Figs. 5C and S7C). Both initial leaf detritus and rhizome/root litter were dominated by γ-proteobacteria, with the *Vibrio* genus comprising the largest fraction of the group. Over the course of the experiment, however, the abundance of γ-proteobacteria consistently declined, although the abundance of taxa belonging to the *Alteromonadaceae* family increased between day 0 and 7 for leaf detritus samples (Fig. 5). During the same period, aerobic α-proteobacteria, mainly including members of the *Erythrobacter* and *Rhodobacteraceae*, were dominated the community on the leaf detritus samples (Figs. 5A and S7A). Conversely, rhizome/root tissues became dominated by anaerobic bacteria including *Clostridia, Bacteroidia* and the δ-proteobacteria, which were primarily composed of *Desulfovibrio* and *Desulfobacteraceae* (Figs. 5B and S7B). Lastly, PICRUSt analysis was used to predict the functional capacity of the bacterial communities from the Kyoto
Encyclopedia of Genes and Genomes (KEGG) Pathway Database via the KEGG Orthologies based on the metagenomic data of closely-related taxa (Langille et al. 2013). Leaf detritus-associated bacterial communities showed over-representation in several KEGG categories relative to rhizome/root samples. These included aromatic and non-aromatic amino acid metabolism and xenobiotics metabolism (Fig. S8A), suggesting a greater capacity of bacteria associated with the rhizome/roots to degradation of aromatic compounds than those associated with leaf detritus.

Microbial remineralization leaf detritus

During the three months of monitored decomposition, leaf detritus decay included the loss of lipids and proteins (Table 1, Fig. 3), but was primarily dominated by loss of carbohydrates, which resembled cellulose (Kobayashi et al. 2011) in the $^{13}$C-NMR ‘loss spectra’, i.e. the spectra of the compounds lost during decomposition (Fig. S4A; raw spectral intensities in Table S3). The microbial communities associated with leaf detritus also shifted with decomposition. Despite the absence of a significant leaching phase, leaf detritus supported high abundances of putative $r$-strategists, including members of the $\alpha$-proteobacteria and several copiotrophic groups within $\gamma$-proteobacteria, i.e. Alteromonadaceae, Vibrio sp. (Table 2) (Lauro et al. 2009; López-Pérez et al. 2012; Nelson and Carlson 2012; Pedler et al. 2014), suggesting relatively active decomposition of the leaf detritus.

Over the three-month period of decomposition, there were increases in $\alpha$-proteobacteria, including members of the Rhizobiales and Hyphomicrobiaceae, as well as Actinobacteria, Myxococcales and Cytophagia (Table 2, Figs. 4A and S7A). The increased abundances of known $K$-strategists, such as Cytophagia and Actinobacteria, suggest the breakdown of more complex organic matter (Pasti et al. 1990; Rosselló-Mora et al. 1999).
During the course of the experiment, leaf detritus bacteria also showed an increased capacity for anaerobic energy metabolism (Fig. S8B), including sulfur and methane metabolism. Despite oxygen profiles showing that the leaf detritus litter bags were generally oxic throughout the experiment, the observed increases in anaerobic metabolism could be a result of an increasing presence of anoxic microniches within the bags, resulting from the lowered oxygen concentrations and collapse of the leaf detritus during degradation. Under field conditions, sedimentation rate and burial would likely influence both microbial functional groups and decomposition patterns. Taking into account that these conditions were not incorporated in this experimental design, the C remineralization and microbial community data suggest that the primarily aerobic leaf degradation was occurring during the second, active decomposition phase. Additionally, the evidence for shifting microbial communities described above suggests that there may a transition to the slower refractory phase occurring as labile compounds become depleted or the microbial populations lack the capacity to degrade the leaf biomass further.

