# **Dynamics of refractory carbon in seagrass meadows**

Stacey Marie Trevathan-Tackett

PhD by Research

University of Technology Sydney

2016

## **Certificate of Original Authorship**

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student:

Production Note: Signature removed prior to publication.

Date: 21 April 2016

## Acknowledgements

Thank you to my supervisors, Peter Ralph and Peter Macreadie, and the many coauthors, Justin Seymour, Tom Jeffries, Daniel Nielsen, Jeff Baldock, Jon Sanderman, Joh Howes, Alex Thomson, Bojana Manojlovic, Caitlin Wessel, Just Cebrián and Andy Steven, for your time, training, guidance and critiques that helped make these chapters into top-notch pieces of science. Thank you to all my PhD mates who supported me during the past four years and never failed to remind me there is a world outside the lab. Lastly, this thesis would also not have been accomplished without my personal graphic designer, fieldwork assistant and comedian, Randy Tackett.

## Preface

This thesis has been prepared in publication format, whereby each chapter represents a manuscript ready for submission to a peer-reviewed journal. Therefore, there will be a degree of redundancy across chapter introductions and methodologies. As of yet, no individual chapter has been accepted for publication in a peer-reviewed journal. Therefore, the citations and manuscript follow ESA's Ecology Journal formatting for research articles, with the exception of Chapter 4, which is prepared as a note (results and discussion are combined).

Two published papers were produced in association with my PhD, but are not a part of this thesis. These two articles, which are sited within the thesis chapters, are attached as appendices at the end of the thesis.

Trevathan-Tackett, S.M, P.I. Macreadie, P. Ralph, and J. Seymour. 2014. Detachment and flow cytometric quantification of seagrass-associated bacteria. Journal of Microbiological Methods **102**:23-25.

Trevathan-Tackett, S. M., J.J. Kelleway, P.I. Macreadie, J. Beardall, P. Ralph and A. Bellgrove. 2015 (2015). Comparison of marine macrophytes for their contributions to blue carbon sequestration. Ecology **96**:3043-3057.

# **Table of Contents**

Certification of Authorship	i
Acknowledgements	ii
Preface	iii
Table of Contents	iv
List of Illustrations and Tables	v
Abstract	xi
Introduction	1
Chapter 1: Assessing the organic composition of seagrasses for their capacity to	
contribute to long-term carbon sequestration: A global survey	17
Chapter 2: Microbial-driven seagrass remineralisation influenced by temperature	and
sediment chemistry	69
Chapter 3: Long-term microbial remineralisation of seagrass under natural field	
conditions	108
Chapter 4: Microbial priming effect as a mechanism for enhanced CO <sub>2</sub> release in	coastal
sediments	139
Chapter 5: Small-scale seagrass habitat loss affects quantity and quality of sedime	entary
carbon	153
Synthesis, Conclusions and Outlooks	182
Literature Cited	204
Appendix Table 1 with Literature Cited	225
Appendix Publications (attached pdfs of articles, separate page numbering)	

# List of Illustrations and Tables

### Introduction

Figure I1: Conceptual model of the multi-variable approach used to investigate th	e
dynamics of refractory carbon in seagrass meadows	.6
Table I1: Review of seagrass decomposition studies and the variables and factors	
measured or analysed in each study	. 9
Table I2: Review of the seagrass decay rates (percent per day) under oxic and ano	oxic
conditions	. 13

Table 1.1: Summary of structural carbohydrate and fibre content reported from the	
literature for seagrass tissue types2	1
Table 1.2: Summary of the seagrass samples collected	4
Figure 1.1: Map of seagrasses collected	9
Figure 1.2: Principal components analysis of TGA data for all samples shown by	
climatic region and tissue type	3
Figure 1.3: Representative thermograms for each tissue type	34
Figure 1.4: Proportion of thermal intervals (TI) of total organic matter (OM) and OM	M as
percent of the total mass across all tissue types	5
Figure 1.5: Linear regressions of the strongest relationships (adjusted $R^2 > 0.09$ )	
between TGA intervals and latitudes	7
Figure 1.6: Principal components analysis of molecular mixing model predictions fr	rom
NMR analysis for seagrass tissue types	9
Figure 1.7: FTIR spectroscopy loadings for climatic zone and tissue type	-1
Figure 1.8: Principal components analysis of FTIR analysis shown by climatic region	)n
and tissue type4	2

