Title

Development of Novel Stable Isotope Approaches to Evaluate Carbon Flow in a Restored Coastal Wetland in Southern California

Authors

Dillon, Jesse
Whitcraft, Christine

Publication Date

2012-01-18

California Sea Grant College Program
Final Report

R/ENV-215
7/1/2010–12/31/2011
Development of Novel Stable Isotope Approaches to Evaluate Carbon Flow in a Restored Coastal Wetland in Southern California

Jesse Dillon
562-985-4824
jesse.dillon@csulb.edu

Christine Whitcraft
562-985-4820
cwhitcra@csulb.edu

I. Project Hypotheses

We hypothesized there would be differences in the microbial and infaunal communities primarily responsible for degrading macrophyte vs. microalgal carbon. Specifically we predicted that:

> *The invertebrate community degrading macrophyte-derived carbon will be significantly different from those degrading algal-derived carbon.*
>
> *The microbial communities (bacteria and fungi) degrading macrophyte-derived carbon will be significantly different from those not using this carbon source.*

II. Project Goals and Objectives

Our overall objective was to determine the fate of primary production in a restored southern California salt marsh and specifically to assess the importance of macrophyte vs. microphytobenthic algal derived carbon in determining infaunal trophic structure. In addition, we wanted to identify the bacteria and fungi responsible for macrophyte (plant) carbon degradation and utilization. Lastly, we wanted to demonstrate the use of a novel molecular ecology technique, DNA stable isotope probing (SIP), to elucidate active microbial carbon cyclers. Traditional approaches use molecular tools to sequence sediment and make an assumption that proximity relates to function; however, if DNA SIP is successful in salt marsh sediment, it could be a useful tool for evaluating other functions of microbiota in these systems.

III. Briefly describe project methodology

We used natural abundance stable isotope analyses to describe components of the salt marsh sediment food web in addition to an isotopic enrichment experiment where microphytobenthic algae and marsh cordgrass (*Spartina foliosa*) were labeled with ^{13}C and ^{15}N respectively. We then followed the enriched signature of the plant vs. algal material into infauna invertebrate consumers. In addition, we performed a lab-based SIP experiment to identify microbial carbon utilizers of ^{13}C-labeled lignocellulose to model usage recalcitrant carbon available *in situ*.

IV. Describe Project and Accomplishments towards meeting goals and objectives

As of Dec. 31, 2011, we have completed all of our experimentation/data collection and much of our data analyses. Final analyses are ongoing and will be completed in the next 6 months as Ms. Darjany, the Seagrant Trainee associated with the grant completes her M.S. degree. Following methodological testing, preparation, and waiting for access to the ecologically sensitive field site in 2010, in early 2011 we performed a lab and field based thirty-day experiment to test our first hypothesis and collected infauna and microbial samples at both a 0 and 30 day time point. *Spartina* plants in the Huntington Beach Wetland Conservancy nursery that had been labeled with ^{15}N-ammonium sulfate in July 2010 were checked for δ15N enrichment (3305 $^{o}/_{oo}$) in January 2011. The enriched *Spartina* blades were harvested the morning of the start of the experiment and redeployed to the sediment surface in litter-bags (large mesh finch bags to evaluate decomposition, Nitex mesh to test for leaching). These bags were deployed in Talbert Marsh in Huntington Beach between 2/14-3/16/2011. During the initial setup day, algal mats were also labeled with ammonium ^{13}C-bicarbonate. Invertebrate infauna, sediment, and algal samples were taken at time 0 and time 30 from labeled algal mats and under labeled *Spartina* bags. After collection, infauna were sorted to species, counted, allowed to evacuate their guts for 24 hours, dried, and were sent to U.C. Davis Stable Isotope Facility. Microbial degradation from litter-bags was calculated from bag weight loss and estimated to be 2.05g +/- 0.19 and finch bag degradation was calculated to be 1.48g +/- 0.72 suggesting that both microbes and invertebrates are important degraders of *Spartina* in the marsh. These differences in *Spartina* loss were not significantly different between Nitex and Finch bag treatments (P=0.08), suggesting that exclusion of invertebrates (microbes only) may not decrease decomposition in the marsh, although these experiments need to be repeated to confirm this. In the nitrogen labeling experiment, we observed high nitrogen enrichment of two groups of insect larvae (Psychodidae and Chironomids) from labeled *S. foliosa* (Figure 1). In contrast, five additional groups had elevated ^{13}C signatures in the algal labeling experiment, suggesting a broader algal carbon food web. Interestingly, the two gastropod groups (*C. californica* and Aceteocina) and a crustacean showed high ^{13}C signatures even though they were sampled from the ^{15}N-macrophyte labeling experiment. This seems contradictory since they did not show an elevated ^{15}N signature but suggests that they must have moved there after previously grazing on the ^{13}C labeled algal mats.

