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In situ ^{13}C tracer experiments elucidate carbon translocation rates and allocation patterns in eelgrass *Zostera marina*

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ABSTRACT: *Zostera marina* L. carbon dynamics have been intensively studied, yet *in situ* translocation rate estimates remain elusive, particularly for north temperate seagrasses. To better understand carbohydrate synthesis, allocation and use in *Z. marina*, we conducted summer and winter *in situ* ^{13}C labeling experiments and measured the δ^{13}C of bulk tissue and individual carbohydrate compounds. Leaf tissue exhibited immediate isotope enrichment within hours of the tracer pulse. As the isotope ratio of the leaf tissue decreased over a period of days, enrichment became more evident in the belowground tissues (rhizome and roots) not directly exposed to the ^{13}C-dissolved inorganic carbon label, indicating that non-structural carbohydrate was translocated belowground. Rhizome δ^{13}C increased for up to 2 wk after the ^{13}C label pulse. The isotopic enrichment of glucose, fructose and sucrose were similar and significantly greater than the enrichment of myo-inositol. Maximum enrichment occurred in the glucose pool, with leaf tissue at 258 ± 61‰ and rhizome tissue at 55.1 ± 28.8‰ during the July 2004 labeling period. Leaf excess ^{13}C loss rates (4 d average) were on the order of 11% ^{13}C d^{-1}, while the excess ^{13}C accumulation rate in the rhizome combined with roots was about 1.5% ^{13}C d^{-1} (4 d average). Whole plant excess ^{13}C loss rates as a result of respiration, detrital production and exudation ranged between about 8.8 and 10% ^{13}C d^{-1} (4 d average). The results provide important estimates of carbon uptake and translocation that can be used to better understand whole plant carbon budgets as well as the transfer of seagrass-derived carbon to other trophic communities.

KEY WORDS: ^{13}C tracer · Translocation · Carbohydrate · *Zostera marina* · Compound-specific isotope analysis · Carbon dynamics

INTRODUCTION

Seagrass habitat is widely recognized as a critical component of healthy marine ecosystems and the focus of intense interest for conservation and restoration (Kenworthy et al. 2006, Orth et al. 2006, Heck et al. 2008, Short et al. 2011). Although many factors influence seagrass growth and production rates (e.g. light, temperature, CO_2 availability, etc.), photosynthesis and whole plant carbon balance ultimately determine the survival of a plant. Seagrass carbon dynamics and photosynthetic physiology have received intense attention over the last 3 decades. Reviews devote a substantial amount of text to these and related topics (e.g. Hemminga & Duarte 2000, Touchette & Burkholder 2000, Larkum et al. 2006a), and there have been many refinements and advances since the early synthesis publications on seagrass biology and physiology (McRoy & Helfferich 1977, Phillips & McRoy 1980). Understanding seagrass car-

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bon dynamics and balance is necessary to protect and restore this valuable ecosystem engineer. Although many studies have examined photosynthetic rates (how much carbon is fixed) and how large the storage reserves are (non-structural carbohydrate [NSC]), remarkably few studies have examined how much carbon is moved around within a plant and which compounds are mobilized. However, recent work has begun to explore seagrass translocation and clonal integration of ramets for a variety of species (Zimmerman & Alberte 1996, Boschker et al. 2000, Marbà et al. 2002, Kiswara et al. 2005, Dean & Durako 2007, Prado et al. 2008, Collier et al. 2010). Understanding whole plant carbon balance is critical to the maintenance and protection of the water quality conditions necessary to conserve and restore the habitat.

Recent efforts to evaluate and predict where seagrass populations may be effectively conserved or restored have relied on coupled biological–physical models with integrated hydrodynamic, water quality and seagrass plant models. This coupled modeling approach was used in Laguna Madre, Texas, USA (Dunton et al. 2003) and is currently being used in Puget Sound, Washington, USA (R. Thom pers. comm.). The development of sophisticated seagrass production models based on carbon balance requires a detailed understanding of the complex physiology of carbon fixation, mobilization, storage and loss processes and their feedbacks with multiple environmental drivers (Burd & Dunton 2001, Zimmerman 2003, Eldridge et al. 2004, Miller et al. 2007, Devereux et al. 2011). Experiments that address ecological or physiological questions can often provide data useful to model development (e.g. rate estimates and constraints) and provide critical information for validation of model predictions.

Seagrass growth results from the difference between gross photosynthesis and losses due to respiration and exudation. Storage of NSC can act as a buffer to provide energy to meet metabolic demands during periods of low photosynthesis due to low light, low temperature or anthropogenic stresses (Herzka & Dunton 1998, Cabello-Pasini et al. 2002). Annual patterns of high NSC during summer and low NSC during winter/spring have been reported from several seagrass species (Dawes & Lawrence 1980, Pirc 1985, Burke et al. 1996). Sucrose is the dominant storage carbohydrate in most seagrass species, accounting for up to 90% of the NSC pool; other carbohydrates include fructose, glucose, trehalose and complex polysaccharides such as cyclitols (Drew 1978, 1983, Touchette & Burkholder 2000, Larkum et al. 2006b, Vichkovitten et al. 2007). The importance of starch as a seagrass carbohydrate storage product is highly variable among species (Touchette & Burkholder 2000, Vichkovitten et al. 2007). Some studies have examined the biochemical pathways leading to various carbohydrate products (see review by Touchette & Burkholder 2000); however, very few present rates of carbon translocation, and several of those are unpublished (Harrison 1978, Drew unpubl., cited in Larkum et al. 2006b).

Stable isotopes provide a powerful tool for evaluating pathways and transformations. Natural abundance stable isotope studies have been used in seagrass research primarily as a technique for understanding the fate of seagrass-derived carbon (Lepoint et al. 2004). The few historical tracer studies that have been conducted (typically with radioisotopes) provided valuable information on carbon transport (Bittaker & Iverson 1976, Harrison 1978) and dissolved organic carbon (DOC) exudation (Wetzel & Penhale 1979). More recently, stable isotope tracer experiments have been used to evaluate carbon and nitrogen acquisition and transport (Lepoint et al. 2002, Marbà et al. 2002). Advances in stable isotope methodology (e.g. separation chemistry and coupled gas chromatography-combustion-isotope ratio mass spectrometry [GC-C-IRMS] technology) permit compound-specific isotope analyses (CSIA), which provide a new tool for understanding seagrass organic carbon dynamics (Boschker et al. 2000, Jones et al. 2003, Lepoint et al. 2004, and others). For example, isotope enrichment studies have recently been combined with CSIA to evaluate the coupling between seagrass carbon exudation and sediment bacterial communities (Boschker et al. 1998, Holmer et al. 2001, Kaldy et al. 2006). However, we are unaware of seagrass studies combining isotope tracer methods with compound-specific analyses to evaluate the fate of translocated sugars in plant carbon allocation over time scales of days to weeks.

In this study, we use the isotope tracer-CSIA approach to examine carbohydrate dynamics within *Zostera marina* growing in the northwestern United States. Our goal was to examine the patterns of carbon allocation and to estimate translocation (transport from leaves to belowground components) rates of fixed carbon *in situ* under winter and summer conditions. We conducted 3 ^{13}C enrichment experiments including a laboratory-based preliminary experiment and 2 *in situ* field experiments. We measured the bulk tissue isotope ratio and used CSIA to measure the $\delta^{13}C$ of individual carbohydrate pools. Using these data, we were able to determine minimum ^{13}C translocation rates for a field population of *Z. marina*.

