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ORIGINAL RESEARCH

Response of Primary Producer Communities to Short-Term Nutrient Additions in a Pacific Northwest Estuarine Tidal Wetland

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Abstract While the detrimental effects of excess nutrients have been documented for tidal wetlands in many locations, the response of the primary producer community to nutrients in Pacific Northwest wetlands is unknown. We investigated the response of emergent macrophytes and sediment microalgae to 15 months of nitrogen and phosphorus addition in a Pacific Northwest estuarine tidal wetland. While both primary producer communities responded to nutrients, increased plant growth in fertilized plots resulted in less light reaching the sediment and suppressed growth of the benthic algal community. Heights of the two dominant plants, *Distichlis spicata* and *Triglochin maritima*, were significantly greater in plots fertilized with nitrogen. The relative cover of *Distichlis spicata* was significantly lower in fertilized plots while cover of *Triglochin maritima* was significantly higher in plots fertilized with nitrogen and phosphorus. Increased plant growth in fertilized plots resulted in significantly lower light levels at the sediment surface. The benthic algal community responded to these lower light levels, with biomass being significantly lower in all fertilized plots and community structure being significantly different in plots fertilized with nitrogen and phosphorus compared with all other treatments. Species richness and diversity was significantly higher in all fertilized plots. The rapid response of primary producer communities to nutrient additions indicates that Pacific Northwest tidal wetlands might be sensitive to eutrophication brought on by increased development in the coastal region.

Keywords Tidal wetland · Macrophytes · Benthic microalgae · Diatoms · Fertilization · Pacific Northwest

Introduction

Nutrient cycling is an important ecosystem function provided by wetlands (Craft 1996; Zedler 2003; Hansson et al. 2005). However, a disruption of ecosystem functioning can result when nutrient loads surpass the system's capacity for assimilation of nutrients (e.g., Deegan 2002). Recent studies along the Atlantic Coast of the U.S. documented that long term nutrient loading in salt marshes leads to ecosystem collapse as plants shift away from producing below ground biomass that holds sediments in place (Deegan et al. 2012). While there has been extensive research on the response of primary producers to nutrients along the Atlantic coast and in southern California (e.g., Valiela and Teal 1974; Boyer and Zedler 1999; Wigand et al. 2003), it is presently unknown how tidal wetlands of the Pacific Northwest will respond to nutrients. Currently, many Pacific Northwest tidal wetlands face minimal impact by human activities. However, coastal populations are projected to rise (NOAA 2013), likely increasing anthropogenic nutrient loads to these wetlands.

The primary producer communities in tidal wetlands are diverse, with most wetlands having well-developed emergent macrophyte, sediment microalgae, and bacterial components. There has been much work examining how abiotic and biotic factors interact to shape the emergent macrophyte community (e.g., Valiela and Teal 1974; Bertness and Ellison 1987; Pennings et al. 2005). Increasingly, the importance of benthic algae to the primary production of tidal wetlands and the larger estuary has been documented (Haines 1977; Zedler 1980; Sullivan and Moncreiff 1990; Pinckney and Zingmark 1993; Kwak and Zedler 1997). The benthic algal community is also

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instrumental in mediating the movement of nutrients between the water column and the sediments (Sundbäck and Granéli 1988; Sundbäck et al. 1991). Despite the importance of benthic microalgae, the environmental factors controlling this community in tidal wetlands are less well understood, particularly in Pacific Northwest systems.