**Microbial remineralization of rhizome/root tissue**

The rhizome/root decomposition process captured both leaching and microbial remineralization phases of decomposition. The leaching phase resulted in the rapid loss of up to 50% of the rhizome/root biomass during the first two days of the study (Fig. 2). While this degradation was faster than what is typically reported for belowground biomass (4 days - 2 weeks; Josselyn et al. 1986; Vichkovitten and Holmer 2004), rhizome/roots from the smaller seagrass *Halophila* have been shown to lose ~ 70% wet weight after 4 days under anoxic conditions (Josselyn et al. 1986). According to the molecular mixing model derived from the NMR spectra, the leachate mass consisted of approximately 69% carbohydrates, 13% lignin, 8% protein, 7% carbonyls and 3% lipids. This is typical of seagrass leachate, including
carbohydrates and amino acids (Maie et al. 2006; Torbatinejad et al. 2007; Vichkovitten and Holmer 2004). The loss of leachate was matched by an increased capacity of the bacteria to degrade starch, sucrose and amino sugars, as shown from the predicative function PICRUSt analyses (Fig. S8C). The rhizome/root-associated bacterial communities also displayed an over-representation of genes involved in several metabolic pathways including glycan, and nitrogen and methane metabolism (i.e. energy metabolism in Fig. S8A). This period of leaching also supported high rates of sulfate reduction, similar to rates reported near seagrass rhizospheres (Marbà et al. 2006), as well as an increase in both bacterial abundances (Fig. S5) and diversity (Fig. S6). In addition to sulfate reduction, several taxa that were abundant during the leaching stage were from the γ-proteobacteria and putative anaerobic bacteria and fermenters in *Clostridiales* and δ-proteobacteria, suggesting these taxa were competitive for leachate metabolism.

After the leaching phase, the microbial remineralization phase between days 2 and 84 were similar to decay patterns of other rhizome/root decomposition studies (0.0022-0.0031 d⁻¹; Fig. 2; Fourquarean and Schrlau 2003; Kenworthy and Thayer 1984). During the same time period, there was an increase in %N (Fig. S3) and a loss of carbohydrates (Table 1, Fig. 3), which closely resembled cellulose according to the NMR loss spectra (Fig. S4B; Kobayashi et al. 2011; raw spectral intensities in Table S3). This data suggest that once labile compounds are consumed, the active bacterial decomposition of seagrass belowground tissues occurs at a relatively consistent rate under anoxic conditions. After three months, there was a marked increase in *Spirochaetes* and δ-proteobacteria (Table 2, Figs. 5B and S7B). Among the δ-proteobacteria, there was a large decline in *Desulfovibrionaceae* between days 7 and 84 (~65% to 20%) with a concomitant diversification among other sulfate reducers (Fig. S7B). Additionally, genes involved in lipid biosynthesis and the production of other secondary metabolites, including some antibiotics and flavonoids, were significantly
over-represented in the PICRUSt analysis (Fig. S8D). These results suggest that the later stage of decomposition had a strong shift toward other \( K \)-strategist anaerobes like \textit{Bacteroidales, Spirochaetes, Desulfosarcina}, which have been linked to cell wall degradation in both saltmarsh (Darjany et al. 2014) and seagrass ecosystems (Roth and Hayasaka 1984). While the abundance of sulfate-reducing taxa were still dominant on the rhizome/root tissues, the decrease in sulfate reductions rates suggest that either the anaerobe metabolic efficiency was reduced because of limited sulfate electron donors or the compounds remaining were not usable by the sulfate reduction pathway (Arndt et al. 2013). The breakdown of carbohydrates, primarily as cellulose, and relative increase in elemental N, as observed in the rhizome/root incubations, is characteristic for preferential consumption of non-N compounds in seagrass detritus (Harrison 1989). The increased degradation of the cellulose component of lignocellulose in combination with the presence of select bacteria capable of this kind of enzymatic breakdown indicates that decomposition was occurring the later stages of the study (day 84). Based on previous coastal biogeochemical studies, we would expect that preferential lignin degradation by bacteria, could occur during future stages of remineralization beyond the timeframe of this study (i.e. > 3 months; Benner et al. 1987; Benner et al. 1986). While \textit{Actinobacteria} and the Proteobacteria seem to be key groups involved in lignin degradation (Brown and Chang 2014; Kameshwar and Qin 2016), the taxa involved in anaerobic degradation of lignin in natural coastal sediments are yet to be identified.

