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Salt marsh restoration surprise: A subordinate species accumulates and shares nitrogen while outcompeting salt marsh dominants

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Selectively planting native species could guide ecosystem development toward wetland restoration targets, once we understand how influential species function, alone and in combination. Knowing that *Triglochin concinna* (arrow grass, Juncaceae) accumulates N in its perennial roots, we asked how it would influence N dynamics on an excavated salt marsh plain at Tijuana Estuary, in southern California. We hypothesized that it would (a) accumulate N in roots and shoots, (b) reduce biomass of other marsh plain plants or, alternatively, (c) share N with neighbors as its litter decomposed and released N. We used ^{15}N stable isotope enrichment to quantify N transfer between *Triglochin* and the marsh plain's seven-species halophyte assemblage in field and greenhouse experiments. We also examined the effect of *Triglochin* on individual marsh plain species' biomass and N accumulation. *Triglochin* had low shoot biomass ($0.96\pm0.5\,\text{gm}^{-2}$ in field plots and $17.64\pm2.2\,\text{gm}^{-2}$ in greenhouse pots), high root:shoot ratios (4.3 in the field and 2.0 in the greenhouse), and high tissue N content ($1.9\pm0.2\%$ in the field and $1.7\pm0.1\%$ in the greenhouse). Two productive perennials, *Sarcocornia pacifica* (pickleweed) and *Frankenia salina* (alkali heath), outgrew *Triglochin;* yet these biomass dominants produced 44%–45% less shoot biomass in greenhouse pots with *Triglochin* than without. However, we did not find this reduction in the field where roots were unconfined. In the greenhouse, δ^{15}N values were higher for species grown with ^{15}N-enriched *Triglochin*, indicating that this species made N available to its neighbors. The δ^{15}N values for plants grown in the field exceeded background levels, also indicating that the marsh plain assemblage took up N released by *Triglochin*. We conclude that *Triglochin* can influence the restoration of salt marsh vegetation by accumulating N and releasing its tissue N to neighbors as leaves and roots decompose, while simultaneously reducing the biomass of neighbors. The seasonally deciduous *Triglochin* is low in shoot biomass, yet competitively superior in N uptake. Because this often-ignored species has limited tidal dispersal, we suggest restoration plantings, including tests of its ability to facilitate diversity where *S. pacifica*, the marsh plain dominant, might otherwise form monocultures.

Introduction

Coastal wetlands have been degraded for centuries but are being restored globally to regain valued ecosystem services (biodiversity support, erosion control, wildlife habitat, productivity, trophic support, and cultural uses; Gann et al., 2019). In southern California, urbanization, pollution (e.g., 2021 Huntington Beach oil spill), sea level rise, storm surges, and global warming Thorne et al., 2018; Armitage, 2021) continue to threaten remnant and restored salt marshes. Mitigation for impacts involves restoration (and creation) of tidal marshes and endangered species habitat, with strict criteria, but uncertain outcomes (Doherty et al., 2011; Adam, 2019).

Field experiments in wetland remnants and on newly graded marsh plains have revealed halophyte traits and methods for restoration (Zedler, 2001; Bonin and Zedler, 2008; Janousek et al., 2019). Understandably, research has focused on dominant plant species, especially *Sarcocornia pacifica* Standl. (*Salicornia virginica* L.) and the dynamics of nitrogen (N), which is often limited in restored marshes (Boyer and Zedler, 1998; Boyer et al., 2001; Ryan and Boyer, 2012; Cott et al., 2018). One method for non-mycorrhizal species to acquire N when planted in nutrient-poor substrates is N transfer from N-rich sources to N-poor tissues (Haystead et al., 1988; Moyer-Henry et al., 2006; He et al., 2009; Teste et al., 2015).

Non-dominant species can play a critical role in restoring N dynamics (Sullivan et al., 2007). Species with high N tissue content can accumulate biomass over time and strongly influence nutrient dynamics through seasonal uptake and release (Berendse, 1994). In southern California, *Triglochin concinna* Davy (Juncaceae, hereafter *Triglochin*), could be playing such a role. This subordinate species has perennial roots and rhizomes, with deciduous shoots. Its leaves emerge in fall and reach peak biomass by late spring; it flowers and fruits between January and June, and shoots die back by midsummer (Sullivan and Noe, 2001). *Triglochin* expands clonally and from seed (Keer and Zedler, 2002). In the nearby experimental "Tidal Linkage" marsh, *Triglochin* monocultures had low biomass ($7.0 \pm 1.9 \, \mathrm{g \, m^{-2}}$, Callaway et al., 2003), low cover and height (~10–15 cm), and minimal layering (Keer and Zedler, 2002). In greenhouse pots, *Triglochin* had high N tissue content (17–$52 \, \mathrm{mg \, g^{-1}}$), high N uptake rates and roots that concentrated N (Sullivan and Zedler, 1999; Callaway et al., 2003; Sullivan et al., 2007; Morzaria-Luna and Zedler, 2014). Canopy cover of *Triglochin* and *S. pacifica* were negatively correlated in the field (Morzaria-Luna et al., 2004); and in a greenhouse experiment, *S. pacifica* and *Triglochin* competed, especially with prolonged stress (low water level and N supply) (Morzaria-Luna and Zedler, 2014). These observations and experimental results suggested a detailed study of its capabilities in mixed restoration plantings.

We hypothesized that *Triglochin* could influence community dynamics in a newly planted tidal restoration site by (a) accumulating N in roots and shoots, (b) reducing biomass of other marsh plain plants or, alternatively, (c) sharing N with neighbors as its litter decomposed and released N. We expected *Triglochin* to develop a "nitrogen reservoir" in a mostly-bare restoration site (by accumulating N in roots and shoots), and that it would transfer N to seven other planted species, as its deciduous leaves decompose. We set up a large field experiment in the Model Marsh at Tijuana Estuary in 2001 (Figure 1) and we duplicated the design in 2002 in greenhouse pots, where we could simulate tidal flushing. The greenhouse experiment served as a back-up, because in 2000 the Model Marsh lost over 5,000 plantings because it was not connected to tidal influence on schedule (see Zedler et al., 2003).
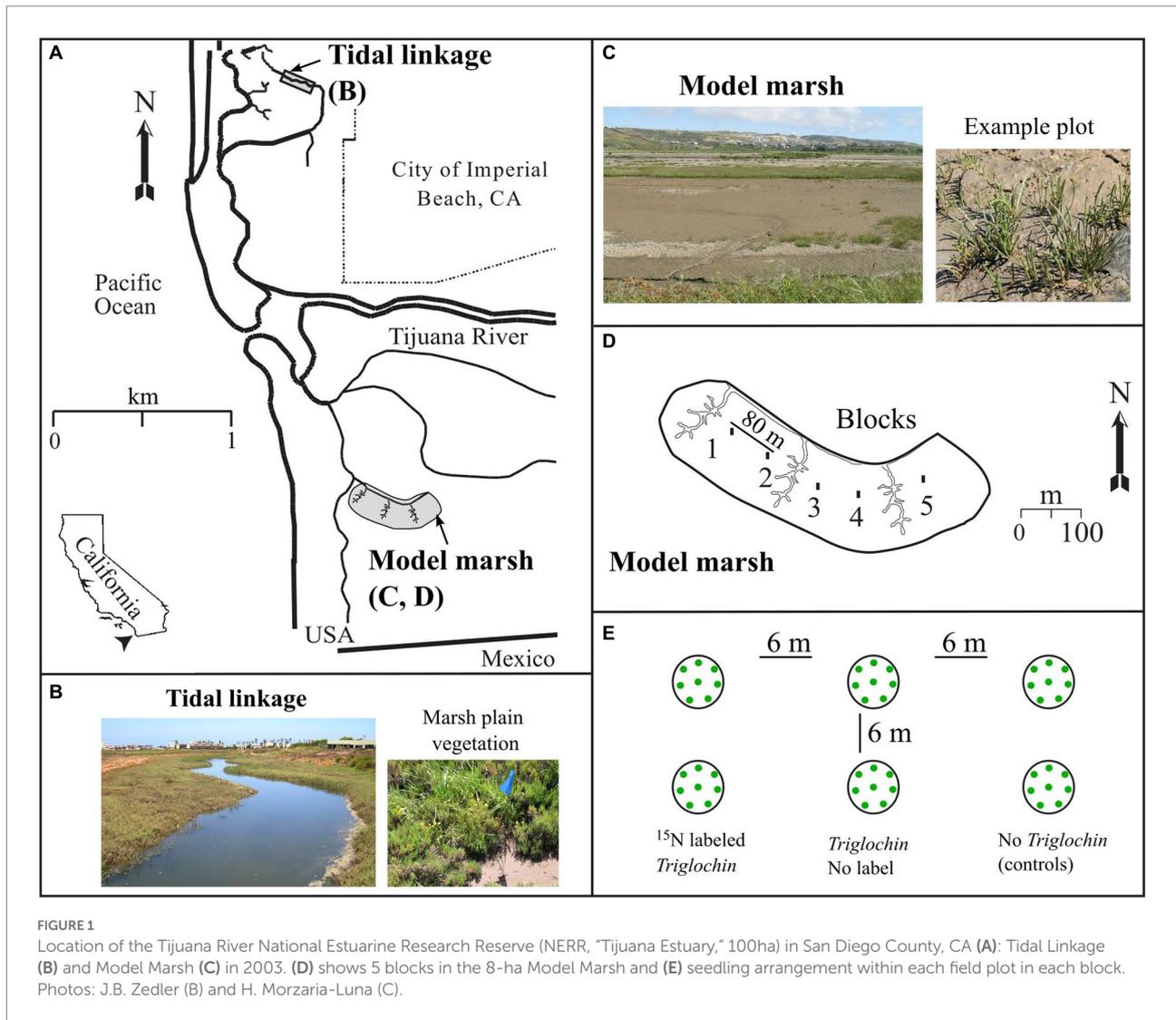
Methods

Southern California's Mediterranean-type climate allows a year-round growing season for most salt marsh species, although there is generally a slow-growth period during cool winters. Rainfall and flooding occur in winter months and evaporation is high during the warm, dry summer. As a result, soil salinities are high (40–$100 \, \mathrm{g \, L^{-1}}$). The annual precipitation averages 26.6 cm; the average annual high temperature is 21°C and the average low 12.9°C (Zedler et al., 1986; Zedler, 2009).