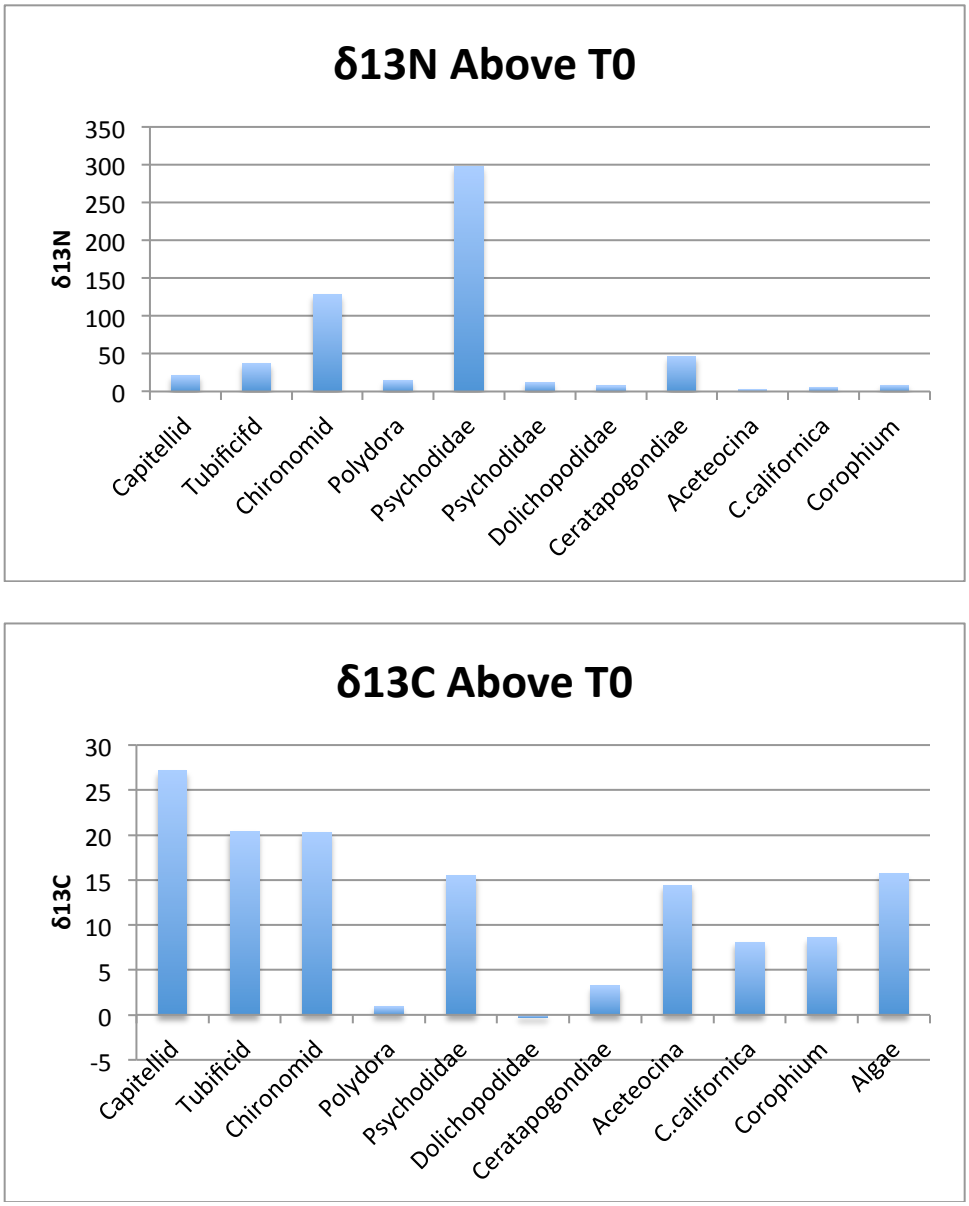

Figure 1. Stable isotope values of nitrogen (top) and carbon (bottom) above background (T0) values after 30 days of incubation with enriched substrates.

In parallel to field experiments, a laboratory experiment was set up for thirty days to identify bacteria and fungi consuming macrophyte material. Talbert Marsh sediment was amended with labeled ^{13}C- lignocellulose (experimental incubations) or ^{12}C-lignocellulose (control incubations). After incubation, genomic DNA was extracted from samples and ultra-centrifuged for 40 hours. These samples were then fractionated in 425 µl aliquots and measured for density to confirm a gradient. Density measurements show successful separation of "light" and "heavy" fractions (Figure 2) indicating that the ^{13}C-labelled DNA can be separated from unlabeled DNA (i.e., DNA from cells not taking up lignocellulose).

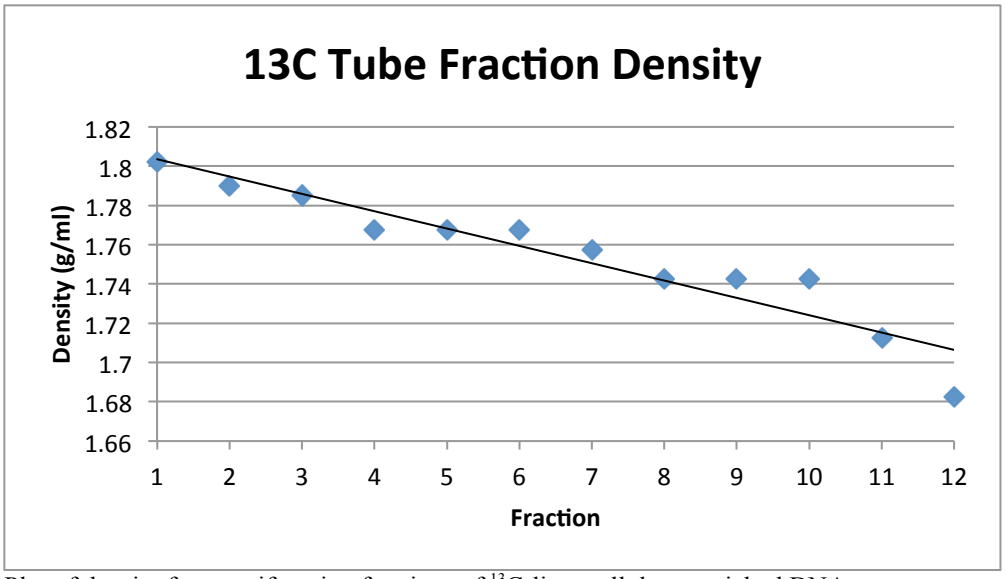

Figure 2. Plot of density for centrifugation fractions of ^{13}C-lignocellulose-enriched DNA.

The bacterial 16S rRNA sequences from each fraction were successfully amplified and analyzed for genetic differences using terminal restriction fragment length polymorphism (T-RFLP), a DNA fingerprinting approach commonly used in SIP experiments. Relative abundance data of organismal taxonomic units were analyzed using clustering analyses to identify key fractions to pool for representation of a heavy and light fraction (Figure 3).

