



# Restoration of Cordgrass Salt Marshes: Limited Effects of Organic Matter Additions on Nitrogen Fixation

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## Abstract

The extensive loss of tidal marsh habitat surrounding the San Francisco Bay has led to numerous restoration projects to restore ecosystem function. Native cordgrass (*Spartina foliosa*) is important for sediment accumulation and nesting habitat for endangered California Ridgway's Rail (*Rallus obsoletus obsoletus*), and because salt marshes are typically nitrogen-limited ecosystems, previous restoration studies in California have used nitrogen fertilizers to increase plant growth. This study compared the use of two low-nitrogen, high-carbon fertilizers (*Macrosystis pyrifera* (kelp) and sodium alginate) to stimulate N<sub>2</sub>-fixing bacteria inhabiting the *S. foliosa* rhizosphere in a historic and recently restored marsh in San Francisco Bay. Alginate increased N<sub>2</sub>-fixation, though there was no observed *S. foliosa* growth response. Kelp suppressed N<sub>2</sub>-fixation, and increased the foliar nitrogen content of *Salicornia pacifica* in mixed *Spartina*-*Salicornia* stands. The restored marsh had less than 0.5 μM phosphate in porewaters, suggesting phosphorus, not nitrogen, limits *S. foliosa* growth in this marsh. Alginate increased rates of N<sub>2</sub>-fixation and may promote plant growth in marshes exhibiting nitrogen limitation. Phosphate limitation was a surprise considering numerous previous studies indicate nitrogen as the primary limiting nutrient of tidal marsh plants. We recommend first assessing nutrient stoichiometry when considering manipulations to promote plant growth in restored marshes.

**Keywords** *Spartina* · Cordgrass · Restoration · Nitrogen fixation · Sediment · Porewater

## Introduction

Tidal marshes in California have been highly impacted by human activities, with a 90% loss in the San Francisco Estuary (SFE) during the twentieth century (Nichols et al. 1986). Restoration of tidal marshes in the SFE has been underway for decades (Williams and Faber 2001; Boyer and Thornton 2012), with projects ranging from less than an acre to the multiple projects within the 6000-ha South Bay Salt Ponds Restoration Project (see <https://www.ecoatlas.org> and <http://www.southbayrestoration.org>). *Spartina* (cordgrass) species are often targeted in restoration projects as they provide cover, nesting, and foraging habitat for birds and insects

(Boyer and Zedler 1996, 1998; Gratton and Denno 2005). *Spartina* stands also reduce sediment erosion and can raise the level of the marsh surface, through the reduction of water flow and subsequent sediment deposition (Leonard and Luther 1995; Fredrichs and Perry 2001; Leonard and Reed 2002), an important characteristic given the projected rise in sea level and limits to upslope migration due to coastal human development. In the SFE, restoration of the native cordgrass, *Spartina foliosa*, is currently underway, following the control of a highly invasive hybrid of Atlantic cordgrass (*Spartina alterniflora*) and *S. foliosa*, which had spread for decades through much of the central and south bay (Ayres et al. 2004). Now, for several years, scientists and managers have been seeking the best methods to restore native cordgrass (<http://www.spartina.org>; W.J. Thornton master's thesis, in review), including ways to promote dense, tall stands to support nesting by the endangered California Ridgway's Rail (*Rallus obsoletus obsoletus*).

Tidal marshes on both the Atlantic and Pacific coast of the United States are typically considered to be nitrogen-limited, and numerous studies have demonstrated that short stands of

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cordgrass in both salt and brackish marshes grow taller when supplied with nitrogen (e.g., Gallagher 1975; Mendelsohn 1979; Boyer and Zedler 1996; Trnka and Zedler 2000; Crain 2007; Langley et al. 2009). However, studies also suggest that phosphate may limit bacterial communities in sediments (e.g., Sundareshwar et al. 2003), highlighting the complex nature of biogeochemical cycling in salt marsh systems. Further, sustained inputs of nitrogen do not necessarily promote persistent cordgrass marsh structure or function. For example, in sandy restored salt marshes in southern California, nitrogen fertilizer (urea) increased *S. foliosa* height (Gibson et al. 1994; Boyer and Zedler 1998), but effects did not persist even following multiple years of additions (Boyer et al. 2000; Lindig-Cisneros et al. 2003); hence, the short cordgrass did not provide adequate nesting habitat for the endangered southern California subspecies of Ridgway's Rail (Zedler 1993). In addition, long term nitrogen inputs can lead to height growth without corresponding root growth, which can ultimately lead to decreased marsh elevation (Langley et al. 2009) and even loss of marsh acreage through channel slumping (Deegan et al. 2012).

In natural marshes, the nitrogen limitation of *Spartina* species is mitigated by the symbiotic relationship that occurs between *Spartina* and heterotrophic N<sub>2</sub>-fixing bacteria (diazotrophs) in the plant's rhizosphere. Through this mutualistic relationship, the cordgrass receives a source of fixed nitrogen from the bacteria, and the bacteria receive fixed carbon from the plant (Piceno and Lovell 2000; Carpenter et al. 1978; Teal et al. 1979). N<sub>2</sub>-fixation has been shown to be an especially important source of nitrogen in newly created marshes (Currin et al. 1996; Tyler et al. 2003), where sediment organic matter content is low and thus remineralized nitrogen is less available for plants. Nitrogen fixation is energetically demanding and when nitrogen rich fertilizers are applied to salt marsh sediments, rates of nitrogen fixation decrease (Mosemann-Valtierra et al. 2010).