We used ^{15}N stable isotope enrichment to quantify N transfer between *Triglochin* and the 7-species assemblage in both field and greenhouse experiments. We also examined the effect of *Triglochin* on biomass and N accumulation by seven marsh plain halophytes (Table 1). Isotopic labeling has been previously used in marshes to analyze N retention and loss, measure N uptake by plant roots and soil microorganisms (Henry and Jefferies, 2003), analyze internal N transport in clonal plants from the parent ramet to the offspring ramet *via* the rhizome (Do, 2018), trace N transformation pathways, including long-term storage (Erler et al., 2010; Messer et al., 2017), and to identify consumers of marsh plants and algae (Kwak and Zedler, 1997; Levin et al., 2006).

Initially, we obtained background ^{15}N values of the eight common salt marsh plain species from the reference Tidal Linkage (Figure 1). Then, in field and greenhouse experiments, we labeled *Triglochin* plants using ^{15}N enriched fertilizer and incorporated them into assemblages with the remaining 7-species. N transfer was measured as ^{15}N movement from *Triglochin* tissues to other species, and evidenced by an increase in δ^{15}N in marsh plain halophyte tissues relative to background levels. Following the senescence of *Triglochin*, we assessed uptake of ^{15}N by other plants after 14 months' growth in the field and 13 months in the greenhouse.

We investigated N transfer between *Triglochin* and seven other marsh plain species (Table 1) in experimental plantings in the 8-ha restored Model Marsh (32.5458 N, −117.1210 W, Figure 1). The marsh was excavated in 1999 to expose buried salt marsh soil to an elevation range of 0.30–0.80 m NGVD. It was connected to an

FIGURE 1
Location of the Tijuana River National Estuarine Research Reserve (NERR, "Tijuana Estuary," 100ha) in San Diego County, CA **(A)**: Tidal Linkage **(B)** and Model Marsh **(C)** in 2003. **(D)** shows 5 blocks in the 8-ha Model Marsh and **(E)** seedling arrangement within each field plot in each block. Photos: J.B. Zedler (B) and H. Morzaria-Luna (C).

existing tidal channel in February 2000. The sediment was sandy ($75.1 \pm 2.3\%$ sand, $17.3 \pm 1.7\%$ silt, and $7.6 \pm 1.1\%$ clay) and organic matter was $4.5 \pm 0.13\%$ in February 2001 (Zedler et al., 2003; O'Brien and Zedler, 2006). *Spartina foliosa* was planted across the site near the tidal channel.

Background ^{15}N abundance

Background ^{15}N abundance in test species was determined from the literature and from plants collected in the Tidal Linkage (Figure 1; Supplementary Text S1). On December 12, 2002, we collected shoot and root samples from one individual of each species. Plants were refrigerated, taken to the laboratory, and rinsed with deionized (DI) water over a 500-µm sieve to eliminate soil and salts while preserving the root mass. We separated roots and shoots for each plant. In the case of *Frankenia salina*, *Batis maritima*, and *S. pacifica*, any stem fragments with green tissue were classified as shoot throughout the experiment. Tissue N content (%N) and atoms % ^{15}N in shoot and roots were determined directly on dried (60°C for 48h) and ground tissue samples, after milling through a 40 mesh (420-µm openings). Analysis was by a Carla Erba 1,110 Elemental analyzer coupled to a Finnigan MAT 252 Isotope Ratio Mass Spectrometer at the Stable Isotope Ratio Facility for Environmental Research in the University of Utah. Precision (based on internal standards) was $\pm 0.07‰$ for ^{15}N.

Plant ^{15}N labeling

We applied the ^{15}N enrichment approach due to the overlap between *Triglochin*'s isotope natural abundance values (δ^{15}N) and values from other common salt marsh species (Table 2). Additionally, Tijuana Estuary receives sewage overflow during storm events, which is enriched in ^{15}N and results in higher δ^{15}N across the food web relative to natural marshes, which could confound natural abundance levels (Moseman et al., 2004).

TABLE 1 Seven-species assemblage planted with or without *Triglochin concinna* Burtt Davy.

Species	Code	Common name	Growth form	Phenology		
				Germination	Shoot production	Flowering
Batis maritima L.	Bm	saltwort	Perennial trailing succulent	Feb-Apr	Feb-Sep	Jul-Oct
Frankenia salina (Molina) Johnston	Fs	alkali heath	Perennial upright forb	Jan-May	Feb-Sep	Apr-Nov
Jaumea carnosa Gray	Jc	seaside daisy	Perennial trailing succulent	Feb-Apr	Feb-Oct	May-Sep
Limonium californicum Heller	Lc	sea lavender	Perennial basal rosette with taproot	Dec-May	Feb-Oct	Apr-Sep
Salicornia bigelovii Torrey	Sb	annual pickleweed	Annual upright succulent	Nov-Mar	Germination–Oct.	Jun-Oct
Sarcocornia pacifica Standl.	Sv	perennial pickleweed	Short-lived succulent subshrub	Nov-Jun	Feb-Sep	Jul-Dec
Suaeda esteroa Ferren and Whitmore	Se	sea blite	Perennial succulent subshrub	Jan-May	Feb-Oct	Jul-Dec

Phenology from Sullivan and Noe (2001) and Sullivan and Zedler (1999).

Seedlings for the greenhouse and field experiments were grown in 2001 in our greenhouse at San Diego State University, CA. Seeds were collected from the Tidal Linkage in fall–winter 2000, air dried, and stored at 5°C until planting. On November 1, 2001, seeds of all marsh plain species (Table 1), except the annual *Salicornia bigelovii,* were germinated in flats filled with topsoil covered in a ~ 2-cm layer of potting soil. Seedlings were then transplanted individually into sterile 5×5-cm plastic pots and grown for 8 months, watering with freshwater two to three times a week as needed. We germinated *S. bigelovii* seeds 2 months prior to the start of each experiment.

To label *Triglochin* plants, we followed the method proposed by Hart (1990). On August 20, 2002, we transported the 60 largest *Triglochin* plants from San Diego to UW-Madison. Soil was washed off the roots with DI water using a pressure hose. We standardized plant size by removing all but three randomly chosen shoots. Roots were cut to 7-cm length, then 10 large roots were separated and remaining root mass was cut to 2-cm length. Plants were rinsed again in DI water, blotted with paper, weighed, and measured as the length of the tallest shoot.

Triglochin propagules were planted in sterile square plastic pots 9.6-cm tall (112.36 cm² area), filled with sterilized clay soil (35.7% clay, 56.4% silt, and 7.9% sand) similar to marsh plains in southern California (Zedler, 1982). The soil had: 5.6 pH, 5 ppm NO_3, 0.24 ppm P, and 2 ppm K (UW-Madison Soil and Plant Analysis Laboratory). We randomly arranged the pots in plastic trays in the greenhouse (Supplementary Figure S1). We supplemented natural light with 1,000-watt high-pressure sodium lamps in a 14:10-h photoperiod. Trays were filled with 2 g L⁻¹ Proline™ synthetic sea water, to 2 cm below the top of the pots, to maintain soil saturation. We added DI water to offset evaporation and transpiration.