MATERIALS AND METHODS

Isotope enrichment experiments

We conducted 2 laboratory and 2 field ^{13}C isotope enrichment experiments. The laboratory mesocosm experiments were pilot studies (data not presented) used to develop the methods for the field incubations. The first field experiment was conducted during February 2004 to assess carbon distribution in field plants under winter conditions. The field site (Yaquina Bay, Oregon, USA) and *Zostera marina* population used in these experiments have been characterized and described previously (Kaldy 2006, Kaldy & Lee 2007). Based on the results of the laboratory pilot experiment, the seawater media was enriched to ca. 10 000‰ ^{13}C-dissolved inorganic carbon (DIC) using ^{13}C sodium bicarbonate. *In situ* labeling was conducted as follows (Fig. 1). Six replicate shallow plastic tubs were laid out along a horizontal transect in the seagrass bed. As the tide receded, exposed plant leaves were draped over the side of shallow plastic tubs (82 × 38 × 15 cm) containing 20 l of ^{13}C- enriched seawater media. Each replicate tub contained the leaves from 10 *Z. marina* shoots; a plastic surveyor's flag was inserted into the sediment next to each labeled plant for later identification. Care was taken to ensure that the individual shoots were separated by as much distance as possible to increase the likelihood that shoots were physiologically independent. Plants were incubated under ambient light conditions (instantaneous photon flux density [PFD] was 200 to 700 µmol photons m^{-2} s^{-1}) in the enriched media for about 1 h until the rising tide precluded additional labeling. The leaves of the plants were subsequently rinsed and removed from the plastic tubs, and residual labeling water was acidified to release any remaining ^{13}C prior to discarding away from the labeling site. This procedure ensured that the plants remained rooted during the labeling and ^{13}C contamination of sediment did not occur. Plants were collected prior to enrichment, immediately after the enrichment period and at 24, 48, 72, 96 and 408 h. One plant from each of the 6 replicate tubs was collected, rinsed in seawater and separated into leaves, rhizome nodes 1 to 5, roots from nodes 1 to 5 and rhizome + roots > node 5; all tissues were analyzed for bulk isotope ratios. Previous work (Kraemer & Alberte 1993) has shown that metabolic activity in rhizome tissues decreases with distance from the meristem. The plant tissues were then lyophilized and ground to a fine powder using a roller mill. Dissected bulk tissue samples were analyzed using a Europa Scientific Tracermass isotope ratio mass spectrometer (IRMS), which provided δ^{13}C and carbon content (%C) data. All enriched stable isotope analyses were conducted by the Stable Isotope Research Unit at Oregon State University.

Fig. 1. Field ^{13}C tracer enrichment technique. See 'Isotope enrichment experiments' for details

The second field experiment was conducted during July 2004 to assess carbon distribution under summer conditions. The experimental protocol, sampling and analysis were as described above, with the exception that plants were incubated for 2 h in the enriched media and the last sampling occurred 12 d after initial enrichment. During July, the plants were kept moist during the enrichment period using spray bottles with seawater. This was needed to prevent desiccation because the plants were exposed to higher PFD (instantaneous PFD was 500 to 1100 µmol m^{-2} s^{-1}) for a longer time. Subsequent to the bulk tissue determinations, a subset of the samples was extracted, derivatized and analyzed for the isotope ratio of individual sugars, specifically glucose, sucrose, fructose and myo-inositol. Due to resource and tissue limita-

tions, a subset of 36 samples was analyzed for isotope ratio of individual sugars. To provide the best coverage during the time period when we expected isotope values to be maximum, we analyzed 3 tissues (leaf, rhizome and root) from 4 replicate plants collected on Days 1, 2 and 3. Derivatization was carried out using pyridine, hexamethyldisilizane and trimethylchlorosilane as outlined by Cox & Stushnoff (2001). Separation and isotopic analysis was carried out using an Agilent GC interfaced to a PDZ Europa Hydra 20/20 IRMS.

Sugars were quantified using anion exchange high-performance liquid chromatography (HPLC) and a pulsed electrochemical detector. Briefly, dried ground tissue samples were extracted with hot ethanol (80%) and purified using reverse-phase, solid-phase extraction (Phenomenex Strata C18-E cartridges). Extracts were then separated and quantified by HPLC using a Hamilton RCX-10 (250 × 4.1 mm) separation column, with a 50 mM NaOH mobile phase at a flow rate of 1 ml min^{-1}, temperature of 30°C and sample injection volume of 20 µl. Quantification was done by comparison to calibration curves generated by direct injection of pure sugar standards on the HPLC. The contribution of starch to the non-structural carbohydrate pool was not quantified.

Isotope reporting

Throughout the text, we use standard δ notation:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \quad (1)$$

where R is the ratio of ^{13}C to ^{12}C. All $\delta^{13}C$ stable isotope values are reported relative to Pee Dee Belemnite standard; ($R_{standard}$ = 0.0112372). The apparent enrichment factor (Δ) was calculated as:

$$\Delta = \delta_{final} - \delta_{initial} \quad (2)$$

Calculation of excess ^{13}C distribution

To better understand the distribution of the ^{13}C label, the isotope data were expressed as a percent of the total amount of ^{13}C incorporated during the enrichment pulse. Effectively, this calculation normalizes the data to account for variations in plant size and distribution of carbon within the plant components and clearly shows the amount transported to each plant component. The ratio of $^{13}C/^{12}C$ in the sample was calculated from the isotope ratio of the sample (δ):

$$R_{sample} = \left(\frac{\delta}{1000} + 1\right) R_{standard} \quad (3)$$

The fractional abundance (A) of ^{13}C relative to $^{13}C + ^{12}C$ is calculated as:

$$A = \frac{R_{sample}}{R_{sample} + 1} \quad (4)$$

The amount of ^{13}C in the sample (leaf, rhizome or root) is calculated as:

$$^{13}C = A \times B \times \frac{\%C}{100} \quad (5)$$

where B is the biomass of the component (g dry wt) and %C is the amount of carbon in the component (expressed as a percent). The isotope sampling was destructive; therefore, different plants were used for each sampling time, and these plants had different biomass. To account for these differences, average biomasses were used in the calculation for each of the plant components during the February and July experiments (Table 1). The amount of ^{13}C in the whole plant was calculated as:

$$^{13}C \text{ whole plant} = A_{leaf} B_{leaf} \frac{\%C_{leaf}}{100} + A_{rh} B_{rh} \frac{\%C_{rh}}{100} + A_{rt} B_{rt} \frac{\%C_{rt}}{100} \quad (6)$$

where subscripts refer to plant tissues, leaf, rhizome (rh) and root (rt). The excess ^{13}C incorporated into the whole plant during the experiment was calculated as the difference between the ^{13}C in the whole plant immediately after enrichment and the ^{13}C prior to enrichment. The percentage of excess ^{13}C in each of the plant components was calculated as the differ-

Table 1. *Zostera marina*. Tissue biomass and carbon content (%C) (mean ± SE) of plant components during the February and July experiments. Values presented represent mean values for replicate shoots during each experiment

Experiment	Tissue	Biomass (g dry wt)	%C	Sample size
February 2004	Leaf	1.64 ± 0.08	27.9 ± 0.41	42
	Rhizome	0.213 ± 0.013	30.7 ± 0.29	42
	Root	0.064 ± 0.007	17.6 ± 0.54	42
July 2004	Leaf	1.65 ± 0.09	26.9 ± 0.36	42
	Rhizome	0.247 ± 0.018	33.1 ± 0.25	38
	Root	0.111 ± 0.019	21.4 ± 0.81	41

ence of the ^{13}C in the plant component at time (t) and ^{13}C in the plant component prior to the enrichment divided by the total amount of ^{13}C incorporated into the plant during the experiment.

For the July experiment, the contribution of sucrose, fructose and glucose to the observed bulk excess ^{13}C was estimated for the rhizome and leaf tissue. The amount of ^{13}C in the carbohydrate was calculated as:

$$^{13}C \text{ in carbohydrate compound} = A \times B \times C_c \quad (7)$$

where A is the fractional abundance calculated using the isotope ratio of the carbohydrate compound and Eqs. (3) & (4), B is the biomass of the component (g dry wt), and C_c is the amount of carbon in each carbohydrate compound in the component (g C g^{-1} dry wt). To calculate the excess ^{13}C incorporated in the carbohydrate compound, we needed to subtract the value of ^{13}C prior to enrichment; unfortunately, we did not have these data, so we assumed that the isotope ratio of sucrose, fructose and glucose prior to enrichment was equivalent to the isotope ratio of the bulk tissue for each component prior to enrichment. We based this assumption on the results of Benedict et al. (1980), who found that the isotope ratio of leaf tissue of the seagrass *Thalassia testudinum* (−11.4‰) was similar to the isotope ratio of glucose and fructose in the leaves of *Thalassia* (−11.5‰). To calculate the fractional contribution of each carbohydrate, the amount of ^{13}C in the carbohydrate compound (Eq. 7) was divided by the excess ^{13}C in the bulk tissue (Eq. 5). For these calculations, we used the observed biomass and %C for the specific plant component for which we had the isotope ratio of the carbohydrate compounds rather than the average values.

