Journal of Experimental Marine Biology and Ecology 507 (2018) 53–60

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Nitrogen uptake and allocation estimates for *Spartina alterniflora* and *Distichlis spicata*

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ARTICLE INFO

Keywords:
Nitrogen uptake
Nitrogen-15
Salt marsh
Spartina alterniflora
Distichlis spicata

ABSTRACT

Salt marshes have the potential to intercept nitrogen that could otherwise impact coastal water quality. Salt marsh plants play a central role in nutrient interception by retaining N in above- and belowground tissues. We examine N uptake and allocation in two dominant salt marsh plants, short-form *Spartina alterniflora* and *Distichlis spicata*. Nitrogen uptake was measured using ^{15}N tracer experiments conducted over a four-week period, supplemented with stem-level growth rates, primary production, and microbial denitrification assays. By varying experiment duration, we identify the importance of a rarely-measured aspect of experimental design in ^{15}N tracer studies. Experiment duration had a greater impact on quantitative N uptake estimates than primary production or stem-level relative growth rates. Rapid initial scavenging of added ^{15}N caused apparent nitrogen uptake rates to decline by a factor of two as experiment duration increased from one week to one month, although each experiment shared the qualitative conclusion that *Distichlis* roots scavenged N approximately twice as rapidly as *Spartina*. We estimate total N uptake into above- and belowground tissues as 154 and 277 mg $N \cdot m^{-2} \cdot d^{-1}$ for *Spartina* and *Distichlis*, respectively. Driving this pattern were higher N content in *Distichlis* leaves and belowground tissue and strong differences in primary production; *Spartina* and *Distichlis* produced 8.8 and 14.7 g biomass $\cdot m^{-2} \cdot d^{-1}$. Denitrification potentials were similar in sediment associated with both species, but the strong species-specific difference in N uptake suggests that *Distichlis*-dominated marshes are likely to intercept more N from coastal waters than are short-form *Spartina* marshes. The data and source code for this manuscript are available as an R package from https://github.com/troyhill/NitrogenUptake2016.

1. Introduction

Nitrogen (N) enrichment in coastal ecosystems is a chronic and widespread phenomenon (Paerl et al., 2002), and one that may be exacerbated by changing precipitation regimes (Sinha et al., 2017). Salt marshes occur along terrestrial margins and serve as buffers, retaining and transforming N before it reaches coastal waters (Craft et al., 2009) and can contribute to cultural eutrophication. The N-buffering capacity of salt marshes reflects the combined effects of microbial conversion of N to gaseous forms, sediment accumulation, and plant uptake of mineral N into organic tissues.

Microbial denitrification converts bioavailable forms of N to less reactive gaseous compounds (e.g., Koop-Jakobsen and Giblin, 2010; Yang et al., 2015) that can be readily exported to the atmosphere. The magnitude of denitrification is estimated at ~25% of annual plant N demand (White and Howes, 1994). Burial of N in deposited mineral sediment is a function of cation exchange capacity and sediment accumulation rates, both of which are variable and potentially impermanent (Anisfeld and Benoit, 1997). Nitrogen is also taken up by plants and stored in organic tissue during the growing season, when it is, in roughly equal parts, released to coastal waters or translocated to belowground plant structures for future re-use (Hopkinson and Schubauer, 1984) or long-term burial in dead organic tissues (White and Howes, 1994). This understanding of plant N cycling suggests that approximately half of annual plant N demand is supplied by inorganic N imported in coastal waters or produced by local N fixation.

In salt marshes from the northeastern United States to the Gulf Coast, short-form *Spartina alterniflora* Loisel and *Distichlis spicata* (L.)

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https://doi.org/10.1016/j.jembe.2018.07.006
Received 15 December 2017; Received in revised form 19 July 2018; Accepted 21 July 2018
0022-0981/ Published by Elsevier B.V.

Greene (hereafter *Spartina* and *Distichlis*) can co-occur and behave as dominants on the marsh platform. Both species are typically N-limited (Smart and Barko, 1980) and respond positively to N enrichment throughout their ranges, increasing their biomass and dominance (Levine et al., 1998; Pennings et al., 2002). To the extent that these species differ in N uptake capacity, environmental changes affecting their dominance may have ancillary effects on ecosystem-scale nutrient interception.

Nutrient enrichment itself can affect competition between these species, potentially signaling disparate N uptake capacities. Community ecology theory suggests that relief from nutrient limitation may lead to *Spartina* to outcompete *Distichlis*, as competition shifts from nutrients to light (Levine et al., 1998). However, nutrient enrichment experiments have shown *Distichlis* increasing in dominance at the expense of *Spartina* (Fox et al., 2012; Pennings et al., 2002). The nature of the changing N regime - whether additional N is delivered as a "press" through a rising baseline level or in episodic pulses - may also play a role in enrichment effects on species cover and the ability of marsh plants to capture N. Understanding N uptake dynamics in these two species may provide insight into competitive outcomes under eutrophic conditions, and can provide a basis for estimating the nutrient-interception implications of shifts in dominance, regardless of cause.

The use of ^{15}N as a tracer is a common means of investigating N uptake dynamics. This approach is particularly useful when N stocks are poorly constrained or where production and N uptake may be loosely coupled. As a methodological tool ^{15}N is powerful, but cost and labor requirements often limit applications to a single time interval. Time periods used for ^{15}N studies vary widely, from two days (Mozdzer et al., 2014) to three months (Oczkowski et al., 2015) to as much as seven years (White and Howes, 1994). Because uptake rates are calculated based on the amount of ^{15}N accumulated and the time elapsed, tracer studies using different time intervals have differential sensitivity to temporal variation in N uptake, and at least over long time periods emphasize different processes, as initial plant-microbe competition gives way to translocation dynamics and leaching rates (White and Howes, 1994).