We fertilized *Triglochin* pots with Technigro™. ¹⁵N natural abundance was 0.368 ± 0.002 atoms % ¹⁵N, so we aimed to enrich plant tissue to 1.102 atoms % ¹⁵N. The water-soluble Technigro™ fertilizer has a 20 N: 9 P: 20 K ratio, trace elements, and 8.5% N (as $CO(NH_2)_2$) and 11.5% NO_3 (as KNO_3). This fertilizer was enriched with 99% ¹⁵N ammonium nitrate (NH_4NO_3), which was 35% N (Isotec, Inc. Miamisburg OH). Salt marsh sediments release mineralized nitrogen predominantly as ammonium, which is the main source of nitrogen uptake by marsh plants (Gardner et al., 1991; Chambers et al., 1992). We applied the enriched fertilizer to each pot at a rate of 5 g N m⁻² by dissolving the fertilizer in 240 ml of water. The fertilizer was added in two 2.5 g N m⁻² applications on September 9 and on September 23, 2002. Following fertilization and algal proliferation, we treated each pot with ZeroTol^{ZT}, a broad spectrum algicide containing 27% H_2O_2, in a 1:100 concentration, on September 25, then repeated on September 27 with a 1:300 concentration. On October 7 the plants were transplanted to larger plastic pots, 14-tall × 15.2-cm diameter (153.4-cm⁻² area), filled with sterilized clay soil. We covered each pot's soil with Perlite© to limit algal growth. After transplanting, we applied another dose of ¹⁵N enriched fertilizer (15 g m⁻², mixed as described above); each pot was irrigated with 2 ml of the liquid mixture on October 25 and on November 1. In total, each plant received 0.133 g N ¹⁵N enriched.

On November 22, we began to salt-harden plants and eliminate freshwater algae. Over 2 weeks, we applied dilute seawater in increasing concentrations: 4, 6, 15, and 35 g L⁻¹ every 3 days. In between seawater additions, we added DI water and monitored soil surface salinities as interstitial water squeezed through filter paper onto a refractometer. Once soil surface salinities were > 35 g L⁻¹ salt, pots were watered with DI water only. On December 3, plants were removed from the pots and we carefully washed each plant over a 350-μm sieve with DI water to clean the root mass.

We blotted the labeled 25 *Triglochin* plants, obtained wet weight, and measured the height of the tallest shoot to analyze ¹⁵N

TABLE 2 ANOVA results for the Model Marsh field and greenhouse experiments.

Experiment	Parameter	Factor	ANOVA
Model marsh field experiment	Shoot biomass	± *Triglochin* treatments	n.s.
		Species	$F = 77.42$, $p < 0.001$
	Root biomass	± *Triglochin* treatments	n.s.
		Species	n.s.
	Root:shoot ratio	± *Triglochin* treatments	n.s.
		Species	$F = 28$, $p < 0.0001$
	$\delta^{15}N$	Background levels	n.s.
		± *Triglochin* treatments	$F = 6.582$, $p = 0.0116$
		Species	$F = 143.8$, $p < 0.001$
	N tissue content	± *Triglochin* treatments	n.s.
		Species	$F = 5.23$, $p = 0.01$
Greenhouse experiment	Shoot biomass	± *Triglochin* treatments	$F = 22.16$, $p < 0.001$
		Species	$F = 5.44$, $p < 0.000$
	Root biomass	± *Triglochin* treatments	n.s.
		Species	$F = 96.45$, $p < 0.001$
	Root:shoot ratio	± *Triglochin* treatments	$F = 6.14$, $p = 0.02$
		Species	n.s.
	$\delta^{15}N$	Species	$F = 143.8$, $p < 0.01$
		± *Triglochin* treatments	$F = 13.49$, $p < 0.0001$
	N tissue content	Species	$F = 30.002$, $p < 0.01$
		± *Triglochin* treatments	n.s.

label uptake. of these, 20 *Triglochin* plants were analyzed whole and 5 were separated into green shoots, senesced shoots, inflorescences, and roots. Then, they were then dried at 60°C for at least 48 h and weighed. We determined Tissue N content and atoms % ^{15}N as with the background samples (as above). We determined recovery of the ^{15}N label following Cabrera and Kissel (1989) as:

$$\text{Recovery} = \frac{\text{moles N plant} \times \left(\text{atom } \%^{15}\text{N sample} - \text{atom } \%^{15}\text{N background level} \right)}{\text{moles N fertilizer} \times \left(\text{atom } \%^{15}\text{N in fertilizer} - \text{atom } \%^{15}\text{N background level} \right)}$$

The remaining labeled *Triglochin* plants were held in DI water at 5°C for use in the field and greenhouse experiments.

Field experiment design

To test for N uptake and N transfer from *Triglochin* to seven halophytes, we set up a complete block experiment on December 11–12, 2002 on the unvegetated marsh plain of the Model Marsh surface ~20 m away from the edge of the *S. foliosa* plantings (Figure 1). The 5 blocks were ~80 m apart, and each block encompassed four 0.04-m^{-2} plots. In each plot, we planted one individual each of *S. pacifica*, *F. salina*, *Jaumea carnosa*, *S. esteroa*, *L. californicum*, *B. maritima*, and 4 *S. bigelovii* seedlings in a circle, with position assigned randomly. Four *S. bigelovii* seedlings *were* planted to compensate for their small size. In each block, we established 4 plots ±*Triglochin* ($n = 2$), i.e., 2 control plots with no *Triglochin* (−*Triglochin*; extrapolated density = 273 plants m^{-2}) and 2 plots +Triglochin, planted with ^{15}N-labeled *Triglochin* plants (extrapolated density = 328 plants m^{-2}). The ±*Triglochin* plots were

6 m apart. Each +*Triglochin* plot held a pair of plants with approximately the same wet weight (36.49 ± 0.66 g). We assessed mortality on January 6 and replaced dead plants. Strong winter storms in 2003 during a mild El Niño/Southern Oscillation delivered >16 cm of heavy rainfall (NWS 2004), which dislodged *S. bigelovii* seedlings. After storms ceased, we replaced missing seedlings on January 30 and again on April 11, 2003. No further seedlings were replaced, due to transpiration stress as temperatures rose to 27°C in April (NOAA, 2003).

We determined soil salinity from 5-cm deep cores collected next to the treatment plots on December 12, 2002, May 19, 2003, and March 23, 2004; interstitial water was minimal, so we mixed soil pastes by drying soil samples at 60°C, mixed with DI to make a saturated soil paste (Richards, 1954), and expressed the filtrate onto a refractometer. The experiment was terminated March 23–26, 2004.

Prior to harvesting, we counted survivors (with green tissue). We collected live and dead shoots on the surface of the plot. We extracted roots using a corer 22 × 25 cm (diameter × length). Cores were taken to the lab and washed over a series of four

stacked 500-μm mesh sieves to collect roots. Shoots and roots were rinsed with DI water to remove soil and salts and then stored at 5°C. Individual species' rhizomes were separated from the root mass with dissection needles; fine roots were combined for each plot. Shoot and root samples were oven-dried at 60°C for 2 days and weighed to obtain dry weight. We analyzed whole plant %N and atoms % ^{15}N (shoot and root tissue combined) from all plants in +*Triglochin* plots as described in the previous section. The N pool for each plot was calculated based on root, shoot, algal turf, and fine root biomass and tissue N content. Root:shoot ratio was calculated per plot and by species.

Greenhouse experiment design

We repeated the design of the field experiment except that we had to divide treatment replicates between two UW greenhouses to fit available space. Shading differed somewhat and one greenhouse averaged 7°C higher in ambient temperature. Evapotranspiration and soil saturation were not measured but appeared to vary with temperature.

We grew seedlings for 20+ pots in San Diego and flew them to UW-Madison, then transplanted them to sterilized plastic pots 22 × 22 cm (diameter x length) filled with sterilized clay soil. Pairs of Triglochin in each pot weighed on average 59.85 ± 1.28 g. To introduce microflora, we inoculated each pot with a small amount (~10 g) of marsh soil taken from a natural marsh in Tijuana Estuary. We assessed mortality after 2 weeks, when only *S. bigelovii* plants had died and were replaced. Three subsequent replants all died within a week, so we stopped replanting after March 16, 2003, when transpiration stress made *S. bigelovii* seedling survival unlikely.

To salt-harden seedlings, starting January 2, 2003 we watered pots with artificial Proline™ seawater (2, 4, 6, 15, and 35 g L^{-1} salt). In between seawater additions, we watered with DI water. Once soil surface interstitial salinities were > 35 g L^{-1} salt, pots were watered with DI water only. On April 8, plants were placed inside 11-L plastic containers and assigned to each of two greenhouses. Starting May 11, we alternated between dry and wet soil by filling containers with either 3 or 1.5 l of DI water every 2 weeks. We used 1,000-watt high pressure sodium lamps to supplement natural light in a 14-h photoperiod.

Triglochin produced seeds that germinated continually, so we maintained pot density by pulling up seedlings on October 17, December 4, December 18, 2003, and February 1, 2004. Seedlings were left on the soil surface to maintain each pot's N pool. On October 17, *Triglochin* plants were pruned of standing dead biomass and the canopy of the other species was shaken to remove dead tissue. We fragmented this plant biomass manually and returned it to the pots. We measured interstitial soil surface salinity on June 26, August 8, September 19, 2003, and February 20, 2004. As possible, soil was returned to each pot after measuring salinity. Salinity in the bottom 5 cm of each pot was measured on February 20, 2004 while

harvesting the experiment. Litter and *Triglochin* seedlings present at the end of the experiment were collected separately for each pot. We processed plant material as with the field experiment (see above), to determine whole plant biomass (shoots + roots), %N and atoms % ^{15}N for +*Triglochin* treatments and for six plants from −*Triglochin* pots (three from each UW greenhouse). We pooled *Triglochin* seedlings to provide enough material for %N and ^{15}N analysis. The N pool for each pot was calculated based on root, shoot, litter, fine root biomass, and tissue N content. Root:shoot ratio was calculated per plot and by species.