The decline in excess ^{13}C (expressed as percent of ^{13}C incorporated) in the whole plant and leaves over time were fitted using a first-order exponential decay function (parameter definitions: see Table 2):

$$C = C_0 + De^{-kt} \quad (8)$$

and the accumulation of excess ^{13}C in the rhizomes and roots were fitted using a first-order exponential rise to maximum value function (C_m):

$$C = C_m(1 - e^{-k't}) \quad (9)$$

Curve fitting was calculated using the mean value of percent of excess ^{13}C incorporated of the 6 replicate values for each plant component (Table 2) and was performed using SigmaPlot (version 11.0, Systat Software). Fig. 2 shows a conceptual model for the change in excess ^{13}C in plant components as a function of time. The slope of excess ^{13}C of the whole

Table 2. First-order exponential models of loss of excess ^{13}C in *Zostera marina* whole plant and leaves (Eq. 8) and accumulation of excess ^{13}C in roots and rhizome (Eq. 9) during the February and July 2004 experiments. Models are fit using data collected during the experiment (17 and 12 d in February and July experiments, respectively). C_0: amount of ^{13}C retained in the whole plant or leaves as $t \to \infty$; D: difference between the y-intercept and C_0; k: loss rate of ^{13}C (d^{-1}); k': accumulation rate of ^{13}C (d^{-1}); C_m: maximal accumulation of ^{13}C in the below ground components

Month Component	Coefficient (mean ± SE)	p	Model R^2
February			
Whole plant	C_0 = 63.93 ± 2.06	0.001	0.98
	D = 49.22 ± 4.18	<0.01	
	k = 0.314 ± 0.051	<0.05	
Leaf	C_0 = 54.55 ± 1.71	0.001	0.99
	D = 60.40 ± 3.53	<0.005	
	k = 0.322 ± 0.035	<0.05	
Rhizome	C_m = 8.46 ± 0.47	<0.001	0.97
	k' = 0.242 ± 0.029	<0.005	
Root	C_m = 1.24 ± 0.24	<0.05	0.62
	k' = 0.500 ± 0.257	0.14	
Rhizome + root	C_m = 9.51 ± 0.68	<0.001	0.96
	k' = 0.277 ± 0.043	<0.01	
July			
Whole plant	C_0 = 50.70 ± 4.01	<0.005	0.96
	D = 50.54 ± 4.99	<0.005	
	k = 0.397 ± 0.085	<0.05	
Leaf	C_0 = 43.35 ± 3.46	<0.005	0.97
	D = 57.13 ± 4.26	<0.001	
	k = 0.385 ± 0.063	<0.01	
Rhizome	C_m = 6.80 ± 0.94	<0.005	0.82
	k' = 0.333 ± 0.103	<0.05	
Root	C_m = 0.47 ± 0.06	0.001	0.73
	k' = 4.17 ± 16.82	0.816	
Rhizome + root	C_m = 7.34 ± 1.06	<0.005	0.87
	k' = 0.352 ± 0.117	<0.05	

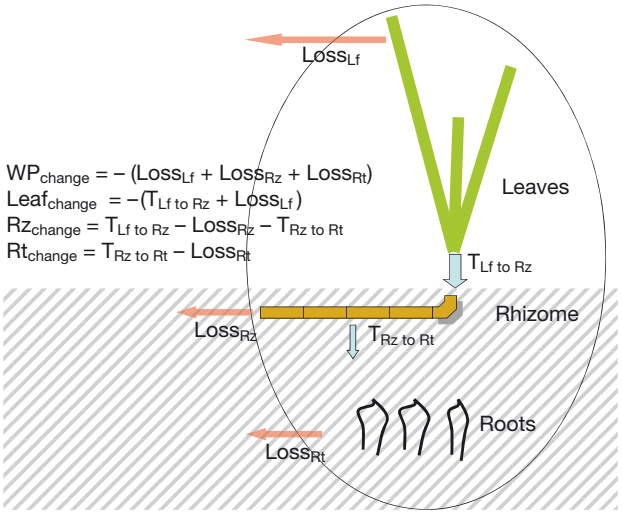

Fig. 2. *Zostera marina*. Carbon allocation and translocation. WP = whole plant; T = translocation; Lf = leaf; Rz = rhizome; Rt = root; Loss = respi-ration, detritus, herbivory, etc.

plant as a function of time (dC/dt) provides an estimate of whole plant losses, including respiration, detrital production and exudation. The dC/dt of the leaves provides an estimate of the sum of the net translocation from the leaves to the rhizome and leaf losses. The dC/dt of the rhizomes provides an estimate of net transport from the leaf to the rhizome minus losses, including rhizome losses and transport from the rhizome to the roots. The dC/dt of the roots as a function of time is an estimate of net transport from the rhizome to the root minus root losses. The dC/dt of rhizomes + roots as a function of time is an estimate of net transport from the leaf to the rhizome minus losses in the root and rhizome. The time-averaged loss or accumulation rate of excess ^{13}C was calculated as:

$$\text{Loss or accumulation rate} = \frac{1}{b-a}\int_a^b \frac{dC}{dt}\,dt \quad (10)$$

where a and b are the time limits of integration.

Other data collected

Surface irradiance was measured during the field experiments using a Li-Cor data logger (LI-1400, Li-Cor) with input from a cosine-corrected (2π) sensor. In addition, water temperatures were measured every 15 min using a YSI 6600 datasonde (Yellow Springs) deployed about 2 km from the study location.

RESULTS

During the February *in situ* experiment incubation, irradiance averaged 14 mol photons m^{-2} d^{-1}, with water temperatures ranging between 9.3 and 10.7°C. Plants exhibited small variations in the initial isotope ratio of the organs, with leaf and rhizome having similar isotope ratios of −11.16 ± 0.38 and −11.09 ± 0.35‰, respectively, while roots were −12.93 ± 0.74‰ (Fig. 3a). After 1 h pulse enrichment, leaf tissue was enriched by about 35‰, and after 24 h, leaf tissue was enriched by almost 40‰ (Δ = 39.8‰) (Fig. 3a). Rhizome δ values increased over the course of the experiment, with maximum value of 11.91 ± 12.20‰ 17 d after the enrichment pulse (Δ = 22.9‰). Root $\delta^{13}C$ values followed similar patterns and trajectories, although after 4 d of increasing $\delta^{13}C$ values (maximum 11.97 ± 6.35‰, Δ = 24.9‰) there was a decline by Day 17 to 5.71 ± 6.66‰ (Fig. 3a); however, this decline was not statistically significant (t-test, $p > 0.05$).