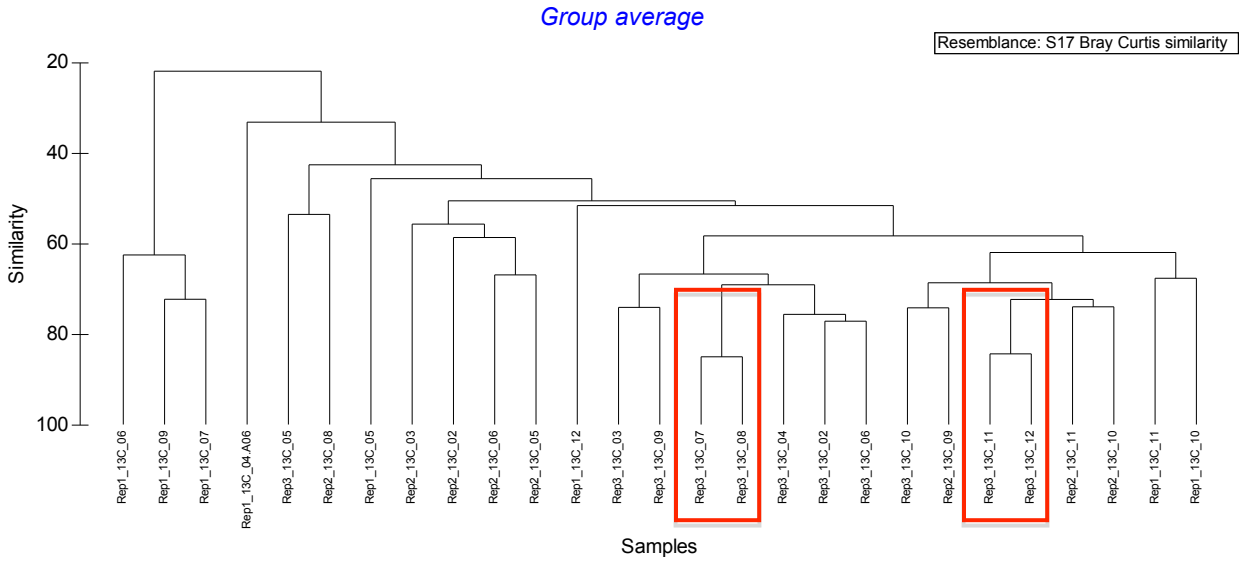

Figure 3. Cluster analysis of all triplicate fractions using Bray Curtis similarity indices of relative abundance values. Red boxes indicate labeled (7/8) and non-labeled (11/12) fractions that were pooled and used for molecular analyses.

In addition, these same fractions were analyzed using PRIMER software to elucidate general differences in relative abundance of bacterial species (indicated by base pair number)(Figure 4). Light fractions 12 and 11 clustered together as well as 7 and 8. These

fractions were chosen to represent the corresponding a labeled heavy (i.e., lignocellulose utilizer) and unlabeled light fraction.