Data analysis

We quantified the isotopic composition relative to the ^{15}N/^{14}N atmospheric ratio. Results are expressed as parts per thousand (‰) differences from the corresponding standard:

$$\delta = \left[\left(R\text{sample} / R\text{standard} \right) - 1 \right] \bullet 1,000$$

higher δ values indicate a greater proportion of the heavy ^{15}N isotope.

Differences among ± *Triglochin* treatments in shoot and root biomass, root:shoot ratio, %N, and δ^{15}N values were analyzed using a two-way ANOVA with interactions. Total shoot biomass excluded litter and total root biomass excluded fine root weight; we also eliminated *Triglochin* from +*Triglochin* treatments to assess its effect on the 7-species assemblage. We specified block and greenhouse effects as factors. We analyzed individual species performance between plots and pots ± *Triglochin* using a multistratum ANOVA with the dependent variable (shoot and root biomass, root:shoot ratio, %N, and δ^{15}N) nested within plots or pots.

Dependent variables that did not meet the assumptions of ANOVA were transformed: biomass was transformed using x' = $\sqrt{x + \tfrac{3}{8}}$ to stabilize the variance, and root:shoot data were log-transformed (Zar, 1999). Tukey's Honest Significant Difference (HSD) was computed for post-hoc pairwise multiple comparisons. Statistical analyses were performed using R statistical framework (R Development Core Team, 2012) and the package MASS (Venables and Ripley, 2002). Means ±1 SE are reported in the text.

Results

All results are deposited in the Knowledge Network for Biocomplexity repository.[1] Table 2 summarizes all results from two-way ANOVAs on shoot and root biomass, root:shoot ratio, %N, and δ15N values.

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1   https://knb.ecoinformatics.org/view/doi:10.5063/F1V12367

TABLE 3 Shoot and root stable isotope ratios (‰) and N concentrations (%) from marsh plain halophytes.

Source	Species	Location	n	Habitat	δ^{15}N	%N
Shoot biomass						
This study	*Batis maritima*	Tidal Linkage	1	Marsh plain	−0.2	0.8
	F. salina				5.7	1.1
	Jaumea carnosa				4.2	1.1
	Limonium californicum				6.1	1.3
	Suaeda esteroa				6.3	1.6
	Sarcocornia pacifica				5.8	0.8
	Triglochin concinna				6.7	4.5
Page (1995)	*S. pacifica*	Carpinteria Marsh	4	Marsh plain	9.5 ± 0.0	2.3 ± 0.3
	S. pacifica			High marsh	6.9 ± 0.6	1.5 ± 0.3
	S. pacifica			Marsh edge	16.8 ± 4.2	2.9 ± 0.2
Kwak and Zedler (1997)	*S. pacifica*	Tijuana Estuary	4	Marsh plain	11.0 ± 1.2	
	S. pacifica	San Dieguito Lagoon	2		14.2 ± 0.6	
Harding and Stevens (2001)[a]	*F. salina*	Elkhorn Slough	6	Marsh edge	4.9 ± 0.8	
	S. pacifica				6.7 ± 0.6	
Cloern et al. (2002)	*S. pacifica*	San Francisco Bay	49	Creek edge	13.4 ± 0.5	
Root biomass						
This study	*B. maritima*	Tidal Linkage	1	Marsh plain	5.9	1.2
	F. salina				−0.1	0.7
	J. carnosa				−2.0	0.5
	L. californicum				−1.1	0.7
	S. esteroa				3.9	0.7
	S. pacifica				−2.6	0.6
	T. concinna				4.4	2.9

Comparison of data from plants collected in the restored Tidal Linkage of Tijuana Estuary and other studies (all sites in California, USA). For $n > 1$ data are mean values (± 1 SE).
[a]indicates values were estimated from published graphs. See Table 1 for species names.

Background ¹⁵N abundance at the tidal linkage (planted in 1997)

Delta values from shoot samples were low and ranged from −0.2 to 6.7‰ (Table 3). Triglochin shoots had the highest δ^{15}N value and *B. maritima* was depleted. Root samples of *F. salina*, *J. carnosa*, *L. californicum*, and *S. pacifica* were depleted relative to shoots. The δ^{15}N values found for *F. salina* and *S. pacifica* were lower than records from other sites (Table 3); we found no published information for other species. Tissue N contents in shoots (Table 3) were highest for *Triglochin* (4.5%), and ranged from 0.8 to 1.6% for other species. N content in *Triglochin* root tissue was also higher (2.9%) than for other species (~0.7%).
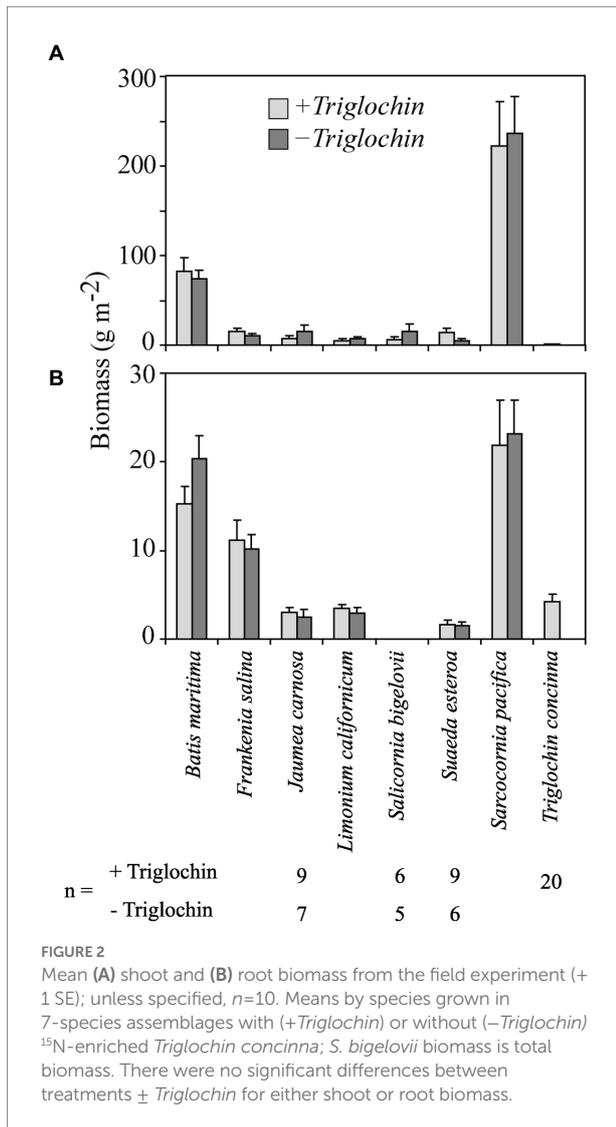
Triglochin labeling

Biomass of *Triglochin* plants increased 20.3 ± 1.0 g wet weight by the end of the labeling process. The mean δ value in labeled tissue was 656 ± 13.2‰ and N tissue content was 1.9 ± 0.3%. There was no difference in δ^{15}N values between tissue types but N content in senesced shoots was 0.9 ± 0.01 compared to 2.2 ± 0.05% in live tissue. The recovery rate of the ¹⁵N label was on average 14.4 ± 0.3%.

Field experiment at the Model Marsh

Two years after it was opened to tidal flows, the soil surface salinity did not vary between blocks and it averaged 56.4 ± 2.5 g L⁻¹ in December 2002, 72.4 ± 6.5 g L⁻¹ in May 2003, and 70.1 ± 4.9 g L⁻¹ in March 2004, reflecting seasonal differences.

Plant survival at the end of the experiment, in 2004, was high: 100% for *B. maritima, F. salina, and S. pacifica;* 65% in −*Triglochin* plots and 74% in +*Triglochin* plots. 75% for *J. carnosa* and *S. esteroa*, but only 14% for *S. bigelovii*. Four of the *S. bigelovii* plants collected were natural recruits. At the end of the experiment, only one −*Triglochin* plot and three +*Triglochin* plots still contained the original species assemblage.

Unless specified, total plot biomass for +*Triglochin* treatments considers all plants, including *Triglochin*; also, "roots" includes rhizomes throughout. Biomass did not differ between ± *Triglochin* treatments, despite different planting density ($n = 10$ in −*Triglochin* vs. $n = 12$ in +*Triglochin* plots). Scaling from 0.04 m⁻² plots, mean shoot biomass was 363.6 ± 41.3 g m⁻² in −*Triglochin* and 352.44 ± 58.1 g m⁻² in +*Triglochin* plots. Shoot biomass varied among species (Figure 2; $F = 77.42$, $p < 0.001$; Supplementary Table S1). *S. pacifica* was the dominant; with 57.6 ± 3.7% of all shoot biomass per plot. Although *Triglochin*

FIGURE 2
Mean **(A)** shoot and **(B)** root biomass from the field experiment (+ 1 SE); unless specified, n=10. Means by species grown in 7-species assemblages with (+*Triglochin*) or without (−*Triglochin*) ^{15}N-enriched *Triglochin concinna*; *S. bigelovii* biomass is total biomass. There were no significant differences between treatments $\pm$ *Triglochin* for either shoot or root biomass.

plants were still alive at the end of the experiment, most had little shoot biomass ($0.96 \pm 0.5\,g\,m^{-2}$) and showed evidence of herbivory.