While nutrients are essential for the growth of primary producers, it is not expected that all primary producer types will respond similarly to nutrient additions as they have different nutrient requirements and different abilities to uptake and store nutrients. To date, most of the work on tidal wetland primary producer response to nutrients has focused on the emergent macrophyte community. The early work of Valiela and Teal (1974) established the paradigm of nitrogen limitation in salt marshes. Since then, the tidal wetland macrophyte response to nutrient amendments has been well documented in the literature, with studies first focusing on the response of a single species (e.g., Mendelssohn 1979; Smart and Barko 1980) and then community shifts resulting from nutrient induced reversal of competitive hierarchies (e.g., Levine et al. 1998; Pennings et al. 2002). While most studies have examined the importance of nitrogen in shaping the macrophyte community, there has been some work examining the interactive effects of nitrogen and phosphorus (e.g., Sullivan and Daiber 1974; Patrick and Delaune 1976; Boyer et al. 2001). In contrast, the response of the sediment microalgal community to nutrients has been largely ignored. A few studies in east coast tidal wetlands have demonstrated changes in microalgal diversity, community composition, and biomass in response to changes in nutrient regime (e.g., Sullivan and Daiber 1975; Sullivan and Currin 2000; Parsons et al. 2006). The sensitivity of the benthic microalgal assemblage to nutrients may be regional; work on the Gulf Coast by Sullivan (1981) demonstrated no response to nitrogen additions. While a study in Oregon demonstrated that high levels of nitrogen loading shifted microalgal communities to a single species (Hankin et al. 2012).

Few studies have examined the response of macrophytes and sediment microalgae to nutrient additions concurrently. The response of one primary producer community to nutrients might mask the response of the other community. For example, nutrient enrichment might increase macrophyte growth, causing shading of the sediment surface, decreasing light levels to which microalgae are exposed, and consequently decreasing microalgae growth. Several studies have demonstrated that shading by vegetation can limit benthic algal photosynthesis and biomass (Van Raalte et al. 1976b; Zedler 1980; Baas et al. 2014) or shift community structure (Sullivan and Daiber 1975; Whitcraft and Levin 2007). Alternatively, shifts in macrophyte community composition in response to nutrients may change the sediment microenvironment (Sullivan and Currin 2000) through changes in oxidation of the rhizosphere or symbiotic nitrogen-fixation associations with bacteria, resulting in changes in the microalgae community. Finally, sediment microalgae might quickly absorb excess nutrients from the sediment surface before they have the chance to penetrate to plant roots, resulting in no response of the macrophyte community to nutrient additions. To fully understand the overall response of a tidal wetland to nutrient enrichment, the primary producer communities must be studied together.

Pacific Northwest coastal wetlands exhibit several profound differences from the more studied Atlantic coast and southern California tidal wetlands and therefore it is unclear if paradigms of nutrient limitation developed for other systems will be valid for Pacific Northwest wetlands. Compared to Atlantic coast and southern California salt marshes Pacific Northwest marshes differ in their tidal regimes (mixed semidiurnal tides), are smaller in size, have higher precipitation rates, have perennial river inflows, and have high natural nutrient loads from coastal upwelling and watersheds post-logging being dominated by red alder (*Alnus rubrus* Bong.) (Seliskar and Gallagher 1983; Hickey and Banas 2003; Adamus et al. 2005; Naymik et al. 2005; Mitsch and Gosselink 2007). Compared to their southern California neighbors, these wetlands have lower salinity, lower temperatures, and higher ambient nutrient levels. In addition, Pacific Northwest tidal wetlands have diverse macrophyte assemblages with less conspicuous zonation and highly productive algal assemblages (Adamus et al. 2005; Weilhoefer et al. 2013; Janousek and Folger 2014). Finally, these wetland lack both *Spartina alterniflora* Loisel. and *Spartina foliosa* Trin. *S. alterniflora* is considered an invasive species on the west coast (Saarela 2012) and is only found in estuaries where it was planted to control erosion or has escaped management activities. Due to these physical and biological differences, it is unclear if primary producer communities in Pacific Northwest wetlands will respond in a similar manner to the more studied systems. While weak relationships have been observed between sediment nitrogen and both the vegetation community (Weilhoefer et al. 2013; Janousek and Folger 2014) and tidal channel benthic microalgae community in the Pacific Northwest (Weilhoefer et al. 2015), it is unknown whether a critical threshold exists where nutrients shift from being beneficial to detrimental.