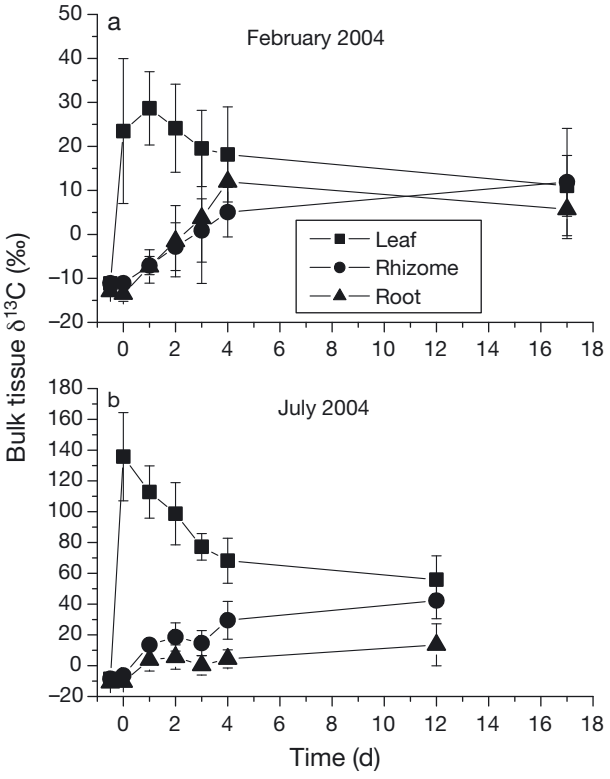

Fig. 3. *Zostera marina*. Time series of bulk tissue isotope ratio (mean ± SD, n = 6) of field plants exposed to an isotopically enriched ^{13}C-dissolved inorganic carbon pool for (a) 1 h during February 2004 and (b) 2 h during July 2004

During the July *in situ* incubation, irradiance averaged 52 mol photons m^{-2} d^{-1}, with water temperatures ranging between 9 and 18°C. Plants had similar initial leaf and rhizome isotope ratios of −8.54 ± 0.69 and −8.47± 0.42‰, respectively, while roots were more depleted (−11.01 ± 0.96‰, Fig. 3b). After a 2 h labeling pulse, leaf tissues were highly enriched, with $\delta^{13}C$ values of 139 ± 29 ‰ (Δ = 148‰). The $\delta^{13}C$ values of leaf tissues decreased by about 50% over the next 4 d (Fig. 3b). The $\delta^{13}C$ values of rhizome tissues increased to a maximum of 42 ± 12‰ (Δ = 50.5‰) 12 d after the pulse, while root tissue reached a maximum of 13.6 ± 13.6‰ (Δ = 24.6‰). Transfer of $\delta^{13}C$ occurred very quickly, with rhizome tissue showing a Δ of 2.1‰ at the end of the 2 h pulse label period. The $\delta^{13}C$ of the rhizome and roots increased throughout the time course of the experiment (Fig. 3b).

To evaluate how the ^{13}C tracer was being transported, we measured the isotope ratio of individual carbohydrate compounds. CSIA of carbohydrates detected and measured $\delta^{13}C$ values for fructose, glucose, sucrose and myo-inositol (Fig. 4). Several minor

unidentified peaks were also observed in the *Zostera marina* extracts, but these compounds were not quantified. In all tissues, myo-inositol was depleted in ^{13}C relative to the bulk tissues. Using pooled leaf and rhizome tissue data (Days 1 to 3), the enrichment of glucose, fructose and sucrose were similar and significantly greater than the enrichment of myo-inositol (Kruskal-Wallis ANOVA on ranks, $p < 0.05$). In the leaf tissue, maximum enrichment was about 258 ± 61‰ in the glucose pool 2 d after the ^{13}C pulse; this was equivalent to Δ = 160‰ relative to the bulk leaf tissue (Fig. 4). The bulk tissue would be expected to have lower enrichment since it is mainly composed of older, non-labeled structural material. In the rhizome tissue, the maximum enrichment was 55.1 ± 28.8‰ in the glucose pool 2 d after the pulse, equivalent to Δ = 36.5 ‰ relative to the rhizome bulk tissue (Fig. 4). Inadequate tissue sample size prevented a thorough evaluation of sugars from root tissue; however, the sucrose pool was enriched to 93.5 ± 60.6‰, equivalent to Δ = 88 ‰ relative to bulk root tissue (Fig. 4).

Using curve fitting and integration (Eqs. 8, 9 & 10), we calculated net translocation rates to belowground tissues and whole plant excess ^{13}C loss rates (Table 3, time-averaged over 4 d). The average excess ^{13}C incorporated into the plants was 16.8 and 58.4 µmol per plant for the February and July experiments, respectively. Time-averaged loss and accumulation rates integrated over the first 4 d of the experiment (a = 0, b = 4) are presented in Table 3. During the February experiment, whole plant decline in excess ^{13}C as a result of loss processes was 8.80 ± 1.30 % d^{-1} (Table 3, averaged over first 4 d). Additionally, by the end of the experiment (17 d) about 64 % of the original ^{13}C tracer was still retained in the plant, probably bound as stored carbohydrate or structural material (Fig. 5). Leaf tissue lost excess ^{13}C at a rate of 10.94 ± 1.09 % d^{-1} (Table 3, averaged over first 4 d) and retained about 55 % of the original tracer at the end of the February experiment (Fig. 5). Rhizome tissue gained excess ^{13}C, with an average accumulation rate of 1.31 ± 0.16 % d^{-1} (Table 3, averaged over first 4 d) and by the end of the experiment had sequestered about 8 % of the excess ^{13}C pulse (Fig. 5). During the summer experiment (July), the whole plant decline in excess ^{13}C was 10.05 ± 1.55 % d^{-1} (Table 3, averaged over first 4 d) with about 53 % of the initial tracer remaining within the plant at the end of the experiment (Fig. 6). Leaf tissue lost excess ^{13}C at a rate of 11.22 ± 1.33 % d^{-1} (Table 3, averaged over first 4 d) and retained about 45 % of the original tracer at the end of the experiment (Fig. 6). Rhizome tissue gained excess ^{13}C throughout the experiment, with an average accumulation rate of 1.25 ± 032 % d^{-1} (Table 3, averaged over first 4 d) and by the end of the experiment had sequestered about 7 % of the excess ^{13}C pulse (Fig. 6). During both experiments, the root tissue sequestered about 1 % excess ^{13}C by the end of the experiment (Fig. 5); however, the curve fitting was not statistically significant due to variability

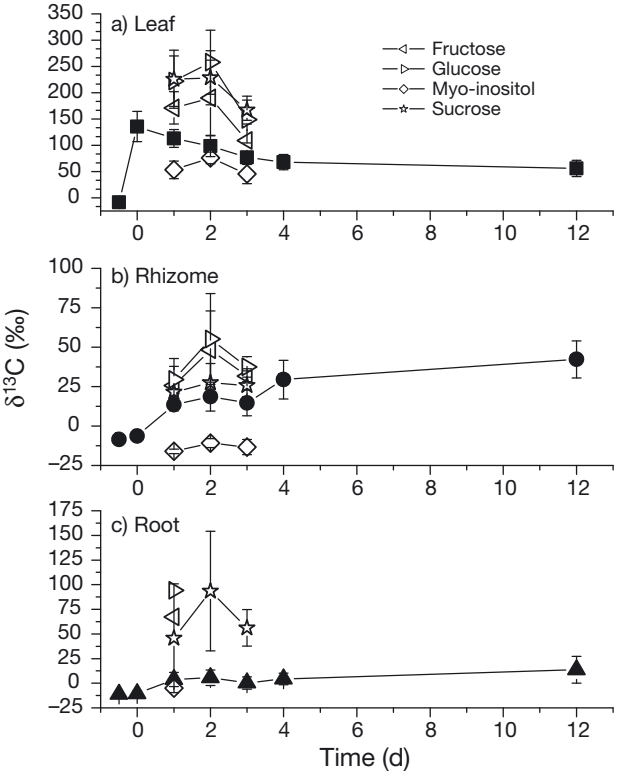

Fig. 4. *Zostera marina*. Time series (mean ± SD, n = 1 to 3) of the $\delta^{13}C$ of fructose, glucose, myo-inositol and sucrose in (a) leaves, (b) rhizomes, and (c) roots relative to the bulk tissue (solid symbols) from the July 2004 experiment

Table 3. *Zostera marina*. Summary of rates (mean ± SE) calculated from the ^{13}C stable isotope tracer experiments. Rates are expressed as the time-average over the first 4 d of the experiment

Process	February	July
Whole plant losses (% d^{-1})	8.80 ± 1.30	10.05 ± 1.55
Leaf losses (% d^{-1})	10.94 ± 1.09	11.22 ± 1.33
Net accumulation in rhizome (% d^{-1})	1.31 ± 0.16	1.25 ± 0.31
Net accumulation in root and rhizome (% d^{-1})	1.59 ± 0.22	1.39 ± 0.35