Figure 1.9: PCA plots and Eigenvalues and Eigenvectors for molecular mixing model and thermogravimetry variables
Figure 1.10: Conceptual model describing the organic matter (OM) quality of seagrasses in different climatic regions, tissue types and taxa
Table S1.1: SIMPER table of significant PERMANOVA pairwise comparisons ofthermogravimetric analysis (TGA) and molecular mixing model (MMM)
Table S1.2: Summary of Principal Components Analyses (PCA) Eigenvectors fromChapter 1
Table S1.3: Raw spectral intensities of main <sup>13</sup> C-CPMAS NMR functional groupsexpressed as % of total spectral intensity for selected samples subsample61
Figure S1.1: Principal components analysis of TGA data for tissue types64
Figure S1.2: Seagrass organic matter quality from thermogravimetric analyses between temperate and tropical regions and across all bioregions
Figure S1.3: Seagrass organic matter quality from thermogravimetric analyses across families for leaf, non-photosynthetic above-ground tissue, rhizome, root
Figure S1.4: Principal components analysis of TGA for Zostera samples67
Figure S1.5: Overlay of the average solid-state <sup>13</sup> C-CPMAS NMR spectra of each tissue type

Figure 2.1: Seagrass mass loss during three months of decomposition and the associated	1
decay rates	
Table 2.1: Percent of macromolecular compound losses from decaying seagrasses   predicted from <sup>13</sup> C-CPMAS NMR spectra	
Figure 2.2: MDS plot of bacterial OTUs grouped by tissue, time and temperature83	
Figure 2.3: Taxonomic shifts of bacterial community at class-level related to	
decomposition time and temperature	

Figure 2.4: Proportion of genera within Alpha- and Deltaproteobacteria for leaves,
rhizome/root and sediment through time and with temperature treatments
Figure 2.5: Statistical Analysis of Metagenomic Profiles (STAMP) of predicted metagenomes from PICRUSt analysis for differences in metabolic pathways between tissue types
Table S2.1: Summary of Analysis of Covariance (ANCOVA) statistics
Table S2.2: Summary table of Analysis of Similarity (ANOSIM) statistics
Table S2.3: Raw spectral intensities of main <sup>13</sup> C- CPMAS NMR functional groups      expressed as % of total spectral intensity for each tissue and treatment throughout      incubation period
Figure S2.1: Temperature recorded over the course of 1 year (May 2013 – May 2014) in a <i>Zostera muelleri</i> meadow in Fagan's Bay, Central Coast, Australia100
Figure S2.2: Seagrass elemental C:N ratios, carbon and nitrogen content101
Figure S2.3: Solid-state <sup>13</sup> C- CPMAS NMR spectra of mass loss through decomposition normalised to organic C loss
Figure S2.4: Diffuse reflective mid-infrared spectroscopy103
Figure S2.5: Oxygen and total sulphide microsensor profiles for 23°C, no nutrient treatments
Figure S2.6: Abundance of bacterial cells associated with seagrass litter105
Figure S2.7: Alpha diversity statistics for the eubacterial communities
Figure S2.8: Statistical Analysis of Metagenomic Profiles (STAMP) of predicted metagenomes from PICRUSt analysis for differences in metabolic pathways throughout decomposition

Table 3.1: Grain size and Corg stocks of the Brisbane Waters Estuary sites
Figure 3.1: Elemental and stable isotope characteristics of the sediments from the
Brisbane Waters Estuary118

Figure 3.2: Box plots of organic matter contribution predictions for the top 20 cm of sediments
Figure 3.3: Proportion of mass remaining of leaf and rhizome/root biomass after two years of decomposition
Figure 3.4: Elemental content of leaf and rhizome/root biomass throughout decomposition
Table 3.2: Decay rates ( $k = d^{-1}$ ) predicted by single-, double- and triple-component decay models for leaves and rhizome/roots from each site
Figure 3.5: MDS plot of the significant shifts in rhizome/root organic matter (OM) quality throughout decomposition
Figure 3.6: MDS plot of bacterial community shifts for each tissue type and sediment throughout decomposition
Figure 3.7: Bacterial communities from seagrass and sediments at different sites and throughout decomposition
Figure S3.1: C:N ratios and $\delta^{13}$ C signatures for the plant reference collected at each of the three sites from this study
Figure S3.2: Decay models fitted to leaf mass loss data137
Figure S3.3: Decay models fitted to rhizome/root mass loss data

Figure 4.1: Cumulative microbial respiration of control and amended treatments fo	r
surface and deep sediments	147
Figure 4.2: Average respiration rates of control and amended treatments for surface	e and
deep sediments	148
Figure 4.3: Estimated contributions of labile (LOC) and refractory (ROC) organic	
carbon sources to respiration	149
Figure 4.4: Organic matter quality of 0-1 cm and 29-30 cm sediments using	
thermogravimetric analysis	151