We conducted a ^{15}N tracer study to improve our understanding of N capture and allocation by *Spartina* and *Distichlis*, and to optimize the application and interpretation of ^{15}N tracer methods. Our first objective was to quantify N uptake and allocation to above- and belowground tissues over a one-month time scale. Based on a zonation paradigm in which plants occupying less stressful, higher-elevation areas more efficiently compete for nutrients (Levine et al., 1998), we hypothesized that *Distichlis* would scavenge N more efficiently than *Spartina*. We further hypothesized that in both species, N would be partitioned rapidly into aboveground photosynthetic tissues. Our second objective was to test the importance of potential determinants of N capture. The determinants we considered were relative growth rates of individual shoots, primary production, root biomass, and experiment duration. We hypothesized that shoot growth rates and root biomass will be critical determinants of N uptake, respectively mediating demand for, and access to, dissolved N.

2. Material and methods

2.1. Experimental design

On 21 June 2016, vegetated salt marsh cores (hereafter "mesocosms") were collected from Colt State Park in Bristol, RI. Mesocosms were collected by inserting modified polyvinylchloride (PVC) coring tubes (35 cm × 10 cm dia.) into the marsh platform. Live aboveground biomass was protected from damage during retrieval using PVC extensions. After extracting the mesocosms from the marsh, mesh netting (0.5 mm) was secured to the bottom of the PVC to prevent sediment loss during the experiment. A total of 30 mesocosms were collected, 15 each from monoculture areas of short-form *Spartina* and *Distichlis* at similar elevations on the marsh platform.

Following collection, mesocosms were transferred to a tidal basin (0.6 m × 1 m dia.) located in an outdoor greenhouse at the US Environmental Protection Agency's Atlantic Ecology Division. Semidiurnal tides were simulated with seawater pumped from Narragansett Bay and timer-operated solenoid valves at the input and outlet of the basin. Using the same system described by Hanson et al. (2016), the water level in the tidal basin was gradually raised from low tide (0.20 m) to high tide (0.45 m; determined by a standpipe) over a four-hour period beginning when the input solenoid was opened at 5 a.m. each morning. Flood tide was followed by a two hour slack tide when the input solenoid was closed, a four hour period of ebb tide drainage (drain solenoid opened), and a two hour slack low tide before the cycle began again. Mesocosms were positioned on a grate to allow drainage, and were inundated to a depth of 0.05 m during high tides. Mesocosms were redistributed inside the tidal basin every three days to homogenize exposure to ambient sunlight.

2.2. Allometry and aboveground production

Every live culm in each mesocosm was tagged and heights were measured to the nearest millimeter on 22 June, 29 June, 6 July, 13 July, and 20 July 2016. Stem heights were also recorded for mesocosms as they were harvested. New shoots were tagged throughout the experiment, and in total 839 unique shoots were tracked. Stem densities were calculated directly from counts of live shoots, and shoot masses were estimated from heights using species-specific allometry equations developed for the marsh where mesocosms were initially collected.

Allometric models were developed from live shoots collected from Colt State Park in May, June, and July 2016. At each sampling event, three 25 × 25 cm quadrats were collected from monoculture areas of short-form *Spartina* and *Distichlis*. Stems were cut at the sediment surface, measured to the nearest millimeter, and a representative subsample of stems were individually dried to constant weight at 50 °C. Masses were modeled as a function of height following Lu et al. (2016), with linear models parameterized using Box-Cox power transformations of biomass (λ) selected to maximize normality of residuals (Box and Cox, 1964). Species-specific allometry equations took the form $mass = e^{(height \cdot a + b)}$ for $\lambda = 0$, and $mass = (height \cdot a + b)^{1/\lambda}$ for $\lambda \neq 0$ (Box and Cox, 1964). These allometry equations were applied to the stem height measurements collected in the greenhouse experiment. Net aboveground primary production (NAPP) was calculated for each mesocosm as the sum of positive live biomass increments at the mesocosm-scale (Milner and Hughes, 1968).

The performance of allometry models was evaluated by comparing predicted biomass with biomass directly measured during mesocosm harvests. By this measure, allometry-based biomass estimates averaged 31 $g \cdot m^{-2}$ (14%) higher than the total observed biomass (Hill et al. (Submitted)). Although the absolute magnitude of the errors was similar between species, the lower total biomass in *Spartina* mesocosms increased the proportional error, making *Distichlis* allometry models more accurate by this measure (8% vs. 19% error).

Estimated shoot masses were used to calculate relative growth rates (RGR; $g \cdot g^{-1} \cdot d^{-1}$); the rate of biomass accumulation per unit of biomass. RGR was calculated for each tagged shoot as the difference between sequential log-transformed shoot masses ($M_{t\text{-}1}$ and M_t in eq. 1) divided by the time interval between measurements (Hunt, 1990). For shoot i over the time period $T_{t\text{-}1}$ to T_t, RGR was calculated as:

$$RGR_{i_t} = \frac{\ln(M_{i_t}) - \ln(M_{i_{t-1}})}{T_t - T_{t-1}} \quad (1)$$

RGR was averaged across all unique stems in each mesocosm. When multiple growth rate measurements were available for an individual shoot, they were averaged to get a single representative quantity for each unique shoot, before calculating a mesocosm average.