Rapid cycling of recently fixed carbon in a *Spartina alterniflora* system: A stable isotope tracer experiment

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The metabolism and carbon dynamics of vegetated ecosystems are shaped by plants, belowground microbes, and their interactions. Ecosystem-level carbon dioxide (CO_2) emissions reflect plant metabolism and microbial decomposition of plant detritus, compounds released from roots, and buried soil organic matter. Plant respiration is an important source of CO_2 emissions as roots can account for 45-60% of belowground respiration in terrestrial systems. Roots release particulate and dissolved organic compounds into the rhizosphere and a portion is decomposed by microbes. Inorganic nutrients released via mineralization can be recycled back to the plant or taken up by other microbes. Soil bacteria affect ecosystem carbon storage by decomposing newly produced organic matter as well as older, buried compounds. Quantifying the fate of new plant production and identifying the sources of organic matter decomposed by soil bacteria through aerobic and anaerobic pathways are key for understanding carbon cycling and storage in vegetated habitats.

In tidal saltwater wetlands, respiration accounts for a large portion of gross primary production and can even exceed fixation during colder months, resulting in net efflux of CO_2. Yet, these systems are globally important carbon sinks (5-87 Tg C y^{-1}). Parsing the respiratory contributions of plants vs. soil microbes and whether bacteria decompose newly produced or buried carbon is difficult since living salt marsh grasses and buried detritus are the main sources of organic matter for respiration and cannot be differentiated based on natural abundance isotopes. In addition, belowground biogeochemistry is complex: tides, bioturbation, and plant metabolism affect redox conditions and carbon is processed by heterotrophic and autotrophic microbes via aerobic and anaerobic metabolisms.

To determine the short-term fate of recently photosynthesized carbon in above- and below- ground plant tissues and soil microbes, we conducted a mesocosm experiment in which individual *Spartina alterniflora* plants were exposed to $^{13}CO_2$ for 0 – 6 h and the isotopic label was traced over 24 h. *Spartina alterniflora*, or smooth cordgrass, is a salt-tolerant herbaceous

perennial common to salt marshes of temperate estuaries. In order to capture short-term carbon transformations (hours – days) via ^{13}C enrichment of specific compounds, we first had to determine the exposure time required to isotopically label phospholipid-linked fatty acid (PLFA) compounds, which are source-specific biomarkers. The optimal exposure time should result in sufficient labeling of compounds for adequate analytical detection but minimize incubation artifacts in the plant and soil system. We predicted the $^{13}CO_2$ label should be applied to *S. alterniflora* for at least 3h to detect measurable changes in the isotopic composition of individual bacteria-specific PLFAs belowground. We also tested two hypotheses regarding plant metabolism and belowground microbial assimilation of recently fixed carbon. First, we hypothesized that substantial fractions of newly fixed carbon are retained in *S. alterniflora* aboveground tissues and lost via respiration within 24h while a smaller fraction is transferred to belowground roots and rhizomes. Second, multiple microbial communities, including anaerobic sulfate reducing bacteria, are coupled to *S. alterniflora* production via organic carbon compounds released by roots.

To follow short-term carbon transformations in a *S. alterniflora* – soil system, we applied ^{13}C-labeled CO_2 to aboveground leaves and chased it belowground into roots and bacteria-specific lipids. Plant mesocosms experienced $^{13}CO_2$ for 0, 1, 3, or 6h. Incorporation of $^{13}CO_2$ by plants and soil microbes was measured immediately after the incubation (Day 0) and 24h later (Day 1) (Fig. 1).

Results from our experiment demonstrated that fractions of CO_2 fixed by *S. alterniflora* were transferred to belowground roots and rhizomes and assimilated by soil bacteria within 24 h. Longer periods of exposure to the $^{13}CO_2$ label were important for detecting isotopic shifts of specific PLFA compounds but plant production may have been negatively affected in the 6h incubation treatment. The 3h label exposure period, however, resulted in adequate label application for analytical detection while maintaining environmentally realistic experimental conditions and minimizing artifacts.