Figure 5.1: Down-core variation in organic matter and organic carbon for each plot through time
Table 5.1: Table for OM, Corg and stable isotope and elemental statistical analyses over   time   162
Figure 5.2: Thermograms for the sediment mass loss and rate of change with increasing temperatures
Figure 5.3: Stable isotope and elemental signatures of sediments in comparison with reference organic matter
Figure 5.4: Stable isotope and elemental signatures of sediment samples that significantly changed through time
Figure 5.5: Box plots of the five possible contributions of OM calculated by the mixing model for the samples with significant changes in stable isotopes and C:N through time
Figure S5.1: Historical time lapse of the seagrass meadows at the Johnson's Beach site from Google Earth <sup>®</sup>
Figure S5.2: Differences in % organic matter down-core for each treatment throughout the experiment
Figure S5.3: Remnants of hollow, decaying rhizome tissue from <i>Thalassia testudinum</i> kill plot
Figure S5.4: Histogram of the dry bulk density values for all sediment depths and the depths analysed of C <sub>org</sub>
Figure S5.5: Box plots of predicted organic matter sources for sediments from Bare plots
Figure S5.6: Box plots of predicted organic matter sources for sediments from <i>Halodule wrightii</i> control plots
Figure S5.7: Box plots of predicted organic matter sources for sediments from <i>Halodule wrightii</i> kill plots

Figure S5.8: Box plots of predicted organic matter sources for sediments from 7	halassia
testudinum control plots	180
Figure S5.9: Box plots of predicted organic matter sources for sediments from 7	halassia
testudinum kill plots	181

#### Synthesis, Conclusions and Outlooks

Figure C1: PCA of the bacterial communities from the short-term laboratory and long-
term field decomposition data sets (Chapters 1 and 2)
Figure C2: Comparison of the classes driving the differences in bacterial communities
during initial, early and late-stage seagrass decomposition
Figure C3: Relationship between latitude and decay rates of seagrass
Table C1: R (refractory) index calculated from thermogravimetric analysis for fresh
tissues from the international study (Chapter 1) and the long-term decomposition study
(Chapter 3)
Figure C4: Modified conceptual model for seagrass decomposition based on the new
insights derived from this thesis
Figure C5: Conceptual design illustrating the processes affecting refractory carbon
accumulation and remineralisation with decomposition, the microbial priming effect and
habitat loss

#### Appendix

#### Abstract

The protection and rehabilitation of natural landscapes in order to enhance their role in carbon sequestration is currently a hot topic for scientists and policymakers looking for solutions to reduce atmospheric CO<sub>2</sub> levels. Blue carbon ecosystems (seagrass, mangrove, saltmarsh) have recently been found to match or even exceed the capability of terrestrial ecosystems to sequester carbon. In seagrass habitats, seagrass carbon alone can account for half of the carbon in the top 10 cm of sediment. Litter quality, often measured as refractory carbon content, is one of the main factors that can influence the sequestration and storage of refractory carbon. Yet to-date, there has been little attempt to understand what factors help or hinder refractory carbon preservation in seagrass sediments.

The aim of this thesis was to unravel the processes and factors that influence, and even optimise, the preservation of refractory carbon in seagrass meadows beginning with the refractory carbon content in seagrass tissues, its persistence (or remineralisation) during decomposition and finally, its preservation in sediments and the mechanisms that provoke further remineralisation after burial. To accomplish these aims, a multi-variable approach was taken, which involved assessing the main and interaction effects of biological, chemical and environmental/physical variables on refractory carbon remineralisation and storage.

The results from this thesis revealed that the processes that affect refractory carbon dynamics in seagrass meadows are complex. It was shown that, while inherent refractory carbon content in the tissues can promote sequestration, decomposition was a strong influence on the persistence of refractory carbon. Anoxic conditions and structural complexity of the tissues promoted refractory carbon preservation and were dependent on the microbial communities present. Sheath and stem tissues were considered to be important carbon contributors due to their high refractory carbon content and chance of *in situ* burial. Temperature and the availability of labile organic matter and inorganic nutrients enhanced decay in the short-term under oxic conditions, while physical disturbance and habitat loss caused losses of sediment refractory carbon over the course of months to years depending on the type of disturbance.

In light of these results, a new conceptual model was developed for seagrass decomposition and have highlighted several important avenues of future blue carbon research, including the functional roles of microbes (bacteria, fungi and protists) in carbon remineralisation via bioinformatics and enzymes kinetics, as well as the conversion, or 'up-cycling', of labile carbon to refractory carbon within microbial biomass.