This study aims to examine the concurrent response of tidal wetland emergent macrophyte and benthic microalgae communities to nutrient additions, both nitrogen and phosphorus, in a tidal wetland located in the Yaquina Bay estuary along the central coast of Oregon, U.S.A. We hypothesized the primary producer communities would display phosphorus limitation rather than nitrogen limitation due to naturally high nitrogen levels in this system. In addition, we hypothesized that increased macrophyte growth in response to nutrients would suppress the response of the benthic microalgae community because of light limitation of the microalgal community.

Methods

Study Area

This study was conducted in the Yaquina estuary located on the central Oregon coast of the U.S. The watershed of Yaquina Bay is primarily forested and the Bay contains a large area of wetlands and mudflats. There are approximately 538 ha of wetlands within the estuary of which 331 are tidal marshes (ORDEQ 2005). The dominant source of nutrients to Yaquina Bay varies seasonally; during the wet season (Nov-Apr), nitrate derived from nitrogen-fixing bacteria living in association with red alder in the watershed is the dominant nutrient source. During the dry season (May-Oct), nitrate from oceanic upwelling is the dominant nutrient source (Brown and Ozretich 2009). River flow ranges from 1.3 m^3 sec^{-1} in late summer to 87 m^3 sec^{-1} in the winter, and water temperatures are fairly consistent throughout the year (Brown and Ozretich 2009).

Experimental Plots

This experiment was conducted at one site in the lower Yaquina estuary. Salinity at the site is approximately 30. Four replicates of four fertilization treatments (ambient, phosphorus amended (P), nitrogen amended (N), and phosphorus and nitrogen amended (NP), for a total of 16 plots (1-m^2) were established within the low marsh zone. To limit the confounding influence of tides, all plots were at similar tidal elevation (+2.2 m relative to mean lower low water) and were flooded twice daily by tides throughout most of the year in all but the lowest of high tides. Due to the small size of wetlands in this estuary, only 16 plots could be placed within the low marsh zone limiting replication to four plots of each treatment. Fertilization began in May 2014 and was conducted seasonally throughout the duration of the study. Fertilizer was applied seasonally using slow release fertilizers at a rate of 100-g N m^2 $month^{-1}$ for nitrogen enriched plots and 10-g P m^2 $month^{-1}$. These concentrations were selected because they mimic projected nutrient loading in anthropogenically impacted wetlands of the Pacific Northwest.

Field Data Collection

Macrophyte response to nutrient addition was measured at the level of individual species growth, percent cover, and overall community structure. Individual response was measured as the average height of five randomly selected individuals and height of the tallest individual of each species within the plot prior to fertilization (May 2014), three months after fertilization (August 2014) and after fifteen months of fertilization (August 2015). Because average height and height of the tallest plant showed similar trends, only height of the tallest plant was presented. Emergent macrophyte community structure was measured as percent cover of each species in the 1-m^2 plot on the same sampling dates as height.

Surface sediments were collected from plots at the same time as vegetation sampling for laboratory determination of benthic algal biomass and benthic diatom community structure. Samples were collected by pressing a 2-cm diameter, 0.5-cm thick, plastic delimiter into the sediment surface. The top 1 cm of sediment was scraped out using a metal spatula. Three random samples were collected from each plot to account for heterogeneity of the sediment surface. To ensure that diatoms had migrated to the sediment surface, samples were collected at low tide during midday. The subsamples from each plot were combined and stored in Whirl-Pak ® bags and frozen upon returning to the lab until analysis.

Soil surface temperature, salinity, and conductivity were measured at three random locations within each plot and an average per plot was calculated. Light levels above and below the canopy were measured at three random locations in each plot. Light extinction by the canopy was calculated as: (light above – light below)/light above.