Effect of temperature and oxygen availability on seagrass remineralization

Under elevated temperature, the fitted decomposition rate constant associated with leaf detritus decay was nearly double that obtained for ambient temperature (\( k = 0.0071 \) vs. 0.0038 \( d^{-1} \), respectively; Fig. 2). This enhanced biomass loss coincided with significant loss
in elemental N and increased C:N ratio (Fig. S3), as well as a larger loss of lignin (4.6%), lipids (3.0%) and carbonyls (2.8%; Table 1). Treatments exposed to elevated temperatures showed no significant change in bacterial abundance (Fig. S5), but did lead to increased community evenness (Fig. S6B). Additionally, elevated temperatures led to a significant shift in community composition at the OTU-level (p = 0.001; Fig. 4). Specifically, with the 30°C treatment characterized by an increase in the abundance of *Erythrobacter*, *Marinobacter* and *Clostridia* during the first week. After three months, increased temperatures promoted higher abundances of leaf-associated ammonia-oxidizing Archaea *Nitrosopumilus*, purple non-sulfur bacteria from the *Rhodospiralles* and *Rhizobiales*, but also a decline in some α-proteobacteria including *Loktanella* and N-fixing *Phyllobacteriaceae* (Figs. 5A and S7A).

In contrast to leaf detritus decomposition, the rhizome/root tissue samples exposed to elevated temperature did not significantly differ in biomass loss, elemental and macromolecular content, bacterial abundance or predicted community function compared to control temperatures (Table 1, Figs. 2, S4B, S5 and S6B). However in comparison, elevated temperatures did cause a significant shift in the microbial communities at the OTU-level (p = 0.001; Fig. 4). These shifts were driven by an increase in abundances of already dominant anaerobic classes, such as *Clostridia*, *Bacteroidia* and *Fusibacteria* during the first week, and δ-proteobacteria by day 84 (Fig. 5B). While the δ-proteobacteria assemblages were nearly identical between temperature treatments in the first week, by the end of the experiment higher temperatures promoted an increase in sulfate reducers including *Desulfobacteraceae* and *Desulfarculaceae* at the expense of other groups including *Sulfurimonas* and *Bacteroidia* (Table 2, Figs. 5B and S7B).

Evidence for a temperature-oxygen interaction
Our goal was to assess the effects of elevated seawater temperature and nutrient additions on bacterial remineralization of seagrass biomass to determine how these parameters may ultimately affect C cycling and sequestration processes in coastal ecosystems. Microbial priming, defined as the enhanced degradation of C in response to additional nutrient inputs (Kuzyakov et al. 2000), was not observed as a response to inorganic nutrient additions in this experiment. We did detect a significant enhancement of microbial remineralization of seagrass leaf C under elevated temperatures. This temperature effect was evidenced by ~20% great leaf detritus biomass loss and shifts in bacterial communities, summarized in a conceptual design (Fig. 6). However, the temperature-induced enhancement of remineralization only occurred under oxic conditions suggesting the existence of an important oxygen-temperature interaction involved in C remineralization in seagrass habitats.

It is predicted that climate change induced warming will increase the magnitude and duration of periods of elevated temperature within shallow estuarine habitats (Astles and Loveless 2012). We showed that a 7°C increase in mean water temperature over three months resulted in a near doubling of the aerobic decay rate constants of leaf detritus samples (Figs. 2 and 6). This temperature-enhanced rate of seagrass decomposition has been demonstrated previously where Zostera marina leaves experienced enhanced biomass loss and decline in fiber content (hemicellulose, cellulose, lignin) over 90 days of aerobic decomposition at elevated temperatures (10°C vs. 25°C) (Godshalk and Wetzel 1978). In the current study, elevated temperatures led to a decline in carbonyls, which further indicated enhanced decomposition via the oxidation of the carbohydrate, lipid and protein components (Baldock et al. 2004). According to the mixing model, elevated temperatures also resulted in the loss of lignin from the leaf detritus (Table 1). However, the low aromatic signals in the calculated ‘loss spectra’ (Table S3, Fig. S4) suggested the predicted amount of lignin may be an overestimation and needs to be interpreted with caution since the mixing model was initially
designed for soil (Baldock et al. 2004). Nonetheless, the relative increase in aromatic lignin degradation under elevated temperature coincided with evidence of increased functional capacity for bacterial metabolism of aromatics in leaf detritus samples, including the metabolism by the enzyme cytochrome P450 (Fig. S8A). Notably, this enzyme has been hypothesized to be a potential pathway for bacterial lignin degradation (Brown and Chang 2014). Furthermore, previous studies using compound-specific $^{14}$C-tracing techniques have provided evidence that bacterial communities can degrade lignocellulose aerobically in other blue C habitats within a timeframe similar to that of this study (Benner and Hodson 1985; Benner et al. 1984; Benner et al. 1986). These data suggest that there is the potential for the microbial enzymatic degradation of lignocellulose in these coastal sediments, especially under oxic conditions. Further research using analyses such as enzymatic assays and metatranscriptomics would enhance our understanding of the detailed mechanisms behind the bacterial breakdown of plant-based refractory C in the blue C ecosystems.