Root biomass was 60.6 ± 5.5 in −*Triglochin* and $56.4 \pm 7.2\,g\,m^{-2}$ in +*Triglochin* plots. *S. pacifica* and *B. maritima* contributed 31.7 ± 2.6 and $36.7 \pm 3.1\%$, respectively, of total root biomass within plots (Figure 2; Supplementary Table S1). *Triglochin* root biomass was $4.2 \pm 0.9\,g\,m^{-2}$. Fine root biomass was high in both −*Triglochin,* 33.6 ± 4.4, and +*Triglochin*, $51.4 \pm 8.9\,g\,m^{-2}$ treatments. Root:shoot ratio did not vary either among $\pm$ *Triglochin* treatments. Average root:shoot ratios were 0.4 ± 0.04 in −*Triglochin* and 0.5 ± 0.1 in +*Triglochin* plots. Root:shoot ratio differed among species (Supplementary Table S2; $F = 28$, $p < 0.0001$). *S. pacifica* had the lowest root:shoot ratio (0.09) and *Triglochin* the highest (4.3), largely due to reduced shoot biomass. No litter was found in the plots.

Labeled *Triglochin* plants retained the ^{15}N label after 14 months in the Model Marsh (December 2002–March 2004). Delta values were significantly different between $\pm$ *Triglochin* plots ($F = 6.582$, $p = 0.0116$) and between species ($F = 143.8$,

$p < 0.01$). Delta values for *Triglochin* were 343.1 ± 39.9‰, on average 50% lower than δ^{15}N values in plants immediately after labeling (656 ± 13.2‰). Delta values for other species ranged from 5 to 26‰ (Figure 3) and were consistently higher than background levels for plants of similar age collected in the Tidal Linkage (Table 3). Seedlings that germinated in field plots had high δ15N values (25‰) and 0.4%N. Average tissue N content was highest for *Triglochin*, $1.7 \pm 0.1\%$ N (Figure 3; $F = 5.23$, $p = 0.01$) and was not significantly different from background levels (Table 3). The N pool in +*Triglochin* plots was $5.9 \pm 0.9\,g\,m^{-2}$. The contribution of individual species to the N pool followed biomass patterns; *S. pacifica* accumulated $44 \pm 2.0\%$ of total N.
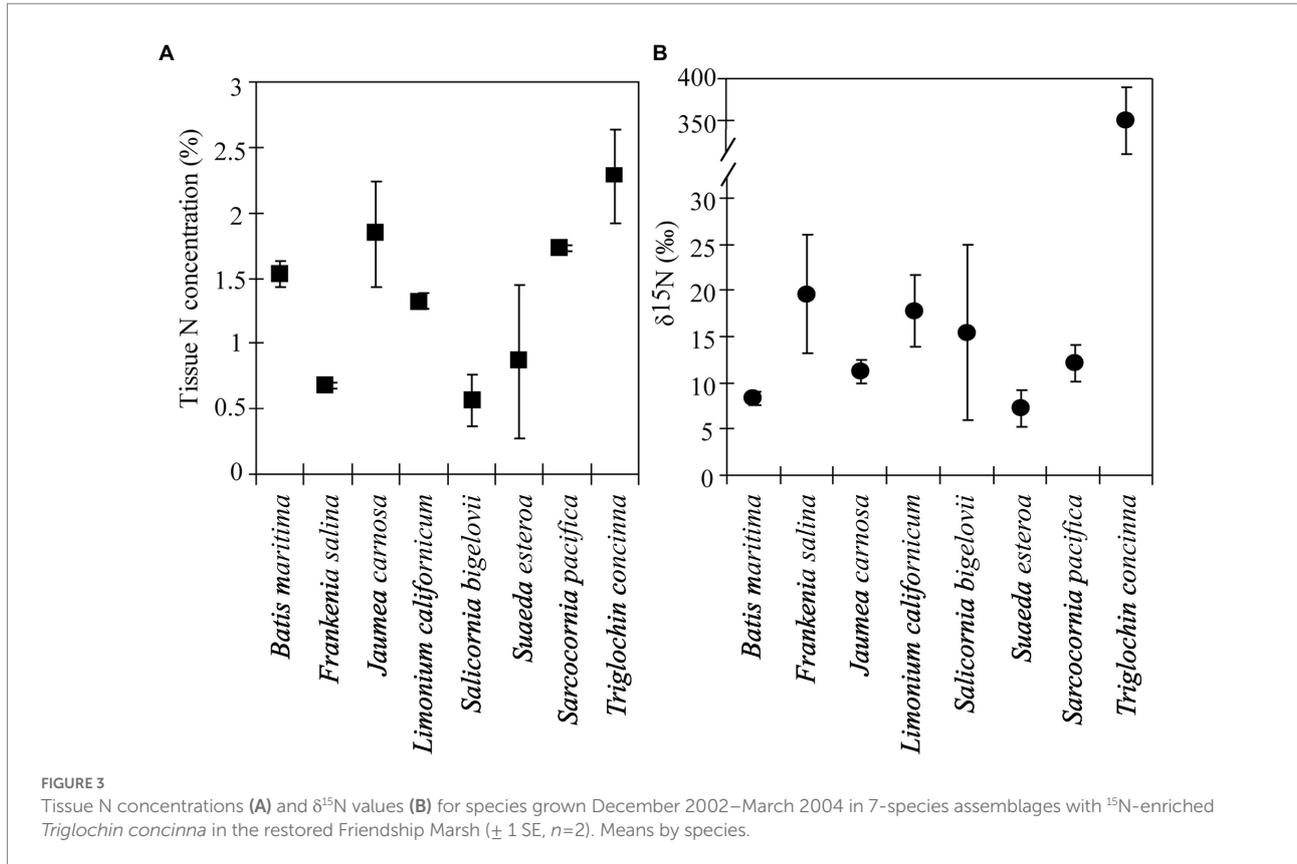
Greenhouse experiment

Growing conditions in the greenhouse differed from the field, in part because pots confined plant roots. Soil surface salinity (5-cm depth) did not vary between pots in the two greenhouses; the average was $66.5 \pm 5.3\,g\,L^{-1}$ in June, $98.5 \pm 2.4\,g\,L^{-1}$ in August, $74.5 \pm 5.3\,g\,L^{-1}$ in September 2003, 53.5 ± 5.9 in February 2004. Salinity in the bottom 5 cm of pots was 13.1 ± 2.3 in the lower vs. $16.4 \pm 2.1\,g\,L^{-1}$ in the less-shaded greenhouse. Still the greenhouse results supported those of the field test.

As in the field, only *S. bigelovii* had a low survival rate (10%); all other plants survived. In both $\pm$ *Triglochin* pots, collected *S. bigelovii* plants were small (< 5 cm tall) and none was branching. At the end of the experiment, only five −*Triglochin* pots still contained the complete species assemblage. One −*Triglochin* pot was contaminated by a *Triglochin* seed that germinated and grew to ~10 cm before being removed at the end of the experiment.

As found in the field, there was a significant overall effect of $\pm$ *Triglochin* treatments on shoot biomass (Figure 4). Mean shoot biomass, scaling from 0.04-m^{-2} pots, was significantly higher in −*Triglochin* pots (373.4 ± 22.9) than +*Triglochin* ($225.8 \pm 21.3\,g\,m^{-2}$; $F = 22.16$, $p < 0.001$). This difference can be attributed to *S. pacifica*, whose shoot biomass was 44% lower in +*Triglochin* than in −*Triglochin* pots (52.5 ± 7.4 vs. $120.4 \pm 18.6\,g\,m^{-2}$; $F = 5.44$, $p < 0.0001$); *F. salina* shoot biomass followed a similar pattern (Figure 4). *Sarcocornia pacifica* showed a significant difference in shoot biomass between $\pm$ *Triglochin* treatments. *Triglochin* shoot biomass was $17.64 \pm 2.2\,g\,m^{-2}$. Litter collected in +*Triglochin* pots was mostly *Triglochin* and *J. carnosa* senesced shoots ($180.7 \pm 23.3\,g\,m^{-2}$); in −*Triglochin* pots litter was mostly *F. salina* and *J. carnosa* senesced leaves ($95 \pm 13.2\,g\,m^{-2}$).