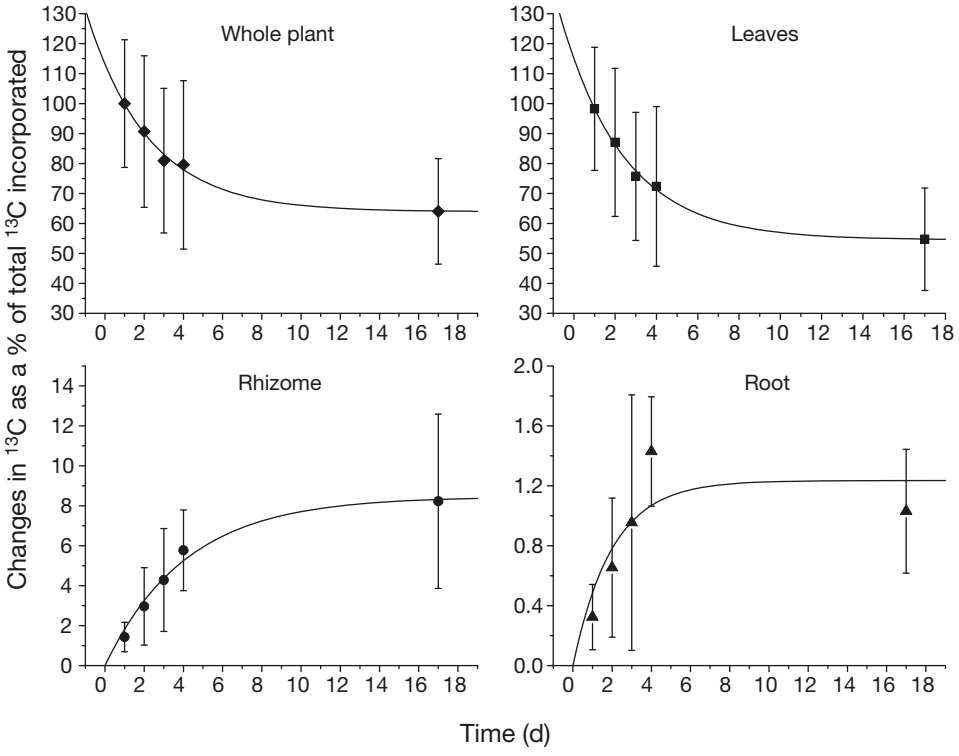

Fig. 5. *Zostera marina.* Time series of the amount of ^{13}C in each tissue (whole plant, leaf, rhizome and root) expressed as the percent of excess ^{13}C incorporated during the pulse label for the February 2004 experiment. Regression equations are presented in Table 2

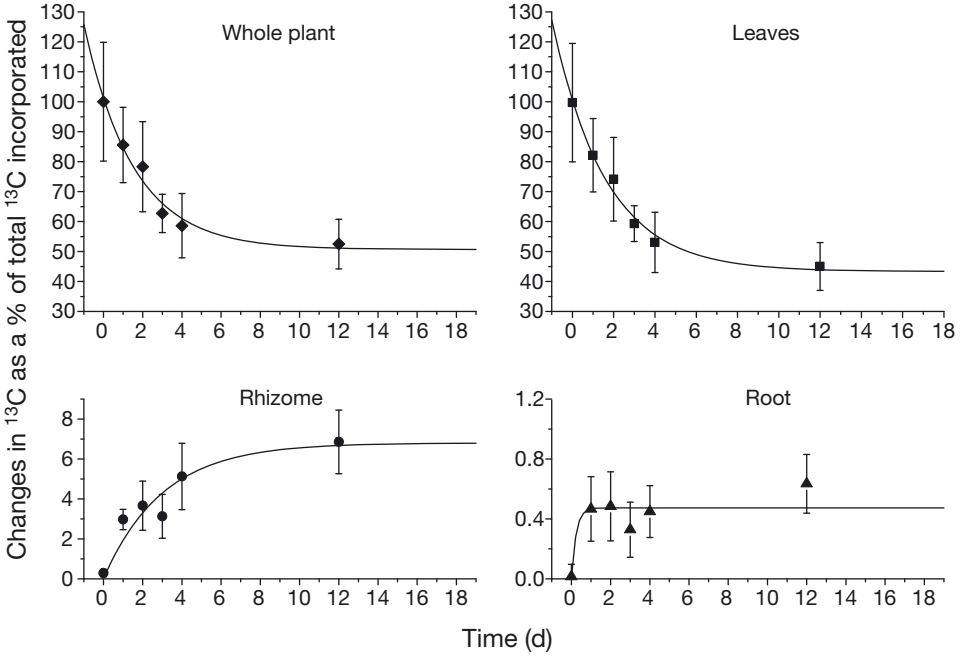

Fig. 6. *Zostera marina.* Time series of the amount of ^{13}C in each tissue (whole plant, leaf, rhizome and root) expressed as the percent of excess ^{13}C incorporated during the pulse label for the July 2004 experiment. Regression equations are presented in Table 2

(Table 2). Additional curve fitting of excess ^{13}C content was performed for rhizome plus root components (Table 2), which resulted in a slight increase in accumulation rates (Table 3).

During the July experiment, the contribution of sucrose, fructose and glucose to the observed excess ^{13}C content of the rhizomes and leaves was calculated for a limited number of samples (n = 7 for rhizome and n = 4 for leaves). During the July experiment, the contribution of fructose and glucose to excess ^{13}C of the rhizome averaged 7.1 ± 1.9 and 4.4 ± 1.1% (average of Days 1, 2 and 3 after enrichment), respectively, with the contribution of fructose increasing as the experiment progressed. During the

July experiment, the sucrose contribution to excess ^{13}C in the leaves averaged 26.1 ±4.2%, while fructose and glucose were minor contributors averaging 1.7 ± 0.3 to 1.6 ± 0.4%, respectively (average of Days 1, 2 and 3 after enrichment). During Days 1 and 2 after enrichment, the sucrose contribution to excess ^{13}C of the rhizomes averaged 32.4 ± 3.5%. On the third day after enrichment, the concentration of sucrose in the rhizome increased almost 3-fold, and as a result the sucrose contribution to the excess ^{13}C of the rhizomes increased to 112 ± 0.1%.

DISCUSSION

Leaf and rhizome tissue exhibited seasonal differences in bulk $\delta^{13}C$ values based on initial tissue $\delta^{13}C$ values, with values about 2.6‰ depleted in February relative to July. These differences may reflect the change in isotopic composition of the DIC pool available to the plants due to freshwater inflow or ocean upwelling rather than any physiological change in carbon acquisition and use. Recent work shows close coupling of ocean and estuarine water column dissolved and particulate constituents, with seasonal shifts during summer and winter conditions (Brown & Ozretich 2009, Brown & Power 2011). Riverine $\delta^{13}C$-DIC tends to be isotopically depleted relative to oceanic DIC (Chanton & Lewis 1999, Kaldy et al. 2005). In the nearshore waters of southern California, the $\delta^{13}C$-DIC of upwelled water has an isotope ratio of about 2‰, while the $\delta^{13}C$-DIC of riverine water is −11‰ (Hinger et al. 2010). Alternatively, light limitation can also result in isotopically depleted $\delta^{13}C$ values (Durako & Hall 1992, Hu et al. 2012).

Root tissue does not appear to be a major sink for recently fixed carbohydrate. Although there were differences among experiments, within each experiment leaf and rhizome tissue exhibited similar initial $\delta^{13}C$ values while roots were lighter. Differences between leaves/rhizomes and roots in the field experiments approached 2 to 2.5‰. These differences may indicate the degree of metabolic discrimination prior to and during transport to these sinks. Alternatively, root tissue is primarily structural carbohydrate, lingocellulose, which is isotopically depleted in ^{13}C relative to other carbohydrates (Benner et al. 1987, Fourqurean & Schrlau 2003). Because roots account for about 2 to 3% of the total plant carbon, differences in isotope ratios over the course of the experiment demonstrate the degree of translocation of enriched carbon (Hemminga et al. 1996).