The lack of a temperature effect on rhizome/root decomposition may have been, in part, due to the greater structural rigidity of the rhizome/roots. In a previous study, it was shown that microbes preferentially utilize the labile storage components in the center of the rhizome (Kenworthy and Thayer 1984). It was only after 170-250 days of decomposition when bacteria began to degrade the outer, more structurally rigid rhizome walls as evidenced by the remaining hollowed rhizome (Kenworthy and Thayer 1984). Based on this observation, we suggest that the bacteria had ample resources and were not limited by the rhizome/root’s rigidity in the relatively short time frame of this study. Alternatively, we hypothesize that the differences in temperature response between tissue types is related to the availability of oxygen during microbial degradation, whereby seagrass leaf detritus decomposed under oxic conditions and belowground tissues under anoxic conditions. These dissimilar conditions also led to the development of different microbial assemblages (Table 2,
Fig. 5). This difference was likely related to the availability of powerful oxygen-dependent enzymes capable of degrading structural carbon as well as related to the slower, less efficient metabolic capacity to hydrolyze organic matter anaerobically (Crump and Koch 2008; Fuchs et al. 2011; Kristensen et al. 1995) under differential temperature regimes.

The interaction between oxygen and temperature observed in this study has not been thoroughly investigated in coastal ecosystems. As such, only one previous study has investigated this relationship in seagrass sediments, and similarly found that effects of elevated temperatures on seagrass remineralization were negligible under anaerobic conditions compared to aerobic conditions (Godshalk and Wetzel 1978). While the mechanism behind these results was not investigated, Pedersen et al. (2011) showed that anaerobic remineralization of a seagrass matte increased with increasing temperature up to 25°C. Afterwards, remineralization rates declined due to limited metabolism and rate of acclimation of slower-growing taxa (e.g. K-strategist microbes) at higher temperatures (Pedersen et al. 2011). The presence of certain bacterial functional groups and the substrate available for remineralization have been shown to be linked to temperature-limited anaerobic remineralization (Roussel et al. 2015; Weston and Joye 2005). Weston and Joye (2005) showed that sulfate reducers, a dominant functional group in this study and marine sediments globally, were highly sensitive to temperature shifts. Specifically, labile C remineralization was elevated at temperatures >25°C initially, but was subsequently resource limited as labile substrates became deplete (Weston and Joye 2005). In addition, increased temperatures of anoxic marine sediments led to a shift in bacterial groups from sulfate reducers, which dominated at < 30-35°C, to fermenters, which were dominant at > 35°C (Roussel et al. 2015). Here, we found no such fermenter-to-sulfate reducer shift in the community. This suggests that the sulfate reducers were still able to out-compete other anaerobic functional groups at elevated temperature, despite not being able to remineralize lignocellulose more efficiently.
under those conditions. In either case of enzymatic or growth rate limitations, anoxic conditions may be providing some ‘protection’ from C remineralization in coastal sediments under elevated temperatures.