Considerable fractions of new production were retained in aboveground leaves, respired, and transferred belowground to roots and rhizomes within 24h. We found that 41±6 – 64±7% of newly fixed carbon was retained in leaves, 2.7±2 – 6.4±2% was transferred to roots, and 30±10 – 55±4% was lost via respiration within 24h. Carbon fate was similar across exposure duration treatments ($p > 0.05$ for all variables), indicating that plant carbon dynamics were unaffected by

incubation time. The fraction attributed to respiration may be a slight overestimate because we did not quantify loss via root exudates. Since small fractions of newly fixed carbon were transferred belowground, our data indicated that new production mainly supported the metabolism of aboveground tissues over short time periods in the summer. Although the fraction of new production transferred belowground was small, bacteria rapidly assimilated the label which indicated that soil microbes were tightly coupled to plant metabolism.

Of the $^{13}CO_2$ fixed in *S. alterniflora* leaves and shoots, very small fractions were transferred belowground and incorporated into PLFAs representing bacteria: Branched PLFA, 10-Methyl $C_{16:0}$ and cy-$C_{17:0}$. Branched PLFAs occur in a range of bacteria while 10-Methyl $C_{16:0}$ and cy-$C_{17:0}$ are considered to be more specific to sulfate reducing bacteria. Sulfate reduction is an important anaerobic metabolic pathway in salt marsh soils, and sulfate reducing bacteria are primarily heterotrophic, although some are mixotrophic or autotrophic. Modest enrichment of 10-Methyl $C_{16:0}$ and cy-$C_{17:0}$ between Days 0 and 1 indicated that plant derived carbon was assimilated by sulfate reducing bacteria. Changes in sulfate reduction rates have been linked to salt marsh productivity and plant life stage, however, this is among the first demonstrations of rapid carbon transfer from *S. alterniflora* to sulfate reducing bacteria.

Transfer of label to heterotrophic soil microbes, including sulfate reducing bacteria, indicated that root exudates were rapidly decomposed and assimilated. This was a short experiment and a longer chase period may have revealed greater contributions of recently produced photosynthetic carbon to soil microbes. Finally, the combined use of stable isotope tracing techniques with isotopic analysis of specific bacterial lipids allowed us to track carbon transformations over short time periods and have the potential to expand our understanding of carbon cycling in vegetated habitats.

The manuscript describing the results from this study was recently published in the journal *Biogeochemistry*. A grant proposal based on this study is currently being considered by Woods Hole Sea Grant.

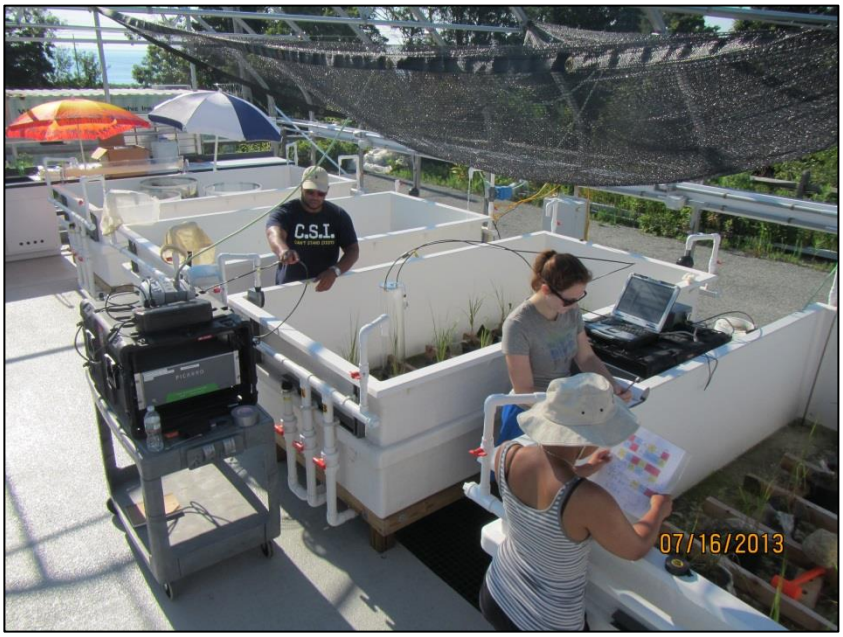

Fig. 1. The mesocosm experiment was conducted in summer 2013 and involved three undergraduates through the WHOI Summer Student Fellow and Woods Hole Partnership in Education programs. One of the undergraduates presented a portion of the results as a poster at the international Ocean Sciences Meeting in 2014.