As expected, the $\delta^{13}C$ of the leaf tissue decreased through time and the $\delta^{13}C$ of both the rhizome and root tissues (that were not exposed to label) increased, indicating that ^{13}C is translocated from the leaves to the rhizome and roots (Fig. 3). Previous studies using radioisotope (^{14}C) labels have clearly shown that translocation is both acropetal (toward the leaf apex) and basipetal (toward the base) and that transport can be detected within hours (Harrison 1978, Wetzel & Penhale 1979, Drew 1983). Although all seagrasses are clonal, rhizomatous plants, the degree of ramet physiological integration appears to be species specific and scales with plant size (Marbà et al. 2006). Many studies have focused on the physiological integration of tropical climax genera (e.g. *Posidonia*, *Thalassia*, *Enhalus*) characterized by large belowground biomass and resource-sharing capabilities (Marbà et al. 2002, Kiswara et al. 2005). At the other end of the spectrum are genera with low belowground biomass and more limited capacity for translocation (e.g. *Halophila johnsonii*, Dean & Durako 2007). *Zostera marina* along the Pacific coast of North America appears to be intermediate to these extremes. Local *Z. marina* plants tend to have long leaf lengths (>100 cm) and only 20 to 40% of total biomass as rhizome + root tissue (Kaldy 2012). Although studies indicate carbon translocation to belowground tissues of *Z. marina* (Wetzel & Penhale 1979, Zimmerman & Alberte 1996), recent work suggests a limited capacity for *Z. marina* to influence sediment biogeochemical processes directly through organic carbon exudation (Boschker et al. 2000, Kaldy 2012). The chemical composition of seagrass-derived dissolved organic constituents in the sediments remains unresolved.

The presence of highly enriched glucose and fructose in *Zostera marina* suggests that these compounds are used as transport sugars. Higher plants typically translocate non-reducing sugars (e.g. sucrose, raffinose, stachyose, etc.), since reducing sugars (e.g. glucose and fructose) contain exposed ketone or aldehyde groups that make them more reactive (Taiz & Zeiger 2002). The major soluble carbohydrates in *Z. marina* are sucrose, glucose, fructose and myo-inositol, which can account for 2.7% of extracted dry weight (Drew 1983). Myo-inositol is involved with the biosynthesis of insoluble carbohydrates such as cellulose (Drew 1983); higher levels would be expected in the meristems, since cell division and growth occur at these locations. Although myo-inositol is important for cell wall synthesis, <1% of photosynthetically fixed carbon is incorporated into this cyclitol (Drew 1978). During short-term ^{14}C

incubations, some tracer was detected in the myo-inositol pool (Drew 1983). Low-level enrichment of the myo-inositol pool is consistent with our observation of much lower $\delta^{13}C$ values than other carbohydrates (Fig. 3), suggesting that this is a slow turnover pool. The isotope ratio of the starch pool was not measured due to sample size limitations. Additionally, previous work indicates that starch generally accounts for <15% of the total non-structural carbohydrate pool in *Z. marina* (Vichkovitten et al. 2007).

Few studies have quantified metabolic losses and translocation rates in seagrasses. Our work indicates that whole plant carbon loss accounts for about 9 to 10% of the excess ^{13}C d^{-1} (Table 3). Whole plant carbon loss includes respiratory losses, detrital formation and DOC exudation, all of which remove carbon from the plant. Rates of leaf carbon loss averaged about 11% d^{-1} (4 d average, Table 3). These loss rates include translocation from the leaves to the rhizome and loss processes in the leaves (such as leaf respiration and DOC exudation). In general, these measured translocation rates for *Zostera marina* are difficult to compare to literature values because of the different methods employed. Most previous studies were of short duration, yielding hourly rates that cannot easily be converted to daily values since translocation may shut down at night due to anoxia (Zimmerman & Alberte 1996). Our data indicate that leaves lose about 10% d^{-1} of their carbon to various processes (4 d average, Table 3) and that about 45 to 55% of the excess ^{13}C incorporated is retained in the leaf tissue at the end of the experiment (12 to 17 d). It is likely that the excess ^{13}C retained in the leaves was bound into storage products such as starch or incorporated into structural material.

Integrated estimates of translocation suggest that rhizome and root tissues accumulate about 1.5% of the excess ^{13}C d^{-1} (4 d average, Table 3), which may be used to build up stored carbohydrate reserves. Interestingly, in both the February and July experiment, about 2 to 3% of the ^{13}C signal was evident in the rhizomes within 24 h of the labeling, suggesting that translocation was initially on the order of 3% d^{-1}. Despite differences in methodology, our results are similar to recent translocation estimates for *Posidonia* spp. from Australia ranging between 3.5 and 4.3% ^{13}C $shoot^{-1}$ d^{-1} (Prado et al. 2008, Table 4). Our estimate of translocation is a minimum value, since it does not account for losses due to respiration and exudation. Recent work indicates that *Zostera marina* rhizome exudation rates account for only 0.4 to 2.6% of gross primary production (Kaldy 2012). The actual translocation to the rhizomes and roots would be the net plus rhizome respiration and DOC exudation from the rhizome, which were not quantified in this study. Although *Z. marina* translocation rates may seem low, ^{13}C tracer studies on terrestrial plants indicate that recently fixed carbon is preferentially respired, as evident by strong enrichment of respired CO_2 (Carbone & Trumbore 2007, Baptist et al. 2009). During the February and July experiments, about 7.5 to 9% of the excess ^{13}C signal accumulated in the rhizomes and roots at the end of the experiment (17 and 12 d, respectively). Although previous studies have demonstrated mobilization of carbon between leaf and rhizome (Zimmerman & Alberte 1996, Boschker et al. 2000, Marbà et al. 2002, Prado et al. 2008), few studies have quantified the incorporation of the isotope signal into structural and non-structural compounds. The amount of carbon translocated to the rhizomes and roots may be an underestimate, since calculations were performed using nodes 1 to 5, and some of the ^{13}C may be transported to other portions of the rhizome. Both rhizome and root $\delta^{13}C$ values continued to increase throughout the experiments (Fig. 3), indicating that at least part of the ^{13}C-labeled pool was mobile for days to weeks. Most previous isotope tracer studies have been short term, on the time scale of several hours (Harrison 1978, Wetzel & Penhale 1979, E. A. Drew unpubl., cited in Larkum et al. 2006b). Our *in situ* experiments evaluated translocation rates over the course of 4 d.

Table 4. Summary of carbon translocation rates obtained from the literature

Species	Tissue	Translocation	Unit (%)	Source
Zostera americana[a]	Shoot	6.5 ± 0.8	per 3 h incubation	Harrison (1978)
	Flower	1.6 ± 0.2	per 3 h	Harrison (1978)
Posidonia oceanica		2.5	per 4.3 h	E. A. Drew (unpubl. data)[b]
Posidonia spp.	Shoot	3.55–4.31	d^{-1}	Prado et al. (2008)
Zostera marina	Seedling	10	d^{-1}	Zimmerman & Alberte (1996)

[a]*Zostera americana* subsequently identified as *Z. japonica* (Bigley & Barreca 1982)
[b]E. A. Drew (unpubl. data), as cited in Larkum et al. (2006b)

Although there were no significant differences in the accumulation rate of excess ^{13}C in the rhizomes during the February and July experiments (Table 3), the rates of carbon fixation and degree of sequestration do appear to exhibit seasonal differences. The apparent leaf enrichment factor (Δ) during the July experiment was almost 4 times greater (and 3.5 times more ^{13}C was incorporated) than that during the February experiment, indicating that substantially more ^{13}C carbon was fixed during July. The differences may be related to the duration of the ^{13}C pulse, 2 h during July and 1 h during February, but it is also likely that there were differences in the photosynthetic and respiration rates, which could account for differences in the amount of isotope incorporated and retained. Seagrass photosynthetic parameters, including those reported for *Zostera marina*, are highly variable and can exhibit annual patterns (Dunton & Tomasko 1994, Cabello-Pasini et al. 2003). Maximum rates of photosynthesis in *Z. marina* from the Yaquina Bay study site range between 85 and 400 µM O_2 g^{-1} leaf dry wt h^{-1} (C. Andersen unpubl. data). Since both experiments were conducted under saturating irradiance and the ^{13}C concentration was the same during both experiments, it seems likely that the photosynthetic rate during July was 1.75 times higher than that during February. The primary environmental factor influencing respiration is temperature (Touchette & Burkholder 2000 and references therein). During the July experiment, water temperatures were up to 8°C higher than those during the February experiment (maximum February water temperature = 10.7°C, while maximum July water temperature = 18.1°C; calculated using water temperature data from the first 4 d of each experiment). Using water temperature data during each experiment and measured respiration rates from other experiments (C. Andersen unpubl. data), the whole plant respiration could have been 14% higher in July than in February. Thus, changes in the balance of production to respiration could account for the observation that 4 d after the initiation of the experiment, 80% of the excess ^{13}C remained in the plant in February, while only 59% remained in the plant in July. Additionally, it is not entirely clear what fraction of this excess ^{13}C remains as sugars that can be remobilized and what fraction has been incorporated to structural or less mobile compounds.