The implications of this oxygen-temperature relationship are significant for future C stocks in blue C habitats. On one hand, enhanced aerobic microbial remineralization under elevated temperatures will have negative effects on C sequestrations rates (1) through reduced C accumulation rates as fresh C is more rapidly remineralized at the sediment surface, and (2) in disturbance scenarios, such as bioturbation, dredging and boat damage, whereby previously anoxic pools of stored C are exposed to oxic conditions, which could amplify the losses of sediment C stocks. Yet, the anoxic sediment conditions, which begin a few millimeters below the sediment surface, are fundamental and ubiquitous to blue C ecosystems. In undisturbed blue C ecosystems, the reduced remineralization capacity of bacterial communities under anoxic conditions may provide a degree of C protection under warming seawater temperatures, giving these systems a sequestering advantage over ecosystems with predominantly oxic soils or sediments. Here, we demonstrated that substrate quality, bacterial community composition and environmental conditions strongly influence the C remineralization in seagrass ecosystems. These variables likewise control the quantity and quality of recalcitrant seagrass C available for future sequestration. While there is much more to investigate around the biological and physical dynamics of recalcitrant C remineralization, the novel evidence of a temperature-oxygen interaction during the microbial remineralization process necessitates future research on the potential power of disturbance and oxygen availability to enhance or damage blue C sequestration and storage.

Acknowledgements
We would like to thank Stacey Ong-Benyamin, Randy Tackett and all the volunteers involved in this project. We want to acknowledge the contributions of Janine McGowan and Bruce Hawke for providing training and assistance in the acquisition and processing of NMR and FTIR spectra. This study was financially supported by the Climate Change Cluster at UTS and the CSIRO Marine and Coastal Carbon Biogeochemistry Cluster. PM was supported by an Australian Research Council DECRA Fellowship (DE130101084). JRS was supported by Australian Research Council Future Fellowship (FT130100218).

Author Contributions

STT, PR, ADLS, DN, JRS and PM conceived the project and experimental design. STT and DN ran the project, and analysed and interpreted the microsensor data. STT, JS, JB and JH analysed and interpreted the FTIR and NMR data. STT, JRS, and TCJ analysed and interpreted the microbial abundance and omics data. STT wrote the manuscript, and all authors took part in editing and commenting on the manuscript.

The authors declare no conflict of interest.

Supporting information is available at FEM Microbiology Ecology’s website.
Literature Cited


Blum LK, Mills AL. Microbial growth and activity during the initial stages of seagrass decomposition. *Marine Ecology Progress Series* 1991;70: 73-82.


Stocker T, Qin D, Plattner G et al. IPCC, 2013: climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. 2013.


Figure 1: Oxygen and total sulfide microsensor profiles for 23°C, no nutrient treatments. (A-B) Control, sediment samples, (C-D) rhizome/root samples, and (E-F) leaf samples. Boxes indicated the location of the litter bags for leaf and for rhizome/root samples. Zero on the y-axis represents the sediment surface for control and rhizome/root samples and the litter bag surface for leaf samples. Note the y-axis for (A) and (C) focus only on the top 3 mm of the sediment surface. Values represent mean ± S.E.M (n = 3).

Figure 2: Seagrass mass loss throughout decomposition. Decay rates were calculated using a single exponential decay function. Data represent mean ± S.E.M (n = 4).
Figure 3: Fourier transform (diffuse reflective mid-) infrared spectroscopy. (A) Principal components analysis with PC1 (59%) representing differences among tissue types and PC2 (9%) representing shifts through time. (B) Spectra loadings based on PCA scores. Positive PC1 regression coefficients (black) correspond with greater signals in leaf tissues and negative coefficients for rhizome/root tissues. Positive PC2 regression coefficients (grey) correspond with greater signals in late stages of decay (day 28, day 84) and negative coefficients for early stages of decay (day 0, day 7).
Figure 4: MDS plot of bacterial OTUs by tissue, time and temperature. The communities at day 0 were not significantly different between tissue types. Leaf detritus = triangle, Rhizome/root = circles, Sediment = diamonds, day 0 = crosses for leaf (x) and rhizome/root (+), day 7 = grey, day 84 = black, 23°C = open symbols, 30°C = closed symbols.
Figure 5: Taxonomic shifts of bacterial community at class-level related to decomposition time and temperature. Proportions of each class represents average (n = 3) of each taxa for (A) leaf detritus, (B) rhizome/root tissue and (C) bulk sediment. Parentheses indicated suggested taxonomic assignment by Greengenes. Symbols represent taxa contributing to top 5% dissimilarity in SIMPER analysis between leaf detritus and rhizome/root tissue types (*) and through time for leaf (†) and rhizome/root (‡) samples.