As human developments continue to expand onto existing salt marshes, and new marshes are constructed, managers need restoration methods that both support native species and habitat function. By increasing the availability of fixed carbon to sediment diazotrophs, rather than applying nitrogen fertilizer directly to the marsh, sediment nitrogen fixation could provide a natural source of nitrogen to cordgrass plants. In southern California, low nitrogen compost successfully increased the rate of salt marsh plant establishment and growth in a newly constructed marsh (O'Brien and Zedler 2006). A mesocosm experiment conducted in northern California found that polysaccharide sediment amendments increased rates of sediment nitrogen fixation in the rhizosphere of *S. foliosa* with some evidence of enhanced cordgrass growth (Cohen et al. 2009). Our study was a field-based project, examining the use of two high-carbon, low-nitrogen fertilizers, kelp and a polysaccharide kelp derivative, sodium alginate, to stimulate rhizospheric N<sub>2</sub>-fixation, which could naturally provide

nitrogen for *S. foliosa* growth in a restored tidal marsh. The same experiment was simultaneously conducted in a historic marsh. We hypothesized that the fertilizers would enhance cordgrass growth in both the restored and natural marsh, with the greatest benefit to occur in the sodium alginate amended plots within the restored marsh.

## Methods

### Study Site Description

Located on the western side of San Pablo Bay, in northern San Francisco Bay, China Camp State Park (38°00'43"N, -122°29'12"W) contains both ancient marsh and areas formed more recently from Gold-rush era sediment deposition (i.e., since the late 1800s) (Goman and Wells 2000). The latter includes approximately 20 m of pure *S. foliosa* at the outer edge, followed by *Salicornia pacifica* (pickleweed) dominated marsh plain. Using a randomized complete block design, 1 m<sup>2</sup> plots were set up parallel to the water line, in the middle of the *Spartina* band, with 0.5 m between each plot. The experiment was divided into five blocks to account for variability introduced by the presence of channels in the marsh, with seven treatments repeated once per block.

Guadalcanal Village (38°07'08" N, -122°17'26" W) is located on the northern side of San Pablo Bay, near the mouth of the Napa River and adjacent to Highway 37. This 44-acre marsh was restored to tidal action in 2001, after being surrounded by levees for approximately 60 years. Before being returned to tidal flow, trees and buildings were removed, and the site was graded. Since the levee breach in October 2001, large amounts of sediment have accumulated, creating numerous pools within the marsh. *Spartina foliosa* is found along the edges of the smaller pools, but the site lacks a distinct *Spartina* zone. Plants were most often found as a part of mixed communities of *Salicornia pacifica*, *Distichlis spicata*, *Bolboschoenus maritimus*, and *Cotula coronopifolia*. Due to the complexity of the marsh at Guadalcanal Village, blocks were set up around four pools within the site, with one block per pool, and seven plots within each block.

### Fertilizer Treatments

The same seven treatments were used in each marsh: control, single disturbance control, single kelp addition, single alginate addition, monthly disturbance control, monthly kelp addition, and monthly alginate addition (Table 1). In order to ensure fertilizers remained in the sediments during tidal inundation, channels were dug into each plot to 0.1 m, the depth where the majority of the *Spartina* rhizosphere is located and N<sub>2</sub>-fixation occurs (Whitney et al. 1975), and the fertilizer was placed into the channel and buried.

**Table 1** Description of fertilizer applications and abbreviations

Name	Description
Control0	No manipulation
Control1	Channels dug into plot once (disturbance control for single fertilizer applications)
Kelp1	Kelp (20 g N and 300 g C) applied into channels once
Algin1	Sodium alginate (BDL N and 300 g C) applied into channels once
Control2	Channels dug into plot monthly (disturbance control for monthly fertilizer applications)
Kelp2	Kelp (20 g N and 300 g C) applied into channels monthly
Algin2	Sodium alginate (BDL N and 300 g C) applied into channels monthly

Based on the previous work of Cohen et al. (2009), kelp and alginate were chosen as the fertilizers to apply into the marsh sediments. In June 2007, 1 kg of kelp (0.02 kg of nitrogen and 0.3 kg of carbon) and 0.7 kg of alginate (0.2 kg of carbon; nitrogen was below detection limit (BDL)) was buried into the respective plots. In July and August, the fertilizers were again placed into the monthly kelp and monthly alginate amended plots.

### Sediment Characteristics

In situ oxidation-reduction potentials (ORP) in each plot were measured in November 2007 using an HI 3230 platinum tipped probe and a Hanna HI 98171pH/ORP meter. The probe was inserted to a 1 cm depth followed by a 10 cm depth at three random locations within each plot. In November 2007, one 10 cm deep by 5 cm diameter soil core was collected from each plot, dried at 60 °C, ground, sieved to 1 mm and used for the following analyses. Sediment texture was determined using the hydrometer method (Gee and Bauder 1979), and salinity was measured following the saturated soil paste method of Richards (1954). The pH of the saturated sediment was measured using a handheld pH meter (Hanna HI 98171, probe- HI 1230). Sediment organic matter was quantified by combustion (Craft et al. 1991). Percent carbon and nitrogen were determined using eight random sediment samples from each marsh (i.e., not from the treatment plots) (Costech ECS 4010 Analyzer). Sediment was again collected from the two marshes in February 2011 to confirm previous sediment texture analyses (Gee and Bauder 1979).

### N<sub>2</sub>-fixation

Sediment cores were collected monthly from June through September 2007 to measure rates of N<sub>2</sub>-fixation using the acetylene reduction technique (Capone 1993). Cores were collected from each plot using 30 cm<sup>3</sup> syringes with removed tips. All cores were taken from the base of *S. foliosa* plants, and in order to minimize soil contact with the air, a silicone stopper was inserted into the end opposite the plunger after sediment collection. Beginning in July, due to the large variability in N<sub>2</sub>-fixation rates observed in June, replicate cores were taken from each plot.