Our experiments show that translocation rates to the rhizome were similar between winter (February) and summer (July) experiments, indicating that carbohydrate translocation rates may not exhibit strong seasonal patterns despite other temperature-driven patterns in respiration and production. Carbohydrate concentrations (sucrose + glucose + fructose) in the rhizomes during the July experiment were twice those during the February experiment, suggesting a strong seasonal pattern of storage during this time (Table 5). Numerous papers have noted that stored carbohydrate content is usually higher during the summer months when light is plentiful and that these reserves are drawn down during low light periods typical of winter conditions (Ott 1979, Dunton 1994, Burke et al. 1996, Larkum et al. 2006b). Although we assume sugars were the primary transport and storage compound, some of the fixed ^{13}C may have been incorporated into other unquantified soluble organic compounds such as free amino acids (FAA) and soluble proteins (Invers et al. 2002). The presence of ^{13}C labeled FAA would be reflected in the bulk tissue isotope ratios but not the compound-specific carbohydrates. Since we explicitly focus on ^{13}C-labeled sucrose, glucose, fructose and myo-inositol, the role of FAA and other soluble proteins in whole plant carbon dynamics requires further examination.

The results show the dynamic nature of carbon acquisition and allocation in *Zostera marina*. Stable isotopes and compound-specific isotope analyses proved to be a powerful tool for examining the carbon dynamics within individual plants in the laboratory and in intact ecosystems. Using these tools in a ^{13}C tracer experiment, we were able to quantify minimum translocation rates and empirically evaluate carbon allocation within *Z. marina*. The results provide critical information for the development and parameterization of mechanistic models that can be used to accurately evaluate seagrass ecosystem dynamics and function or to assess stressor-response scenarios for resource managers. Additionally, to cal-

Table 5. Carbohydrate concentrations in *Zostera marina* (mean ± SE) during the February and July 2004 experiments

Experiment	Tissue	Sucrose (µmol g^{-1} dry wt)	Glucose (µmol g^{-1} dry wt)	Fructose (µmol g^{-1} dry wt)	Sample size
February	Leaf	397.7 ± 97.0	67.1 ± 20.3	71.7 ± 24.0	9
	Rhizome	408.9 ± 81.3	58.9 ± 10.8	67.2 ± 15.6	6
	Root	166.8 ± 98.6	73.2 ± 37.1	74.5 ± 39.4	7
July	Leaf	268.5 ± 22.5	32.2 ± 4.2	48.9 ± 11.0	4
	Rhizome	972.6 ± 280.0	119.8 ± 37.6	208.9 ± 57.7	6
	Root	91.0 ± 38.4	8.6 ± 2.0	3.8 ± 1.4	6

culate whole plant carbon budgets (including accumulations, respiratory losses and leakage), it is critical to understand carbon dynamics and translocation rates. Eelgrass growing at this temperate latitude experiences large variations in solar irradiance, temperature, salinity and nutrient loads. Our results show that changes in seasonal temperature and solar irradiance likely alter rates of carbon acquisition and respiration rates, but overall allocation patterns and translocation rates do not vary significantly in response to these environmental drivers.

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LITERATURE CITED

Baptist F, Tcherkez G, Aubert S, Pontailler JY, Choler P, Nogués S (2009) ^{13}C and ^{15}N allocations of two alpine species from early and late snowmelt locations reflect their different growth strategies. J Exp Bot 60:2725–2735

Benedict CR, Wong WWL, Wong JHH (1980) Fractionation of the stable isotopes of inorganic carbon by seagrasses. Plant Physiol 65:512–517

Benner R, Fogel ML, Sprague EK, Hodson RE (1987) Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature 329:708–710

Bigley RE, Barreca JL (1982) Evidence for synonymizing *Zostera Americana* Den Hartog with *Zostera japonica* Aschers. & Graebn. Aquat Bot 14:349–356

Bittaker HF, Iverson RL (1976) *Thalassia testudinum* productivity: a field comparison of measurement methods. Mar Biol 37:39–46

Boschker HTS, Nold SC, Wellsbury P, Bos D and others (1998) Direct linking of microbial populations to specific biogeochemical processes by C^{13}-labelling biomarkers. Nature 407:623–626

Boschker HTS, Wielemaker A, Schaub BEM, Holmer M (2000) Limited coupling of macrophyte production and bacterial carbon cycling in the sediments of *Zostera* spp. meadows. Mar Ecol Prog Ser 203:181–189

Brown CA, Ozretich RJ (2009) Coupling between the coastal ocean and Yaquina Bay, Oregon: importance of oceanic inputs relative to other nitrogen sources. Estuar Coasts 32:219–237

Brown CA, Power JH (2011) Historic and recent patters of dissolved oxygen in the Yaquina Estuary (Oregon, USA): importance of anthropogenic activities and oceanic conditions. Estuar Coast Shelf Sci 92:446–455

Burd, A.B., K.H. Dunton (2001) Field verification of a light-driven model of biomass changes in the seagrass *Halodule wrightii.* Mar Ecol Prog Ser 209: 85–98

Burke MK, Dennison WC, Moore KA (1996) Non-structural carbohydrate reserves of eelgrass *Zostera marina.* Mar Ecol Prog Ser 137:195–201

Cabello-Pasini A, Lara-Turrent C, Zimmerman RC (2002) Effect of storms on photosynthesis, carbohydrate content and survival of eelgrass populations from a coastal lagoon and the adjacent open ocean. Aquat Bot 74:149–164

Cabello-Pasini A, Muniz-Salazar R, Ward DH (2003) Annual variations of biomass and photosynthesis in *Zostera marina* at its southern end of distribution in the North Pacific. Aquat Bot 76:31–47

Carbone MS, Trumbore SE (2007) Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns. New Phytol 176:124–135

Chanton JP, Lewis FG (1999) Plankton and dissolved organic carbon isotopic composition in a river-dominated estuary: Apalachicola Bay, Florida. Estuaries 22:575–583

Collier CJ, Prado P, Lavery PS (2010) Carbon and nitrogen translocation in response to shading of the seagrass *Posidonia sinuosa.* Aquat Bot 93:47–54

Cox SE, Stushnoff C (2001) Temperature-related shifts in soluble carbohydrate content during dormancy and cold acclimation in *Populus tremuloides.* Can J For Res 31: 730–737

Dawes CJ, Lawrence JM (1980) Seasonal changes in the proximate constituents of the seagrasses *Thalassia testudinum, Halodule wrightii* and *Syringodium filiforme.* Aquat Bot 8:371–380

Dean RJ Jr, Durako MJ (2007) Carbon sharing through physiological integration in the threatened seagrass *Halophila johnsonii.* Bull Mar Sci 81:21–35

Devereux R, Yates DF, Aukamp J, Quarles RL, Jordan SJ, Stanley RS, Eldridge PM (2011) Interactions of *Thalassia testudinum* and sediment biogeochemistry in Santa Rosa Sound, NW Florida. Mar Biol Res 7:317–331

Drew EA (1978) Carbohydrate and inositol metabolism in the seagrass *Cymodocea nodosa.* New Phytol 81:249–264

Drew EA (1983) Sugars, cyclitols and seagrass phylogeny. Aquat Bot 15:387–408

Dunton KH (1994) Seasonal growth and biomass of the subtropical seagrass *Halodule wrightii* in relation to continuous measurements of underwater irradiance. Mar Biol 120:479–489