Neither shoot nor root biomass differed between greenhouses. Root biomass did not vary between $\pm$ *Triglochin* treatments (96.9 ± 10.5 −*Triglochin* vs. $102.8 \pm 6.0\,g\,m^{-2}$ +*Triglochin*). *F. salina* root biomass was significantly higher than other species ($54.73 \pm 6.74\,g\,m^{-2}$; $F = 96.45$, $p < 0.001$; Supplementary Table S1), including *Triglochin* root biomass ($35.6 \pm 5.1\,g\,m^{-2}$). Fine root biomass was similar in $\pm$ *Triglochin* treatments (121.1 ± 11.7 in −*Triglochin*

FIGURE 3
Tissue N concentrations **(A)** and δ^{15}N values **(B)** for species grown December 2002–March 2004 in 7-species assemblages with ^{15}N-enriched *Triglochin concinna* in the restored Friendship Marsh ($\pm$ 1 SE, n =2). Means by species.
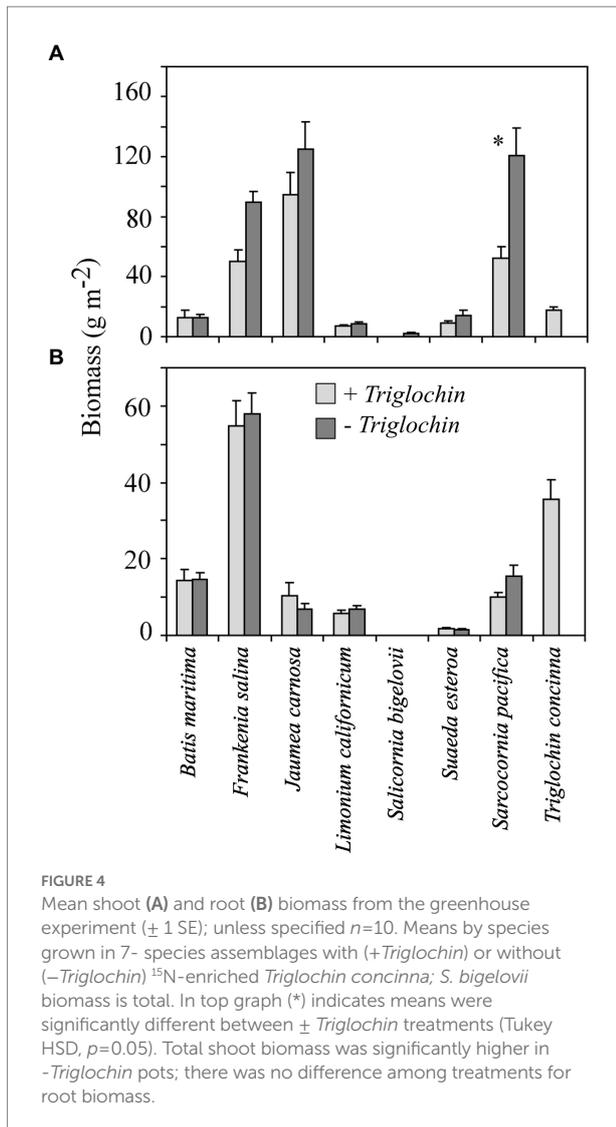
and $147.1 \pm 10.3 \, \text{g m}^{-2}$ in $+Triglochin$). Root:shoot ratio was significantly higher in $+Triglochin$ treatments, average ratio was 0.5 ± 0.04 in $-Triglochin$ and 0.7 ± 0.1 in $+Triglochin$ pots ($F = 6.14$, $p = 0.02$; Supplementary Table S2). *Triglochin* had the highest root:shoot ratio (2.0) and *J. carnosa* the lowest (0.08). Litter production was similar between greenhouses, although litter collected in the cooler greenhouse was moist and appeared more decomposed than litter from the warmer greenhouse.

Delta values were significantly higher for species in $+Triglochin$ pots (Figure 5; $F = 13.49$, $p < 0.0001$; Supplementary Table S3). Delta values in pots $-Triglochin$ ranged between -2.6 and $6.8‰$ and between $231.1 \pm 23.2‰$ for $+Triglochin$ pots. These values for *Triglochin* were on average 65% lower than δ^{15}N values found in plants right after labeling ($656 \pm 13.2‰$). *Triglochin* seedlings that germinated from seeds produced during the experiment had high δ^{15}N values, $158.8 \pm 21.9‰$. Delta values for other species in $+Triglochin$ pots ranged between $4–80‰$. Delta values were highest for *L. californicum* ($89.5 \pm 13‰$) and lowest for *B. maritima* ($15 \pm 23.2‰$). *B. maritima* had a high root:shoot ratio, with little growth aboveground. Delta values for plants grown in $+Triglochin$ pots were consistently higher than background levels and values for other studies (Table 3; Supplementary Table S3). Delta values of litter in $+Triglochin$ pots were lower than for live shoots ($29.1 \pm 34.8‰$); δ^{15}N values in litter from $-Triglochin$ pots were $1.5 \pm 0.4‰$.

There were no differences in whole plant tissue N content between $\pm$ *Triglochin* treatments (Figure 5). There was a significant difference between the two greenhouses ($F = 2.6$, $p < 0.001$); % N was lower in the greenhouse that was warmer and received more light (0.6 ± 0.03 vs. $0.7 \pm 0.1\%$ N). As in the field experiment, tissue N content was highest in *Triglochin* seedlings that germinated during the experiment $1.9 \pm 0.2\%$ ($F = 59.7$, $p < 0.001$); followed by *Triglochin* and *L. californicum* (Figure 5). Percent N in litter was similar in $-Triglochin$, 0.8 ± 0.1, and $+Triglochin$, $0.73 \pm 0.09\%$ N. Tissue N content was positively related to root:shoot ratio ($r = 0.39$, $F = 4.9$, $p < 0.001$); suggesting higher N content in roots than shoots.

Overall, the N pool was lower in $-Triglochin$ than in $+Triglochin$ pots ($F = 8.09$, $p = 0.01$); this difference can be attributed to the addition of *Triglochin* plants. *Triglochin* plants and seedlings accumulated $0.6 \pm 0.03 \, \text{g N m}^{-2}$ (Figure 6). We combined N-pools for the remaining species and we found N accumulation in tissue of the 7-species assemblage was higher in $-Triglochin$ than in $+Triglochin$ pots (2.17 ± 0.08 vs. $1.37 \pm 0.11 \, \text{g N m}^{-2}$; $F = 10.89$, $p < 0.001$). This difference can be attributed to the increase in *F. salina* and *S. pacifica* shoot biomass in $-Triglochin$ pots. There was a significant effect of greenhouse on the N pools among pots; on average, the N pool was on average higher in pots in the cooler greenhouse (4.66 ± 0.12 vs. $3.53 \pm 0.05 \, \text{g N m}^{-2}$; $F = 11.91$, $p = 0.003$).

FIGURE 4
Mean shoot **(A)** and root **(B)** biomass from the greenhouse experiment ($\pm$ 1 SE); unless specified n=10. Means by species grown in 7- species assemblages with (+*Triglochin*) or without (−*Triglochin*) 15N-enriched *Triglochin concinna*; *S. bigelovii* biomass is total. In top graph (*) indicates means were significantly different between ± *Triglochin* treatments (Tukey HSD, p=0.05). Total shoot biomass was significantly higher in -*Triglochin* pots; there was no difference among treatments for root biomass.

Discussion

We learned that *Triglochin* can transfer N to seven other marsh plain species when planted as seedlings into a nearly-bare tidal plain (restored to tidal influence in 2000) as well as when grown in greenhouse pots. Enriched 15N isotopes showed that N from *Triglochin* is transferred to other marsh species (Figures 3, 5). We also confirmed previous experimental findings that *Triglochin* reduces assemblage biomass both above and below ground (Figure 4) and that assemblages with *Triglochin* have greater N accumulation (Figure 6). We found that although *Triglochin* has low biomass, cover, height, and layering in the salt marsh plain (Keer and Zedler, 2002; Callaway et al., 2003; Doughty et al., 2021), it performs two important ecosystem functions, N-storage and N-sharing. Thus, *Triglochin* has similarities with legumes that host biological N fixation and share N (Hamilton, 2012), and with cycads that contribute N to surrounding soils *via* roots and associated N-fixing

cyanobacteria (Marler and Calonje, 2020). Our research also confirmed that isotope enrichment of salt marsh plants is a powerful tool for tracking the fate of N in the ecosystem (Whitcraft, 2007), clarifying ambiguities in natural abundance data, and serving as independent evidence of N transfer (Hart and Lovvorn, 2002). By being conducted in a National Estuarine Research Reserve (Figure 1) that focuses on salt marsh restoration, our realistic test of species-based functioning in the Model Marsh enhances knowledge and practice for restoring Californian tidal marshes.

Triglochin takes up and stores N

A reservoir of N is important where N is limiting, as in newly excavated, formerly buried substrates (Valiela and Teal, 1974; Boyer and Zedler, 1998). We found that *Triglochin* had high uptake of the 15N label, and high N content and retention (Figures 3, 5, 6), likely through recycling or internal translocation. These findings are consistent with previous results showing that *Triglochin* can limit growth and N accumulation of other plants in the 7-species assemblage (Table 1), particularly the biomass dominants *S. pacifica* and *F. salina* (Sullivan and Zedler, 1999; Callaway et al., 2003; Sullivan et al., 2007; Morzaria-Luna and Zedler, 2014). Our results support the hypothesis that *Triglochin* is a superior competitor for N and a functionally important halophyte that dominates N cycling, as proposed by (Zedler and Callaway, 1999; Zedler, 2005).