In the lab, cores were extruded into 125 mL Erlenmeyer Flasks, covered with silicone stoppers, and the headspace of the flask was flushed with nitrogen gas. Acetylene reduction assays (ARA) were carried out using a Shimadzu Flame Ionizing Gas Chromatograph (GC-8A) attached to a Shimadzu C-R6A Chromatopac recorder, with nitrogen as a carrier gas, and hydrogen used for combustion. All injections into the chromatograph were completed using 100 µL gas-tight syringes. Acetylene gas was injected into each bottled core to create a 10–15% headspace concentration as described by Capone (1993). During each ARA, an extra core was collected as a control to which no acetylene gas was injected in order to determine if any ethylene was naturally produced by the core without acetylene addition.

### Porewater Nutrients

Porewater was collected monthly from June through August 2007 from PVC pipe sippers, which collected water at 10 cm beneath the sediment surface. Water was stored unfiltered and frozen for less than six months before being defrosted for 24 h, filtered through GF/F filters and refrozen until analysis on an auto-analyzer. Samples were tested for nitrate + nitrite and phosphate using a Bran and Luebbe Auto-analyser 2.

Ammonium concentrations were determined using a modified fluorometer procedure from Holmes et al. (1999). Due to the small volume of porewater collected, ammonium analyses were conducted using the same water sample on which phosphate and nitrate + nitrite levels had been run. These samples had been frozen and defrosted numerous times, and therefore values obtained should be interpreted with caution.

In February 2011, five replicate cores were collected from each marsh, outside of study plots, in order to determine porewater phosphate concentrations within each marsh. During the initial study phosphate concentrations were found to be very low in the restored marsh, however these samples were not collected to run an accurate phosphate analysis due to the reactive nature of phosphate with oxidized iron (Sundby et al. 1992). To more accurately measure phosphate concentrations, porewater was extracted from each core via centrifugation and filtered through a 0.2 µm filter under a nitrogen environment to minimize oxidation reactions. The samples were then analyzed for dissolved inorganic phosphate on a

Synergy HT BioTek Multi-Mode Microplate Reader following the molybdate blue method (Grasshoff et al. 1983).

## Vegetation

Each month from June through September 2007, the five tallest plants per plot were measured. Total carbon and nitrogen content of leaves was determined using a Costech ECS 4010 analyzer. Three leaves were collected from *S. foliosa* plants in each plot, and in Guadalcanal triplicate *Salicornia pacifica* succulent tips were also collected from each plot.

## Statistics

For China Camp and Guadalcanal, separate two-way Analysis of Variance (ANOVA) tests were used to check for block and treatment effects on each date. For between marsh comparisons, treatment and marsh interactions were tested, and when interactions occurred, between marsh comparisons were run using control plots only. All  $N_2$ -fixation data were log transformed to meet parametric assumptions. When significant differences occurred, Tukey's post hoc test was used, and all data were analyzed using SPSS 15.0 for Windows.

## Results

### Sediment Characteristics

The ORP in surface sediments in the natural marsh ( $-265 \pm 12$  mV) indicated less reduced conditions than those in the restored marsh ( $-322 \pm 9$  mV), while at 10 cm depth ORP was similar between the two marshes (Table 2). Sediment salinity in the natural marsh ( $60 \pm 1$ ) was significantly higher than in the restored marsh ( $37 \pm 9$ ), and sediment pH in the natural marsh ( $5.6 \pm 0.1$ ) was lower than in the restored marsh ( $6.1 \pm 0.1$ ). Elemental analysis of sediment samples revealed no significant difference in percent composition of carbon ( $2.3 \pm 0.5\%$ ) and nitrogen ( $0.22 \pm 0.05\%$ ) between the marshes (Table 2). Percent sediment organic matter was also similar between the natural marsh ( $6.2 \pm 0.9\%$ ) and the restored marsh ( $5.4 \pm 0.9\%$ ). Sediment texture between the

natural marsh in China Camp and the restored marsh in Guadalcanal was similar, though some variability was observed in size fractions between the initial and follow-up sampling four years later (Table 3).

### $N_2$ -fixation

Before fertilization, no difference in rates of  $N_2$ -fixation occurred in the natural marsh (Table 4, Fig. 1). After fertilization,  $N_2$ -fixation rates were highly variable among plots. The highest rate of  $N_2$ -fixation observed in the natural marsh occurred in a monthly algininate plot in early July ( $41 \pm 29$   $\mu\text{mol } N_2/\text{m}^2/\text{h}$ ). On average, August had the highest rate of  $N_2$ -fixation ( $18 \pm 2.6$   $\mu\text{mol } N_2/\text{m}^2/\text{h}$ ), with late July ( $7.7 \pm 1.5$   $\mu\text{mol } N_2/\text{m}^2/\text{h}$ ) and September ( $9.3 \pm 1.4$   $\mu\text{mol } N_2/\text{m}^2/\text{h}$ ) having the lowest. By treatment, lowest rates of  $N_2$ -fixation occurred in kelp-amended plots.