Dunton KH, Tomasko DA (1994) *In situ* photosynthesis in the seagrass *Halodule wrightii* in a hypersaline subtropical lagoon. Mar Ecol Prog Ser 107:281–293

Dunton KH, Burd A, Cifuentes L, Eldridge PM, Morse JW 2003. Concluding report. Effects of dredge deposits on seagrasses: an integrative model for Laguna Madre. US Army Corps of Engineers, Galveston District. http://ww3.swg.usace.army.mil/items/Laguna/special_studies/SeagrassReport.pdf

Durako MJ, Hall MO (1992) Effect of light on the stable carbon isotope composition of the seagrass *Thalassia testudinum.* Mar Ecol Prog Ser 86:99–101

Eldridge PM, Kaldy JE, Burd AB (2004) Stress response model for the tropical seagrass *Thalassia testudinum*: the interactions of light, temperature, sedimentation and geochemistry. Estuaries 27:923–937

Fourqurean JW, Schrlau JE (2003) Changes in nutrient content and stable isotope ratios of C and N during decomposition of seagrasses and mangrove leaves along a nutrient availability gradient in Florida Bay, USA. Chem Ecol 19:373–390

Harrison PG (1978) Patterns of uptake and translocation of ^{14}C by *Zostera americana* den Hartog in the laboratory. Aquat Bot 5:93–97

Heck KL Jr, Carruthers TJB, Duarte CM, Hughes AR, Kendrick G, Orth RJ, Williams SL (2008) Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. Ecosystems 11:1198–1210

Hemminga MA, Duarte CM 2000. Seagrass ecology. Cambridge University Press, Cambridge

Hemminga MA, Huiskes AHL, Steegstra M, van Soelen J (1996) Assessment of carbon allocation and biomass production in a natural stand of the salt marsh plant *Spartina anglica* using ^{13}C. Mar Ecol Prog Ser 130:169–178

Herzka SZ, Dunton KH (1998) Light and carbon balance in the seagrass *Thalassia testudinum*: evaluation of current production models. Mar Biol 132:711–721

Hinger EN, Santos GM, Druffel ERM, Griffen S (2010) Carbon isotope measurements of surface seawater from a time-series site off southern California. Radiocarbon 52: 69–89

Holmer M, Andersen FO, Nielsen SL, Boschker HTS (2001) The importance of mineralization based on sulfate reduction for nutrient regeneration in tropical seagrass sediments. Aquat Bot 71:1–17

Hu X, Burdige DJ, Zimmerman RC (2012) δ^{13}C is a signature of light availability and photosynthesis in seagrass. Limnol Oceanogr 57:441–448

Invers O, Pérez M, Romero J (2002) Seasonal nitrogen speciation in temperate seagrass *Posidonia oceanica* (L.) Delile. J Exp Mar Biol Ecol 273: 219–240

Jones WB, Cifuentes LA, Kaldy JE (2003) Stable carbon isotope evidence for coupling between sedimentary bacteria and seagrasses in a sub-tropical lagoon. Mar Ecol Prog Ser 255:15–25

Kaldy JE (2006) Carbon, nitrogen, phosphorus and heavy metal budgets: How large is the eelgrass (*Zostera marina* L.) sink in a temperate estuary? Mar Pollut Bull 52: 342–353

Kaldy J (2012) Influence of light, temperature and salinity on dissolved organic carbon exudation rates in *Zostera marina* L. Aquat Biosys 8:19

Kaldy JE, Lee KS (2007) Factors controlling *Zostera marina* L. growth in the eastern and western Pacific Ocean: comparisons between Korea and Oregon, USA. Aquat Bot 87: 116–126

Kaldy JE, Cifuentes LA, Brock D (2005) Using stable isotope analyses to assess carbon dynamics in a shallow subtropical estuary. Estuaries 28:86–95

Kaldy JE, Eldridge PM, Cifuentes LA, Jones WB (2006) Utilization of DOC from seagrass rhizomes by sediment bacteria: ^{13}C-tracer experiments and modeling. Mar Ecol Prog Ser 317:41–55

Kenworthy WJ, Wyllie-Echeverria S, Coles RG, Pergent G, Pergent-Martini C (2006) Seagrass conservation biology: an interdisciplinary science for protection of the seagrass biome. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: biology, ecology and conservation. Springer, Dordrecht, p 595–623

Kiswara W, Huiskes AHL, Herman PMJ (2005) Uptake and allocation of ^{13}C by *Enhalus acoroides* at sites differing by light availability. Aquat Bot 81:353–366

Kraemer GP, Alberte RS (1993) Age-related patterns of metabolism and biomass in subterranean tissues of *Zostera marina* (eelgrass). Mar Ecol Prog Ser 95:193–203

Larkum AWD, Orth RJ, Duarte CM (eds) (2006a) Seagrasses: biology, ecology and conservation. Springer, Dordrecht

Larkum AWD, Drew EA, Ralph PJ (2006b) Photosynthesis and metabolism in seagrasses at the cellular level. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: biology, ecology and conservation. Springer, Dordrecht, p 323–345

Lepoint G, Defawe O, Gobert S, Dauby P, Bouquegneau JM (2002) Experimental evidence for N recycling in the leaves of the seagrass *Posidonia oceanica*. J Sea Res 48: 173–179

Lepoint G, Dauby P, Gobert S (2004) Applications of C and N stable isotopes to ecological an environmental studies in seagrass ecosystems. Mar Pollut Bull 49:887–891

Marbà N, Hemminga MA, Mateo MA, Duarte CM, Mass YEM, Terrados J, Gacia E (2002) Carbon and nitrogen translocation between seagrass ramets. Mar Ecol Prog Ser 226:287–300

Marbà N, Hemminga MA, Duarte CM (2006) Resource translocation within seagrass clones: allometric scaling to plant size and productivity. Oecologia 150:362–372

McRoy CP, Helfferich C (eds) (1977) Seagrass ecosystems. Marcel Dekker, New York, NY

Miller HL III, Meile C, Burd AB (2007) A novel 2D model of internal O_2 dynamics and H2S intrusion in seagrasses. Ecol Model 205:365–380

Orth RJ, Carruthers TJB, Dennison WC, Duarte CM and others (2006) A global crisis for seagrass ecosystems. Bioscience 56:987–996

Ott JA (1979) Persistence of a seasonal growth rhythm in *Posidonia oceanica* (L.) Delile under constant conditions of temperature and illumination. Mar Biol Lett 1:99–104

Phillips RC, McRoy CP (eds) (1980) Handbook of seagrass biology: an ecosystem perspective. Garland STPM Press, New York, NY

Pirc H (1985) Growth dynamics in *Posidonia oceanica* (L.) Delile. I. Seasonal changes of soluble carbohydrates, starch, free amino acids and organic anions in different parts of the plant. PSZN I: Mar Ecol 6:141–165

Prado P, Collier CJ, Lavery PS (2008) ^{13}C and ^{15}N translocation within and among shoots in two *Posidonia* species from Western Australia. Mar Ecol Prog Ser 361:69–82

Short FT, Polidoro B, Livingstone SR, Carpenter KE and others (2011) Extinction risk assessment of the world's seagrass species. Biol Conserv 144:1961–1971

Taiz L, Zeiger E (2002). Plant physiology, 3rd edn. Sinauer Assoc, Sunderland, MA

Touchette BW, Burkholder JM (2000) Overview of the physiological ecology of carbon metabolism in seagrasses. J Exp Mar Biol Ecol 250:169–205

Vichkovitten T, Holmer M, Frederiksen MS (2007) Spatial and temporal changes in non-structural carbohydrate reserves in eelgrass (*Zostera marina* L.) in Danish coastal waters. Bot Mar 50:75–87

Wetzel RG, Penhale PA (1979) Transport of carbon and excretion of dissolved organic carbon by leaves and roots/rhizomes in seagrasses and their epiphytes. Aquat Bot 6:149–158

Zimmerman RC (2003) A biooptical model of irradiance distribution and photosynthesis in seagrass canopies. Limnol Oceanogr 48:568–585

Zimmerman RC, Alberte RS (1996) Effect of light/dark transition on carbon translocation in eelgrass *Zostera marina* seedlings. Mar Ecol Prog Ser 136:305–309

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