We demonstrated that a subordinate, low-biomass, species can take up more N than other marsh halophytes in a regional model restoration site. The whole plant N content of *Triglochin* was 1–5 times as high as that of other species in greenhouse and field experiments. Sullivan and Zedler (1999) and Callaway et al. (2003) also found high N in *Triglochin* shoots (1.7%–5.2%). Although waterlogged soils lose N through denitrification, *Triglochin* appears to have a competitive advantage in such soil, because it was able to grow substantial root biomass deep into the 10–12 cm of waterlogged soil at the base of of 38-cm tall greenhouse pots (Sullivan et al., 2007). Plant nitrogen uptake also responds to drought, flooding, salt stress and nitrogen fertilization (Morzaria-Luna and Zedler, 2014; Bai et al., 2017).

Nitrogen has important influences in salt marshes, some of which can be attributed to succulent *Triglochin* spp. with leaves having abundant free amino acids and low cell-wall content, preferred by several bird species as food (Popp and Albert, 1980; Naidoo, 1994). At the same time, herbivory is deterred by cyanogenic glucosides taxiphyllin and triglochinin that *Triglochin* spp. synthesizes (Majak et al., 1980). These compounds contain N (Nielsen and Moller, 1999), and feeding causes the cyanogenic glucosides to degrade rapidly into an aldehyde or ketone, cyanide, and sugar (Jones et al., 2000). Anecdotally, crushing the shoots of this *Triglochin* releases a strong smell that suggests cyanide (Alonso-Amelot and Oliveros, 2000).
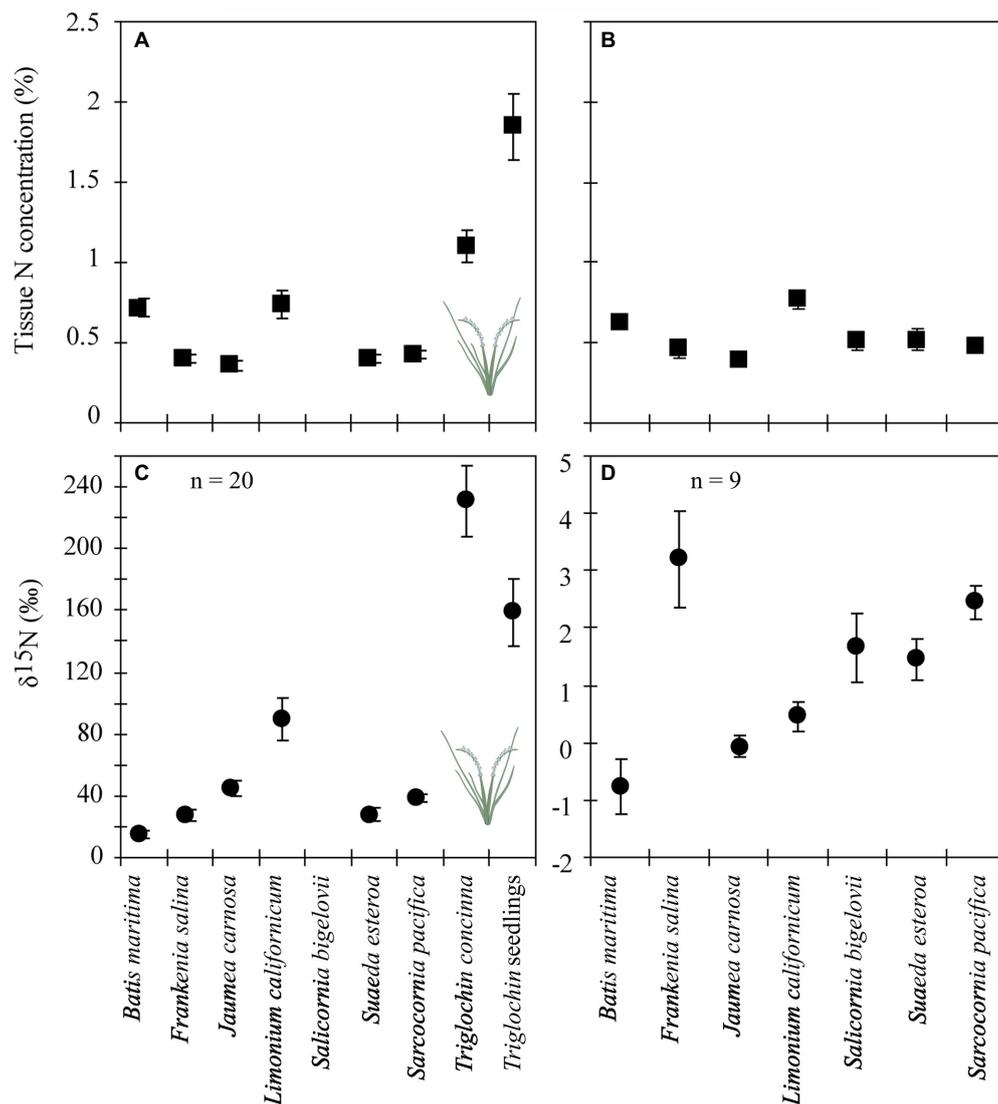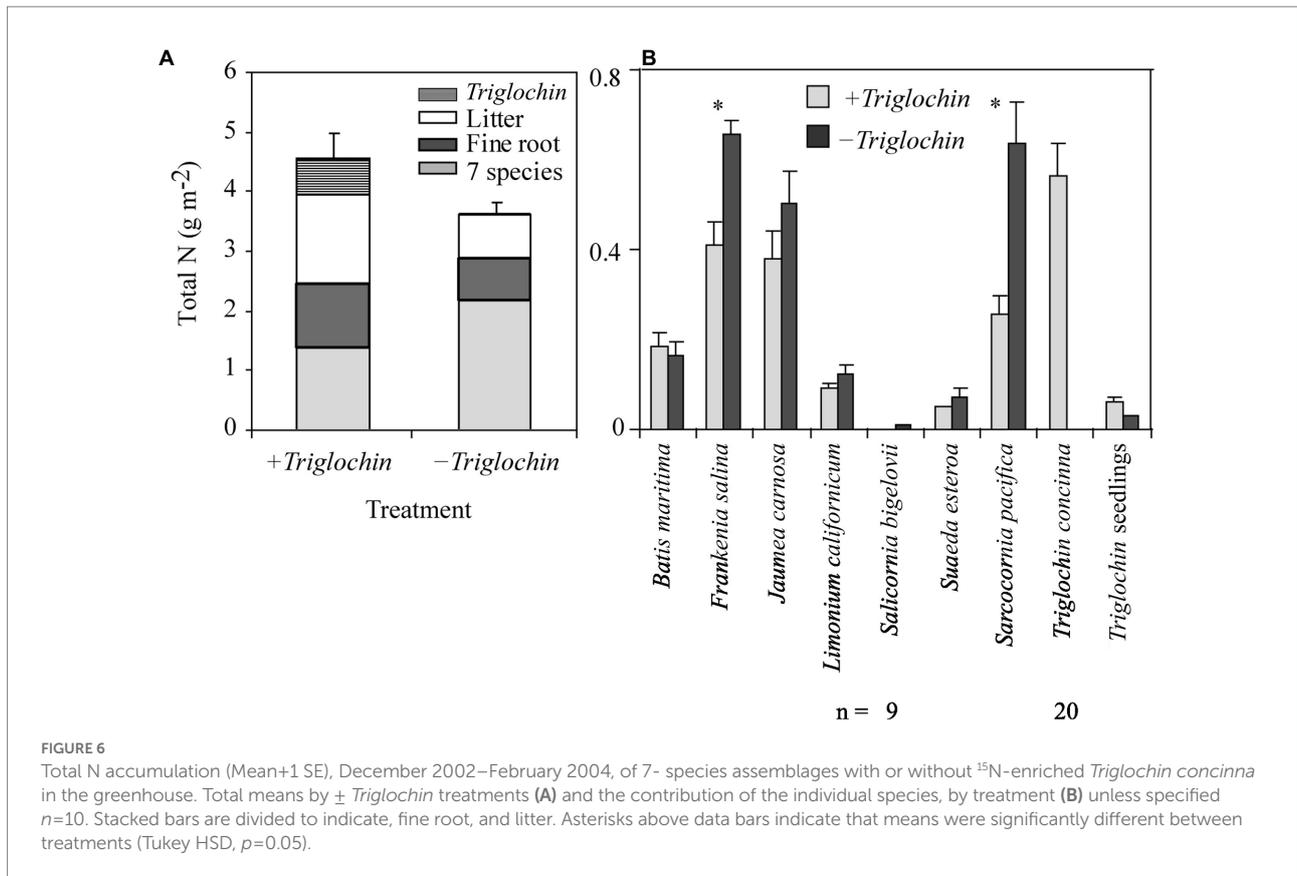
FIGURE 5
Tissue N concentrations (●, **A & B**) and δ15N values (■, **C & D**) for species grown December 2002−February 2004 in 7-species assemblages with N-enriched *Triglochin concinna* (**A & C**) or without (**B & D**). Unless specified, *n*=10. Means by species, including *Triglochin* seedlings that germinated during the experiment. Note different y-axis scales in panels **C & D**.