In the restored marsh,  $N_2$ -fixation rates were similar between sites before fertilizer treatments in early June (Table 4) (Fig. 1). After fertilization, highest rates of  $N_2$ -fixation occurred in algininate-amended plots, with late June ( $100 \pm 80$   $\mu\text{mol } N_2/\text{m}^2/\text{h}$ ) and early July ( $240 \pm 90$   $\mu\text{mol } N_2/\text{m}^2/\text{h}$ ) exhibiting especially high rates. Unlike the natural marsh, no zero rates of  $N_2$ -fixation occurred in the restored marsh. Post fertilization, lowest rates of  $N_2$ -fixation occurred in plots applied monthly with kelp fertilizer. In July, for example, rates of  $N_2$ -fixation in the monthly kelp plot were half that of the control plot. When rates of  $N_2$ -fixation were compared between control plots in the natural and restored marsh, no significant difference in rates of  $N_2$ -fixation was found.

### Porewater Nutrients

In China Camp, porewater ammonium concentrations were significantly higher in the monthly fertilized kelp plots ( $70 \pm 10$   $\mu\text{M}$ ) than the monthly fertilized algininate plots ( $3 \pm 1$   $\mu\text{M}$ ) ( $F_{(5, 28)} = 2.9$ ,  $p = 0.042$ ; Tukey test,  $p < 0.05$ ). When the natural and restored marshes were compared, no interaction was found between marsh and sediment treatment. Between marshes, nitrate + nitrite concentrations were higher in the restored marsh in June, July and September (Table 5). Ammonium concentrations were significantly higher in the

**Table 2** Sediment characteristics (mean  $\pm$  SE) for China Camp ( $n = 35$ ) and Guadalcanal ( $n = 28$ )

	China camp	Guadalcanal	Statistics
Salinity	$60 \pm 1$	$37 \pm 9$	$F(1, 61) = 137$ , $p < 0.001$
pH	$5.6 \pm 0.1$	$6.1 \pm 0.1$	$F(1, 61) = 12.4$ , $p = 0.001$
LOI Org Matter	$6.2\% \pm 0.9\%$	$5.4\% \pm 0.9\%$	Not significant
ORP			
1 cm depth	$-265 \pm 12$ mV	$-322 \pm 9$ mV	$F(1, 33) = 11.07$ , $p = 0.002$
10 cm depth	$-346 \pm 8$ mV	$-328 \pm 9$ mV	Not significant
Total Nitrogen	$0.22\% \pm 0.05\%$	$0.22\% \pm 0.05\%$	Not significant

**Table 3** Comparison of sediment grain size (mean  $\pm$  SE) between 2007 and 2011

		Sand	Silt	Clay
2007	China camp (n = 35)	63% $\pm$ 0.61%	16% $\pm$ 0.52%	21% $\pm$ 0.34%
	Guadacanal (n = 28)	62% $\pm$ 0.53%	16% $\pm$ 0.49%	22% $\pm$ 0.47%
2011	China camp (n = 8)	53% $\pm$ 0.84%	18% $\pm$ 0.86%	29% $\pm$ 0.62%
	Guadacanal (n = 5)	53% $\pm$ 1.7%	15% $\pm$ 0.90%	32% $\pm$ 1.9%

restored marsh in June and in the natural marsh in August and September. Phosphate concentrations were much lower in the restored marsh than the natural one for the duration of the study, and follow-up work to check these values in February 2011 confirmed dissolved inorganic phosphate in the natural marsh ( $10.6 \pm 2.2 \mu\text{M}$ ) to be higher than the restored marsh (BDL,  $<0.5 \mu\text{M}$ ).

### Vegetation Parameters

Based on nitrogen and carbon content of leaf samples, the leaves of *S. foliosa* did not show any effect of fertilizer treatment; however, between marshes, the percent nitrogen and carbon of *S. foliosa* leaves in China Camp (N:  $1.8\% \pm 0.04\%$ ; C:  $42.5\% \pm 0.22\%$ ) was higher than in Guadacanal (N:  $1.6\% \pm 0.04\%$ ; C:  $41.2\% \pm 0.25\%$ ) (Table 6). The mixed

stands within the Guadacanal plots allowed for analysis of *Salicornia pacifica* foliar carbon and nitrogen content; plants in kelp-fertilized plots had a significantly higher percent nitrogen than plants in alginate-treated plots (Fig. 2).

In the natural marsh, treatment height effects were observed in August, where plots with the monthly alginate application were significantly shorter than all other treatments (Fig. 3). In the restored marsh at Guadacanal, there was no significant impact of treatment on *S. foliosa* height (Fig. 3). Between marshes, *S. foliosa* height was compared using the control plots. In June, the natural and restored marshes had similar plant heights; however, for the remainder of the study the natural marsh had taller cordgrass than the restored marsh (Fig. 4).

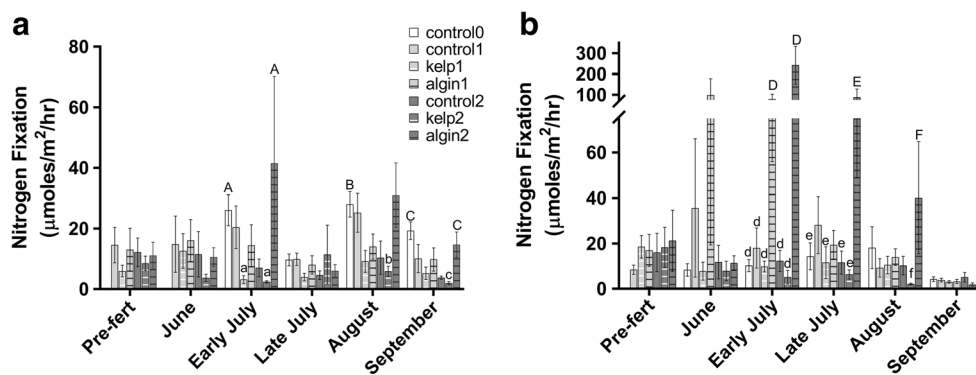
### Discussion

Restored salt marshes in California can lack critical habitat functions of natural systems including tall and dense vegetative cover (Zedler and Callaway 1999; Boyer et al. 2000). *Spartina foliosa* is a vital component to salt marsh function in these marshes, providing organic carbon to the food chain, nesting habitat for endangered Ridgway's Rail and sediment accumulation that helps to maintain and grow marsh elevation (Daehler and Strong 1996; Kwak and Zedler 1997; Zedler and Callaway 1999; Ward et al. 2003; Overton et al. 2014). The purpose of this study was to determine if the height of