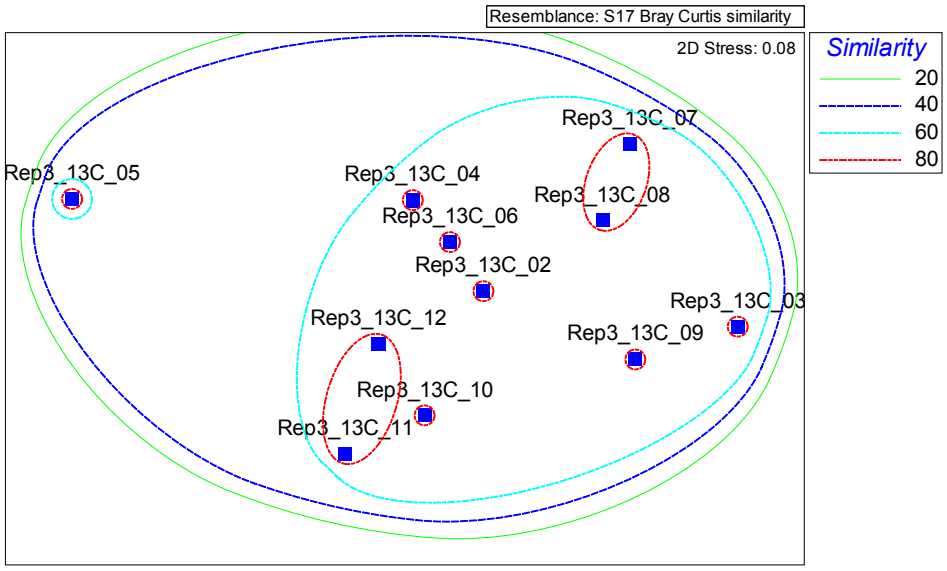

Figure 4. Multidimensional scaling plots showing T-RFLP profiles of fractions from trial ^{13}C lignocellulose incubations. Circles indicate group percent similarities based on Bray Curtis similarity indices.

In addition, the similarity percentage linking each base pair with its abundance in each fraction was highly effective in identifying particular species contributions to each fraction. For example, some representative OTUs such as those shown below were present in the lower "heavy" fractions (7/8), but not in the higher "light" fractions (11/12) (Figure 5).

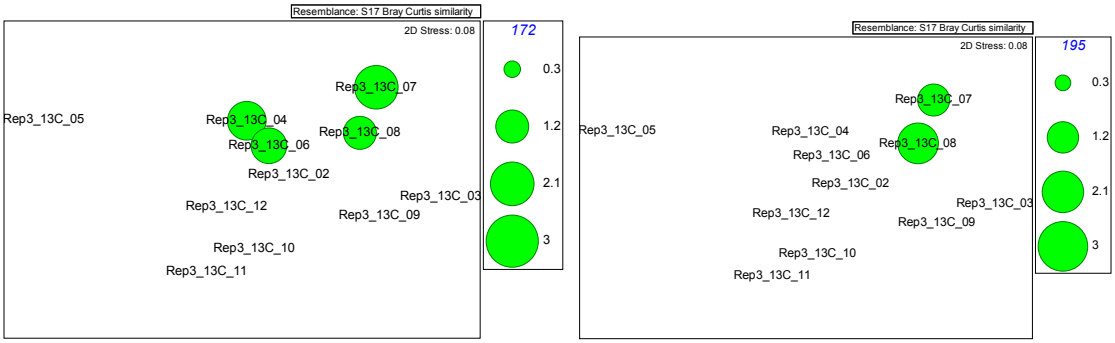

Figure 5. Representative SIMPER results showing clustering of specific OTUs present only in the 7/8 heavy fractions.

Approximately 170 combined Bacterial 16S rRNA genes from both the 7/8 heavy (i.e., labeled) fractions and the 11/12 light (i.e., unlabeled) fractions were successfully cloned and sequenced to compare and identify differences in the two communities. Across both communities there were representatives of the following groups: Gammaproteobacteria, Deltaproteobacteria, Spirochaetes, Bacteroidetes, Chlorobi, Planctomycetes, Lentisphaerae, Gemmatimonadetes, Acidobacteria, Firmicutes, Actinobacteria,

Cyanobacteria and largely uncultured Candidate divisions OP11, TM7, BRC1, and WS3. Community sequence data were analyzed using Mothur, an open source bioinformatics software program. Preliminary results show that labeled and unlabeled bacterial communities were significantly different (P=0.005) based on libshuff analyses, which detect differences in presence/absence of operational taxonomic units (OTUs). Note: because species identification in uncultured bacteria is challenging, OTU assignments based on 97% sequence similarity of 16S rRNA genes are commonly used; these have been shown to roughly equate to species level diversification. Mothur identified 111 total OTUs including 48 in the light fraction, 53 in the heavy fraction, and ten OTUs that were shared by both fractions.