Triglochin shares some of its N with neighbors

Our field and greenhouse experiments showed that seven halophyte species and labeled *Triglochin* had higher δ15N values (Figures 3, 5) than plants grown without labeled *Triglochin.* Values also exceeded previously reported natural abundances (Table 3). Thus, the labeled δ15N from *Triglochin* tissue was transferred to the plant tissue of the other common marsh halophytes. The 15N content in vascular plant tissue is a reflection of the N sources assimilated during the growing season (Shearer and Kohl, 1986; Johannisson and Högberg, 1994). Multiple mechanisms could account for the observed transfer of δ15N label from *Triglochin* to other plants. Although litter collected from +*Triglochin* pots was

predominantly *Triglochin* senesced shoots, litter N content was similar in ± *Triglochin* pots (~0.8%); litter δ15N values in +*Triglochin* pots were also lower (60%) than live *Triglochin* shoots. This is consistent with death, decomposition, and remineralization of *Triglochin* shoots in the sediments and subsequent plant uptake (White and Howes, 1994a,b).

Labeled N could also reach other plant species by root exudates, leaching, or bacterial remineralization (Paynel et al., 2001; Levin et al., 2006), or by simple mass flow or diffusion of ions and simple amino acids along a concentration gradient (Teste et al., 2015). Although we did not measure N or δ15N in the sediment, the rapid transformation of inorganic N to plant organic N is a major mechanism for short-term nitrogen retention (White and Howes, 1994a). Root decomposition could also contribute to

FIGURE 6
Total N accumulation (Mean+1 SE), December 2002–February 2004, of 7- species assemblages with or without ¹⁵N-enriched *Triglochin concinna* in the greenhouse. Total means by ± *Triglochin* treatments **(A)** and the contribution of the individual species, by treatment **(B)** unless specified *n*=10. Stacked bars are divided to indicate, fine root, and litter. Asterisks above data bars indicate that means were significantly different between treatments (Tukey HSD, *p*=0.05).

N transfer; seasonal translocation of N from above to belowground biomass during senescence is potentially important to internal N cycling of other tidal marsh species, like *Spartina alterniflora* (Morris, 1980; Hopkinson and Schubauer, 1984). Physiological integration where N is transferred to ramets in clonal plants, such as *Triglochin*, was recently confirmed in marsh plants (Do, 2018).

Triglochin retains N and can limit neighbor plant growth

While sharing its N, *Triglochin* can also limit the growth of its neighbors. Plants grown in pots +*Triglochin* had reduced shoot biomass; the dominants, *S. pacifica* and *F. salina* were particularly affected. Plants grown in +*Triglochin* pots had on average 44% less *S. pacifica* and 45% less *F. salina* shoot biomass than in −*Triglochin* pots by the end of the greenhouse experiment (Figure 4). These findings are consistent with earlier results (Table 3), Shoot N content in samples collected by Callaway et al. (2003) adjacent to our field plots ranged from 3.1 ± 0.2 to 5.1 ± 0.1% for *Triglochin*, and between 7.7 (*J. carnosa*) and 17.4% (*F. salina*) for the remaining species. Biomass did not differ between ±*Triglochin* in field plots (Figure 2), unlike in greenhouse pots with limited root space.

At least three mechanisms could account for the decreased growth of plants grown with *Triglochin* we observed here: direct competition, allelopathy, and indirect interactions that favor other species. In companion greenhouse and field experiments, we tested for competition between *Triglochin* and *S. pacifica* with varied N and water levels, using biomass to assess outcomes. Although there was evidence of facilitation in a short-term (6 mo.) experiment, the year-long interaction showed competition across the stress gradient (Morzaria-Luna and Zedler, 2014). Species-level traits explained responses of both *Triglochin* (with its high N uptake) and *S. pacifica* (with its large and perennial aboveground biomass). N supply was the only factor that affected outcomes; neither inundation nor time of interaction had any effect. By reducing the growth of its neighbors, *Triglochin* could facilitate canopy gaps, where the annual *S. bigelovii* could persist (Varty and Zedler, 2008). We attribute the observed reduction in shoot growth of plants in +*Triglochin* treatments to competition that we tracked using labeled N, as recommended by McKane et al. (2002). Although %N in plant tissue did not vary between ±*Triglochin* treatments, total N accumulation in *F. salina* and *S. pacifica* was higher in −*Triglochin* pots (0.6 ± 0.03 and 0.7 ± 0.1 g N m⁻²) than in +*Triglochin* (0.4 ± 0.05 and 0.3 ± 0.04 g N m⁻²) suggesting the additional growth of these plants was due to enhanced N availability.

The impact of N released from litter might be highest for *S. bigelovii*, the only annual species on the salt marsh plain (Zedler et al., 1999). N released by *Triglochin* during summer could be taken up by *S. bigelovii* seedlings, with senescing *S. bigelovii*

releasing N in winter and spring, when *Triglochin* can take it up. *Salicornia bigelovii* might also take up higher amounts of N, since it was able to remove 23% of total N applied in a microcosm experiment (Brown et al., 1999). Lindig-Cisneros et al. (2003) found that where N was added to a restored *S. foliosa* marsh, *S. bigelovii* recruited rapidly, reaching biomass of up to $80 \, g \, m^2$. Thus, *Triglochin* could be extremely important in facilitating both the recruitment and establishment of *S. bigelovii* seedlings. Further research could assess the impact of seasonal *Triglochin* litter decomposition on *S. bigelovii* seedlings.

Implications for restoring the salt marsh plant community

Dominant species are often listed as restoration targets, and they are typically defined as those with extensive canopy cover or higher aboveground biomass (Zedler, 2001). In this study, however, we focused on a subordinate species that is hard to find after its leaves have senesced and washed away with the outgoing tide. *Triglochin* is often ignored; here, we confirm that it can dominate assemblages by accumulating N in its roots and shoots, even when it produces very little cover or biomass, and that it can share some of its N with neighbors and simultaneously limit their biomass. We had expected N-sharing or competition, but not both. The evidence, however, is compelling.

N-accumulation by *Triglochin* and subsequent effects on plant growth have immediate implications for the restoration of the plant community composition and its ecosystem functions. The salt marsh plain is dynamic, so species are not likely to remain where they are planted. A clonal species like *Triglochin* could concentrate N over a large area, translocating N between shoots and roots, creating long-lived nutrient-enriched patches and contributing significantly to spatial variability in available N (Baye, 2007; Do, 2018). The differential ability of plant species to compete for nutrients within nutrient-rich patches created by *Triglochin* could shift species abundances and increase diversity (Grime, 1997). As *Triglochin* shoots die back by mid-summer, they could decompose faster and release more nutrients than surrounding species because of their high N content (Quested et al., 2003). In greenhouse pots +*Triglochin*, 32% of the total N pool was contained in the litter (Figure 6).

Restoration is underway in several southern California salt marshes, to help compensate for major historical losses and in anticipation of future needs as sea level rises (SLR) and other anthropogenic impacts (Thorne et al., 2018). Even under the projected "low SLR scenario" for Tijuana Estuary, higher marsh vegetation would be submerged by the end of this century; and under the "high SLR scenario" all tidal marsh habitats would convert to mudflat by 2110 (Thorne et al., 2018). Thus, the upper margins of wetland salt marshes will need to extend into what is now upland. Soils that are low in N and other components (nutrients, mycorrhizae, seed banks) can be amended to support future salt marsh vegetation, but the most efficient plans will be based on knowledge of interactions, such as the ability of *Triglochin* to capture and share N with its native neighbors (Figures 3, 5).

We recommend planting *Triglochin* to increase plant diversity *directly*, adding seedlings and seeds to restoration plantings, and to restored and remnant marshes where *Triglochin* was more abundant historically (1989–2004 data show high variability at Tijuana Estuary (Zedler and West, 2008). We also recommend planting *Triglochin* to increase diversity *indirectly*, by reducing dominance where tidal action is being restored or created. We suggest collecting *Triglochin* seeds during peak months of fruiting (May–June) to build up a reserve for future use, e.g., longevity tests during storage beyond 3 years (Sullivan and Noe, 2001). At the Tidal Linkage, *Triglochin* recruitment was limited even though adult plants were nearby (Lindig-Cisneros and Zedler, 2002). Restoration plantings could omit biomass dominants (especially *S. pacifica*) to allow *Triglochin* to "creep" across the marsh plain with less competition. Additional considerations are needed for large-scale plantings that could be needed following the catastrophic loss of an entire salt marsh (e.g., through erosion, burial, oil or other toxic spills).

We continue to endorse Callaway's (2005) call for "rigorously designed scientific experiments that identify cause-effect relationships for the development of salt marshes." We suggest that including *Triglochin* in restoration can support the development and maintenance of desired ecosystem functions, especially in bare graded substrates and sandy soils, where N is likely to be limiting. We encourage long-term, strategic research and planning as a southern California "insurance policy" for salt marsh restoration.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://knb.ecoinformatics.org/view/doi:10.5063/F1V12367.

Author contributions

HM-L: conceptualization, methodology, field experiments, laboratory analysis, data analysis, data curation, writing—original draft, review and editing, visualization. JZ: conceptualization, methodology, data analysis, writing—review and editing, supervision, project administration, funding acquisition. Both authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.851055/full#supplementary-material

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