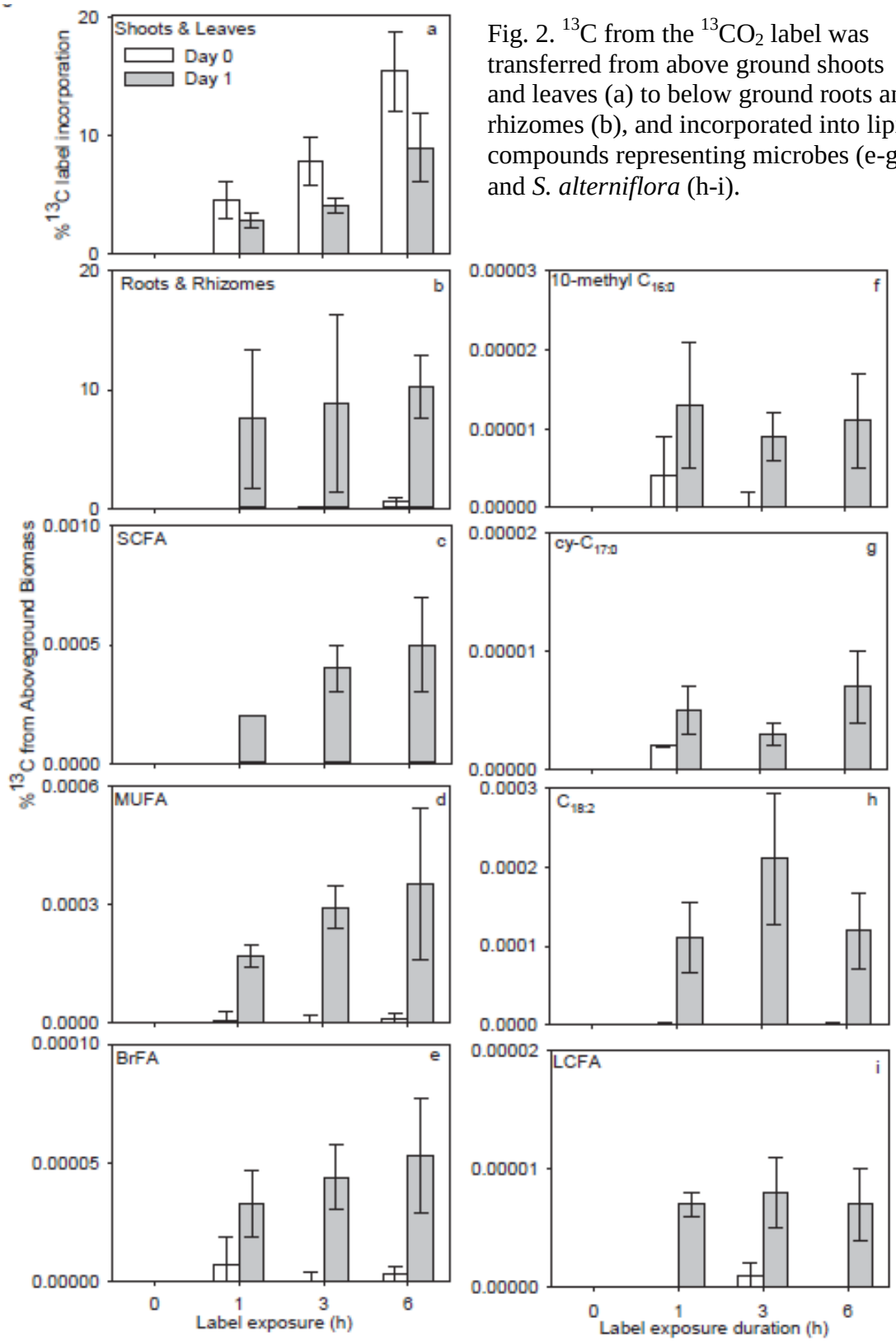

Fig. 2. ^{13}C from the $^{13}CO_2$ label was transferred from above ground shoots and leaves (a) to below ground roots and rhizomes (b), and incorporated into lipid compounds representing microbes (e-g) and *S. alterniflora* (h-i).