Fungal DNA was successfully amplified from the 11/12 light fractions, but was only barely amplifiable from heavy fractions (cloning was attempted but was unsuccessful due to the very faint amplification), suggesting that fungi were present in the sediment sample, but at this point there is little evidence that they utilized the labeled lignocellulose. The unlabeled fungal rRNA amplicons (11/12 fraction) were cloned and sequenced, but have yet to be analyzed. Ongoing efforts are being made to amlify the labeled fractions.

V. Summary

Most models of salt marsh carbon cycling place microbial processes in a "black box", linking microbes with their function only by correlation or spatial association. The goal of this study was to use novel and traditional stable isotope approaches to directly identify key microbial decomposers in a restored salt marsh. As predicted, invertebrates sampled in and around wide-mesh finch bags containing enriched *Spartina foliosa* showed a higher δN value compared to invertebrates in and around the 100µm mesh Nitex bags, which excluded most invertebrates from accessing the nitrogen-enriched *Spartina*. Stable isotopic labeling of both plants and algal mats have revealed diverse but distinct invertebrate communities utilizing these different sources of carbon. Only two groups of invertebrates were substantially labeled in the nitrogen enrichment experiment (utilize macrophyte carbon), whereas 7 groups were labeled in the carbon enrichment experiment (utilize algal carbon). Interestingly, tubificid oligochaetes and capitellid polychaetes showed high algal ^{13}C- enrichment, contrary to previous studies, which found that these organisms are primarily macrophyte detritivores. Our DNA SIP results confirm that as expected, diverse sediment bacteria utilize lignocellulose (macrophyte carbon), although the community of bacterial lignocellose degraders was significantly different from those not utilizing the plant carbon. Overall, this study reveals a diverse population of secondary consumers including both invertebrates and bacteria that degrade salt marsh plant- and algal-derived carbon and suggest that carbon degradation is more complicated than previously understood. To our knowledge, this study is the first to examine both the microbial and infauna consumption of primary production simultaneously in a southern California salt marsh and represents the first successful use of the DNA-SIP method in a salt marsh habitat. These data have served as preliminary data for one Seagrant proposal in 2011 which was declined funding at the full proposal stage, but will also be used in future funding proposals (e.g. NSF). They will also form the basis of Lindsay Darjany's master's thesis, planned defense in summer 2012.

PROJECT MODIFICATIONS:
No significant modifications in the proposed work were made, except for the approved six month no-cost extension.

PROJECT OUTCOMES:
No response

IMPACTS OF PROJECT:
No response

BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS:
No response

ECONOMIC BENEFITS generated by discovery
No response

Issue-based forecast capabilities
No response

Tools, technologies and information services developed
No response

Publications

Conference Papers, Proceedings, Symposia

Conference	2012 International meeting of the Society of Wetland	Location	Orlando, FL	Date	abstract submitted
Title	Characterization of Microbial Carbon Cyclers Using Stable Isotope Approaches	Authors	Lindsay Darjany, Christine Whitcraft and Jesse Dillon		
Conference	112th general meeting of the American Society for	Location	San Francisco, CA	Date	abstract submitted
Title	Characterization of Microbial Carbon Cyclers Using Stable Isotope Approaches	Authors	Lindsay Darjany, Christine Whitcraft and Jesse Dillon		

DISSEMINATION OF RESULTS:
No response

COOPERATING ORGANIZATIONS:
Nongovernment
Huntington Beach Wetland Conservancy

FOR ALL STUDENTS SUPPORTED BY THIS GRANT, PLEASE LIST:
Volunteer Count = 4

Graduate Student Info

Last Name	Darjany	**First Name**	Lindsay	**Middle Initial**	
Contact Email	lindsaydarjany@gmail.com			**Contact Phone**	562-706-4571
Institution	California State University, Long Beach				
Department	Biological Sciences				
Degree Program	M.S. Biology				
Thesis Title					
	Supported by Sea Grant Yes	**Start Date**	7/1/2010	**End Date**	6/30/2011