Figure 6: Summary of microbial decomposition of seagrass leaf detritus and rhizome/root tissues. (A) Aerobic remineralization of leaf detritus with the variables affected by elevated temperature highlighted in red. (B) Anaerobic remineralization of rhizome/root tissues unaffected by elevated temperature. Selected relative transitions in seagrass chemistry and microbial communities are shown for early (day 7) and later (day 84) seagrass decomposition. Figure icons courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).
Table 1: Percent of macromolecular compound losses from decaying seagrasses predicted from $^{13}$C-CPMAS NMR spectra between day 0 and day 84 (i.e., net changes) and between day 28 and day 84 (i.e., progressed decomposition) at both temperature treatments. Data represent means (n = 2).

<table>
<thead>
<tr>
<th></th>
<th>Leaf Detritus, 23°C</th>
<th>Leaf Detritus, 30°C</th>
<th>Rhizome/Root, 23°C</th>
<th>Rhizome/Root, 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>81.7</td>
<td>69.8</td>
<td>74.5</td>
<td>71.1</td>
</tr>
<tr>
<td>Protein</td>
<td>2.26</td>
<td>3.76</td>
<td>6.77</td>
<td>8.02</td>
</tr>
<tr>
<td>Lignin</td>
<td>7.29</td>
<td>11.9</td>
<td>10.3</td>
<td>11.5</td>
</tr>
<tr>
<td>Lipid</td>
<td>2.90</td>
<td>5.83</td>
<td>1.97</td>
<td>2.42</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>5.89</td>
<td>8.64</td>
<td>6.46</td>
<td>7.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Leaf Detritus, 23°C</th>
<th>Leaf Detritus, 30°C</th>
<th>Rhizome/Root, 23°C</th>
<th>Rhizome/Root, 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>69.1</td>
<td>64.4</td>
<td>95.2</td>
<td>100</td>
</tr>
<tr>
<td>Protein</td>
<td>5.25</td>
<td>3.97</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lignin</td>
<td>14.4</td>
<td>16.1</td>
<td>2.18</td>
<td>0</td>
</tr>
<tr>
<td>Lipid</td>
<td>2.65</td>
<td>3.99</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>8.66</td>
<td>11.5</td>
<td>2.56</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2: SIMPER table for taxonomic succession changes during seagrass decomposition at 23 °C and 30 °C. Shifts in succession are indicated by early (E) and later (L) based on the top 10 families driving the dissimilarities between day 7 and day 84, respectively. If family-level taxa were unknown, next taxonomic level were presented.

<table>
<thead>
<tr>
<th></th>
<th>Leaf</th>
<th>23 °C</th>
<th>30 °C</th>
<th>Rhizome/Root</th>
<th>23 °C</th>
<th>30 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-proteobacteria (Class)</td>
<td>L Acidaminobacteraceae</td>
<td>E</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alteromonadaceae</td>
<td>E Bacteroidales (Order)</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cenarchaeaceae</td>
<td>L Campylobacteraceae</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrobacteraceae</td>
<td>E E Desulfaturaceae</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavmeovirgaceae</td>
<td>L L Desulfobacteraceae</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavobacteriaceae</td>
<td>E E Fusobacteriaceae</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemm-2 (Class)</td>
<td>L Fusobacteriales (Order)</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haliangiaceae</td>
<td>L γ-proteobacteria (Class)</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marinicellaceae</td>
<td>L Lachnospiraceae</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oceanospirillaceae</td>
<td>E E Mollicutes (Class)</td>
<td>L</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piscirickettsiaceae</td>
<td>L Moraxellaceae</td>
<td>E</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudoalteromonadaceae</td>
<td>E Parvarchae (Class)</td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychromonadaceae</td>
<td>E E Phycisphaerae (Class)</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodobacteraceae</td>
<td>E Poribacteria (Phylum)</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shewanellaceae</td>
<td>E Pseudoalteromonadaceae</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sva0725 (Acidobacteria Phylum)</td>
<td>L L Psychromonadaceae</td>
<td>E</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrionaceae</td>
<td>E E Spirochaetaceae</td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wb1_P06 (Acidimicrobiales Class)</td>
<td>L Vibrionaceae</td>
<td>E</td>
<td>E</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>