**Table 4** Results of two-way ANOVA on  $\text{N}_2$ -fixation rates by treatment and block for (a) China Camp ( $n = 5$ ) and (b) Guadacanal ( $n = 4$ )

	F statistic	p value	Treatment tukey test results
a.			
Early June	0.530	0.751	–
Late June	1.41	0.267	–
Early July	5.24	0.001	Algin2, Control0 > Kelp2, Kelp1
Late July	1.05	0.419	–
Late August	3.48	0.013	Control0 > Kelp2
Late September	6.07	0.001	Algin2, Control0 > Kelp2
	F statistic	p value	Block tukey test results
Early June	0.985	0.439	–
Late June	11.4	<0.001	D, E > A, B, C
Early July	2.74	0.052	–
Late July	1.35	0.280	–
Late August	2.22	0.097	–
Late September	3.56	0.020	B, D > A
b.			
Early June	0.360	0.868	–
Late June	1.83	0.149	–
Early July	20.4	<0.001	Algin1, Algin2 > all others
Late July	6.03	0.001	Algin2 > Control0, Control2, Kelp1, Kelp2
Late August	3.38	0.021	Algin2 > Kelp2
Early October	1.28	0.314	–
	F statistic	p value	Tukey test results
Early June	3.29	0.050	–
Late June	6.27	0.004	D > A, C
Early July	9.42	0.001	B, D > A
Late July	8.29	0.001	D > A, C
Late August	5.73	0.006	D > A, B
Early October	1.37	0.284	–





**Fig. 1** Mean rates of nitrogen fixation ( $\pm$  SE error bars) by sampling date and treatment for (a) China Camp and (b) Guadalcanal, with treatment codes defined in Table 1. Capital and lowercase letters indicate significant differences within each month. Control0 plots were added after the start of

the experiment in early July at China Camp and in late June at Guadalcanal, no zero rates of nitrogen fixation were measured. Note the change in scale at 100  $\mu\text{moles}/\text{m}^2/\text{h}$

*S. foliosa* in restored marshes could be increased by stimulating heterotrophic  $\text{N}_2$ -fixing bacteria through the use of low nitrogen, high carbon fertilizers, rather than through the application of nitrogen, which can lead to negative impacts on tidal marshes over time (Langley et al. 2009; Deegan et al. 2012). The study was conducted in a natural and a recently restored marsh to compare sediment geochemistry between the two marshes and measure effects of organic amendments on rates of  $\text{N}_2$ -fixation and on vegetative growth parameters.

## $\text{N}_2$ -fixation

Rates of sediment  $\text{N}_2$  fixation varied throughout the study. Maximum rates of  $\text{N}_2$ -fixation occurred in July, which is consistent with previous studies (Piehler et al. 1998 and Tyler et al. 2003), and can be attributed to the combined effect of fertilizer application and the cordgrass having a maximum photosynthate output in midsummer (Whiting et al. 1986). Rates of  $\text{N}_2$ -fixation were highly variable, likely caused by the heterogeneous application of fertilizer in study plots due to the presence of vegetation in all plots prior to fertilization (Langis et al. 1991). Block effects were noted (Table 4) and may be attributed to block proximity to channels within the

saltmarsh, leading to differences in tidal flushing and porewater nutrient exchange (Moffett and Gorelick 2016). While variable, measured rates of  $\text{N}_2$ -fixation were consistent with those found in other *Spartina* systems (Moseman-Valtierra et al. 2016).

Suppressed rates of  $\text{N}_2$ -fixation were observed in kelp-treated plots both in the newly restored and the natural marsh (Fig. 1). Sources of fixed nitrogen are known to inhibit  $\text{N}_2$ -fixation in salt marsh sediments (Yoch and Whiting 1986) both through deactivation of nitrogenase synthesis (Hanson 1977 and Currin et al. 1996) and due to a loss of competitive advantage and subsequent replacement by other bacteria (Sprent and Sprent 1990). The supplied nitrogen from the kelp fertilizer is the likely cause of reduced  $\text{N}_2$ -fixation rates in kelp-amended plots (Fig. 1). In plots receiving a single kelp treatment,  $\text{N}_2$ -fixation rates were similar to control plots in late July, one month post fertilization; this was consistent with work by Moseman-Valtierra et al. (2016) showing that the diversity and community composition of diazotrophs was not altered by nitrogen additions. Previous marsh restoration work has shown that using a kelp-perlite compost can increase plant establishment and survival while increasing the nitrogen content of sediments (O'Brien and Zedler 2006).

**Table 5** Porewater nutrients by month for the natural (China Camp) and newly restored marsh (Guadalcanal). Statistics provided for those data where significant differences were found across all treatments

		China camp	Guadalcanal	Statistics
Nitrate + Nitrite	June	0.730 $\pm$ 0.37	18.4 $\pm$ 7.1	F(1, 47) = 10.5, p = 0.002
	July	1.41 $\pm$ 0.63	23.0 $\pm$ 9.3	F(1, 47) = 6.15, p = 0.017
	August	2.05 $\pm$ 1.2	6.13 $\pm$ 2.3	
	September	0.459 $\pm$ 0.32	8.10 $\pm$ 3.5	F(1, 47) = 29.7, p < 0.001
Ammonium	June	34.6 $\pm$ 6.4	78.7 $\pm$ 13	F(1, 49) = 12.7, p = 0.001
	July	41.6 $\pm$ 7.7	56.0 $\pm$ 8.1	
	August	44.8 $\pm$ 7.9	24.9 $\pm$ 5.3	F(1, 47) = 4.43, p = 0.041
	September	51.1 $\pm$ 7.7	17.3 $\pm$ 5.6	F(1, 46) = 11.4, p = 0.001
Phosphate	June	46.4 $\pm$ 5.0	1.03 $\pm$ 0.46	F(1, 46) = 43.6, p < 0.001
	July	45.6 $\pm$ 4.3	0.253 $\pm$ 0.091	F(1, 46) = 53.6, p < 0.001
	August	463.9 $\pm$ 3.6	0.571 $\pm$ 0.34	F(1, 46) = 75.2, p < 0.001
	September	54.5 $\pm$ 4.8	0.296 $\pm$ 0.66	F(1, 46) = 61.8, p < 0.001

**Table 6** Vegetative parameters for China Camp (n = 35) and Guadalcanal (n = 28) (mean ± SE) with all treatments pooled (no interaction between block or treatment)

	China camp	Guadalcanal	Statistics
<i>Spartina</i> tissue %N	1.8 ± 0.04%	1.6 ± 0.04%	F(1, 62) = 14.7, p < 0.001
<i>Spartina</i> tissue %C	42.5 ± 0.2%	41.2 ± 0.3%	F(1, 62) = 15.6, p < 0.001

While no effect of increased *S. foliosa* growth or increased sediment nitrogen was evident from our study, kelp sediment amendments resulted in a short-term suppression of N<sub>2</sub>-fixation in both the natural and restored marsh, suggesting that relatively high nitrogen organic matter amendments are not a good choice for promoting N<sub>2</sub>-fixation.

Previous mesocosm work demonstrated that rates of N<sub>2</sub>-fixation in *S. foliosa* sediments from the SFE could be increased through the application of low nitrogen organic matter (Cohen et al. 2009). Our in situ work in the natural and newly established marsh showed that rates of N<sub>2</sub>-fixation could be stimulated in *S. foliosa* marshes with preexisting vegetation. However, in the restored marsh, increased rates of N<sub>2</sub>-fixation decreased one-month post fertilizer application, suggesting that it may be necessary to apply organic matter amendments throughout the growing season in order to maintain increased rates of nitrogen fixation in restored marshes.

Control plots exhibited similar rates of N<sub>2</sub>-fixation between the natural and restored marsh, despite differences in salinity between the marshes. This is contrary to previous work by Moseman (2007) which suggests that abiotic factors, such as salinity, are more important in controlling rates of nitrogen fixation than plant species. Previous studies have reported conflicting results regarding rates of N<sub>2</sub>-fixation between natural and restored marshes, with some finding similar rates of N<sub>2</sub>-fixation (Langis et al. 1991) and others showing restored marshes to have higher rates of N<sub>2</sub>-fixation (Currin et al. 1996;

Tyler et al. 2003). There are two main sources of N<sub>2</sub>-fixation in salt marsh ecosystems, cyanobacterial mats and sediment diazotrophs. Often, increased rates of N<sub>2</sub>-fixation in restored marshes are attributed to the presence of cyanobacterial mats (Carpenter et al. 1978; Currin et al. 1996; Tyler et al. 2003), which are associated with bare mudflats. Cyanobacteria do not typically exhibit higher rates of N<sub>2</sub>-fixation when supplied organic matter due to their predominantly autotrophic metabolism (Stewart 1980). Given the observed increase in rates of N<sub>2</sub>-fixation following organic matter additions, sediment diazotrophs are the likely source of nitrogen fixation in China Camp and Guadalcanal. Thus, the type of bacteria present and associated metabolic requirements (i.e., fixed carbon vs sunlight) may be an important consideration in the potential for nitrogen fixation in restored marshes of different ages and degrees of vegetative cover.

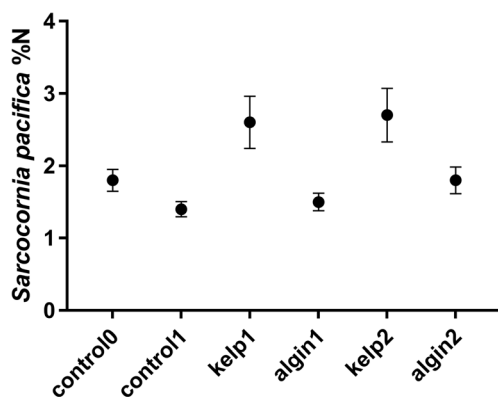
## Vegetation Parameters

Increased rates of N<sub>2</sub>-fixation had no quantifiable impact on measured *Spartina* growth parameters (i.e., leaf nitrogen content, plant height, plant density), similar to a study with organic amendments by Cohen et al. (2009), but in contrast to previous studies with inorganic nitrogen additions (e.g. Covin and Zedler 1988; Gibson et al. 1994; Boyer and Zedler 1998; Tyler et al. 2003). However, Tyler et al. (2007) also reported a lack of *S. foliosa* height response post nitrogen fertilization in the San Francisco Bay, and attributed it to tidal flushing of the nitrogen from sandy sediments before plant uptake. Given the relatively high sand concentration of both marshes in the current study (see below), the lack of *S. foliosa* growth despite increased N<sub>2</sub>-fixation may also be attributable to loss of fixed nitrogen during tidal flushing.

In the restored marsh, where *S. foliosa* and *Salicornia pacifica* were growing together, foliar nitrogen analysis revealed that *S. pacifica* in kelp fertilized plots had significantly higher foliar nitrogen content than plants in other treatment groups (Fig. 2). *Salicornia pacifica* is known to be a superior competitor to *S. foliosa* in its ability to take up available nitrogen (Covin and Zedler 1988) and has been found to outcompete other native species for nitrogen in mixed plant stands (Ryan and Boyer 2012). The increased foliar nitrogen content of *S. pacifica* in kelp fertilized plots suggests kelp may be beneficial to *S. pacifica* over time, and highlights the importance of understanding nutrient competitive advantage between species when conducting restoration work.

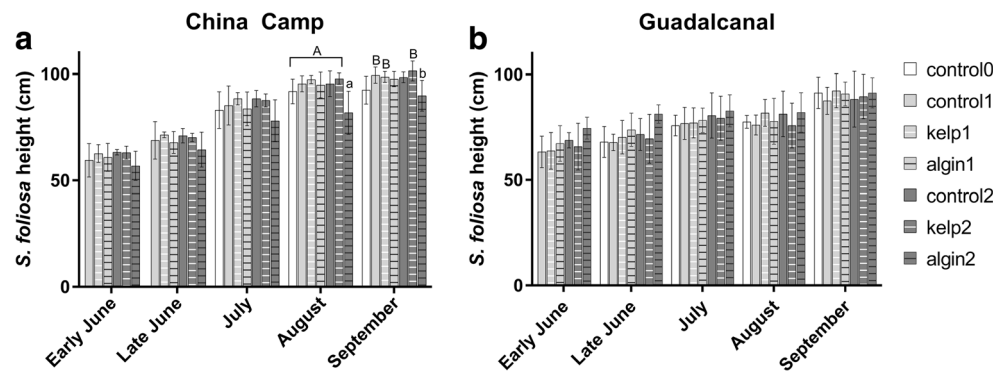
## Sediment Characteristics

The two San Francisco marshes used in this study had similar sediment texture, despite the newly restored marsh being only six years old (Table 3). When sediment texture was measured again four years later, a lower sand fraction was noted in both



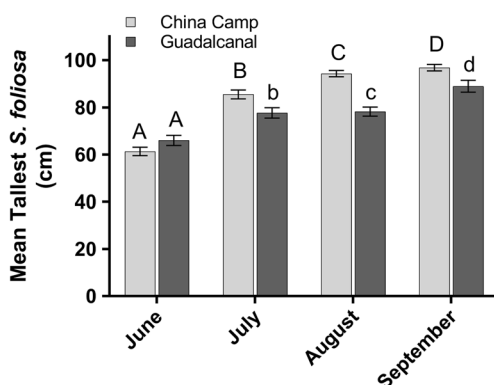
**Fig. 2** Mean *S. pacifica* succulent tissue percent nitrogen content (± SE error bars) for Guadalcanal (n = 24) (F<sub>(6, 18)</sub> = 8.24, p = 0.004; Tukey test, p < 0.05)

**Fig. 3** Mean tallest plant height ( $\pm$  SE error bars) by treatment in (a) China Camp ( $n = 5$ ) and (b) Guadalcanal ( $n = 4$ ). Within China Camp, capital and lowercase letters are used to indicate significant differences in August ( $F_{(5, 30)} = 8.65$ ,  $p < 0.001$ ; Tukey test,  $p < 0.05$ ) and in September ( $F_{(5, 30)} = 4.14$ ,  $p = 0.01$ ; Tukey test,  $p < 0.05$ )



the natural and restored marsh, however both marshes again had similar sediment texture. When compared with other California marshes where *S. foliosa* is native, the two San Francisco marshes from our study were found to have lower clay and higher sand concentrations, comparable to those found in Connector Marsh, a four year old marsh created from dredge material in San Diego (Scatolini and Zedler 1996) (Table 7). In the San Francisco Bay, we suspect texture similarity between the fringing natural marsh that built rapidly along China Camp over the last century (Goman et al. 2008) and the young restored marsh at Guadalcanal is in part due to high riverine sediment supply from the Petaluma and Napa Rivers into the restored and natural marshes, respectively (Neary et al. 2001; Ganju et al. 2013).

In marshes near San Diego, dredged material with a high sand content has been used to create new marshes. The low clay content and associated reduced cation exchange capacity of these sediments has been linked to poor sediment nutrient retention, resulting in shorter cordgrass plants (Boyer et al. 2000). In our study, despite having similar sediment texture, the cordgrass in the natural marsh was significantly taller than the cordgrass in the recently restored marsh (Fig. 4), suggesting that sediment texture is not the factor responsible for producing shorter *S. foliosa* plants in the restored Guadalcanal marsh.



**Fig. 4** Mean tallest plant height ( $\pm$  SE error bars) for control plots in all months for China Camp (June  $n = 12$ , all others  $n = 15$ ) and Guadalcanal (June  $n = 9$ ; all others  $n = 12$ ). Capital and lowercase letters used to indicate significant differences between marshes

Natural marshes typically have higher sediment organic matter content than restored marshes (Craft et al. 1988; Boyer and Zedler 1998), but this did not explain taller plants at China Camp, where sediment organic matter (6.2%) was not significantly higher than at Guadalcanal (5.4%). Callaway et al. (2012) measured organic matter at China Camp in the top 20 cm of cores and found it to be 11.7% ( $\pm 1.92$  SE), higher than that measured in our study. Our cores were from the top 10 cm, and the cores from Callaway et al. (2012) were taken from a transect through the marsh, which likely contained older marsh material, and thus more organic matter, than our cores which were taken from a newer region of the marsh (see above).

The similarities in both sediment texture and organic matter between the sampled region of the naturally-formed marsh at China Camp and the restored marsh at Guadalcanal, suggest that differences in porewater phosphate may be at the heart of differences in *S. foliosa* growth. Phosphate levels in the restored marsh were below detection level ( $0.5 \mu\text{M}$ ). The lack of detectable phosphate in the sediment porewater of the restored marsh, along with measurable levels of nitrate and ammonium, suggests that phosphate, rather than nitrogen, is limiting plant growth in Guadalcanal. This is contrary to our hypothesis that nitrogen is the limiting nutrient in the restored salt marsh. The natural fringing marsh at China Camp had phosphate concentrations ten times higher than those in the restored marsh, consistent with other studies examining the concentration of phosphate in *Spartina* dominated salt marshes (Giblin and Howarth 1984; Negrin et al. 2011).

One reason for low phosphate levels in the restored marsh is sorption onto sediment particles. In oxygenated sediments phosphate is adsorbed to ferric-oxyhydroxides ( $\text{Fe}(\text{OH})_3$ ), rendering phosphate biologically unavailable (Chambers and Odum 1990; Blomqvist and Elmgren 2004); however, under anoxic conditions,  $\text{Fe}(\text{OH})_3$  is reductively dissolved, releasing phosphate into the porewater (Krom and Berner 1980; Jensen et al. 1995; Rozan et al. 2002; Hupfer and Lewandowski 2008). Though the sediments of both marshes were found to be reducing, iron oxides were present on the surface of the marsh at Guadalcanal (personal observation). Increased iron in marsh sediments, combined with the oxic rhizosphere of



**Table 7** Comparison of sediment texture between created and natural marshes in San Diego and San Francisco

Author	Marsh	Marsh age	Sand	Silt	Clay
Gibson et al. 1994	Marisma de Nacion	0 yrs	63%	15%	22%
Scatolini and Zedler 1996	Connector Marsh	4 yrs	38%	35%	27%
Scatolini and Zedler 1996	Sweetwater Marsh	>100 yrs	7%	32%	61%
Boyer et al. 2000	Paradise Creek Marsh	>100 yrs	30%	25–30%	40–45%
Lindig-Cisneros et al. 2003	Connector Marsh	~10 yrs	64%	9%	27%
Current data	China Camp Marsh	>100 yrs	63%	16%	21%
Current data	Guadacanal Marsh	7 yrs	62%	16%	22%

*S. foliosa* would form phosphate sorbing iron oxides, potentially limiting phosphate transfer to the cordgrass. Additionally, iron-phosphate sorption in sediments may explain the low porewater phosphate concentrations observed in the restored marsh. Regardless of the mechanism, the observed low porewater phosphate concentrations in the restored marsh indicate that phosphate, not nitrate, is limiting *S. foliosa* growth in Guadalcanal.

In addition to low phosphate concentrations limiting growth in the restored marsh, sulfide toxicity may have contributed to a lack of plant response to increased rates of  $N_2$ -fixation. Though sulfide concentrations were not directly measured in this study, the ORP of surface sediment in the restored marsh was lower than the natural marsh, and below the potential at which sulfate reduction begins (Mitsch and Gosselink 2000). Sulfide toxicity inhibits ammonium uptake in plants (Koch et al. 1990) and has been shown to affect the kinetic uptake in *S. alterniflora* at concentrations as low as 0.25 mmol/L sulfide (Bradley and Morris 1990). Additionally, the reduced height of cordgrass in the alginate amended plots within the natural marsh may have been caused by the alginate decreasing oxygen penetration into sediments, resulting in higher sulfide production and accumulation (Fig. 3).

Despite the increased rate of  $N_2$ -fixation within alginate fertilized plots, *S. foliosa* height and foliar nitrogen content were not affected. In a mesocosm study by Cohen et al. (2009), alginate fertilizer tended to enhance *S. foliosa* leaf and shoot production (though not significantly), and a lack of plant height response was suggested to be caused by the short-term increase in  $N_2$ -fixation rates. Cohen and Kern (2012) found increased production of new *S. alterniflora* shoots with alginate application: however, even with monthly alginate applications during the summer, and the observed increase in  $N_2$ -fixation in the restored marsh, our in situ study found no change in *S. foliosa* height or shoot number.

In conclusion, sediment physical characteristics were similar between marshes, and though rates of  $N_2$ -fixation were highly variable during the study, application of alginate increased rates of  $N_2$ -fixation, especially in the restored marsh at Guadalcanal, while kelp fertilization suppressed rates of  $N_2$ -fixation. However, despite increased rates of  $N_2$ -fixation, no measurable effect of the increased nitrogen availability was observed in the growth of *S. foliosa* plants. We suspect the

growth of *S. foliosa* at Guadalcanal was suppressed by the low phosphate porewater concentrations, perhaps caused by high iron availability in sediments, and subsequent sorption of phosphate onto iron-rich sediment particles. Evaluating nutrient chemistry and repairing phosphate deficiency needs to be completed before considering the addition of organic matter to promote  $N_2$ -fixation in this restored marsh. This study demonstrates the importance of assessing sediment nutrient stoichiometry when considering manipulations to promote plant growth in restored marsh ecosystems.

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