Laboratory Analysis

Sediment samples were homogenized and divided into two samples, one for benthic algal biomass (total microalgal community) and one for diatom identification. Benthic algal biomass was estimated as chlorophyll-a using the spectrophotometric equations of Lorenzen (1967). Chlorophyll-a concentration was expressed on an areal basis. Sediment samples for diatom analysis were heat digested for 45 min using concentrated sulfuric acid. Samples were rinsed repeatedly with deionized water until the pH was approximately neutral. Diatom material was mounted on slides with Naphrax® high resolution mounting. Transects were scanned until at least 600 diatom valves were identified and enumerated to the species level using a Zeiss Axioscope microscope at 1000× magnification. The primary reference for diatom taxonomy was Krammer and Lange-Bertalot (1986, 1988, 1991a, b, 2000). Many taxa could not be identified using the standard diatom taxonomy literature. No evidence of diatom frustule damage from freezing or thawing was observed.

Data Analysis

Statistical analyses were preformed using R (3.2.2/2015–08-14, R Foundation for Statistical Computing, Vienna Austria). Because we were primarily interested in examining the effects of fertilization on response variables and not the natural variability between years at this site, one-way ANOVA was first used to establish that there were no differences among plots pre-fertilization (May 2014) and then one-way ANOVA was used to examine differences in environmental conditions, plant response (height, relative cover), and algae response (biomass, relative cover) three months post-fertilization (August 2014) and fifteen months post-fertilization (August 2015). We did not use two-way ANOVA analysis because any inter-annual variation in response variables, regardless of whether in response to fertilization, would lead to a significant interaction term. Relative cover data of individual macrophyte species (*Distichlis spicata* (L.) Greene, *Sarcocornia perennis* (Mill.) A.J. Scott, *Triglochin maritima* L.) and dominant microalgae were arc-sine transformed prior to ANOVA analysis. To examine patterns of community structure (macrophytes or benthic microalgae) sites were ordered based on the relative cover of taxa using non-metric multidimensional scaling techniques (MDS; metaMDS in the Vegan package of R). The MDS was performed using Bray-Curtis distance measure. An analysis of similarity (ANOSIM) was used to test for statistical differences in the sediment microalgae community across nutrient treatments (Bray-Curtis distance measure, 999 permutations). Emergent macrophyte and sediment diatom relative abundances were used to calculate several community level diversity metrics, including similarity (Bray-Curtis), dominance (relative abundance of most abundant taxa), taxa richness, and Shannon diversity (H′).

Results

We observed strong responses of both the emergent macrophyte and benthic microalgal communities to 15 months of fertilization. Dominant macrophyte species responded positively to nutrient additions (+N, +NP) at the individual physiological level (height). Fertilization also produced significant changes at the community level, with relative cover of *Distichlis spicata*, the most abundant species, significantly decreasing and relative cover of *Triglochin maritima*, a less common species, significantly increasing. This, in addition to increases in cover of non-halophyte species, resulted in a different macrophyte community post-fertilization. The taller macrophyte canopy resulted in significantly less light reaching the sediment surface in all fertilized plots. Consequently, benthic microalgal biomass was lower in all fertilized plots post-fertilization. Despite this decrease in benthic algal biomass, fertilization significantly impacted benthic algal community structure, with plots fertilized with +NP having a distinct community and all fertilized plots having higher species richness and diversity post-fertilization.

Height of the three dominant macrophyte species, *Distichlis spicata*, *Sarcocornia perennis,* and *Triglochin maritima* responded positively to nutrient additions (Fig. 1). Heights of these species did not differ among plots pre-fertilization or three months post-fertilization. Fifteen months post-fertilization, *T. maritima* and *D. spicata* plants were significantly taller in the +N and +NP compared to ambient or +P treatments (ANOVA $F_{3,\ 12} = 9.92$, $p = 0.006$ and ANOVA $F_{3,\ 12} = 3.78$, $p = 0.04$, respectively). At the end of the study, *S. perennis* plants were taller in all fertilized treatments compared to ambient plots, but these differences were not significant. These three species had similar heights in the ambient and +P plots but *T. martima* and *D. spicata* grew much taller than *S. perennis* in both the +N and +NP plots (TM: 48-52 cm, DS: 47-65 cm). Consequently, *T. maritima* and *D. spicata* created a much taller canopy in +N and +NP plots compared to all plots pre-fertilization.

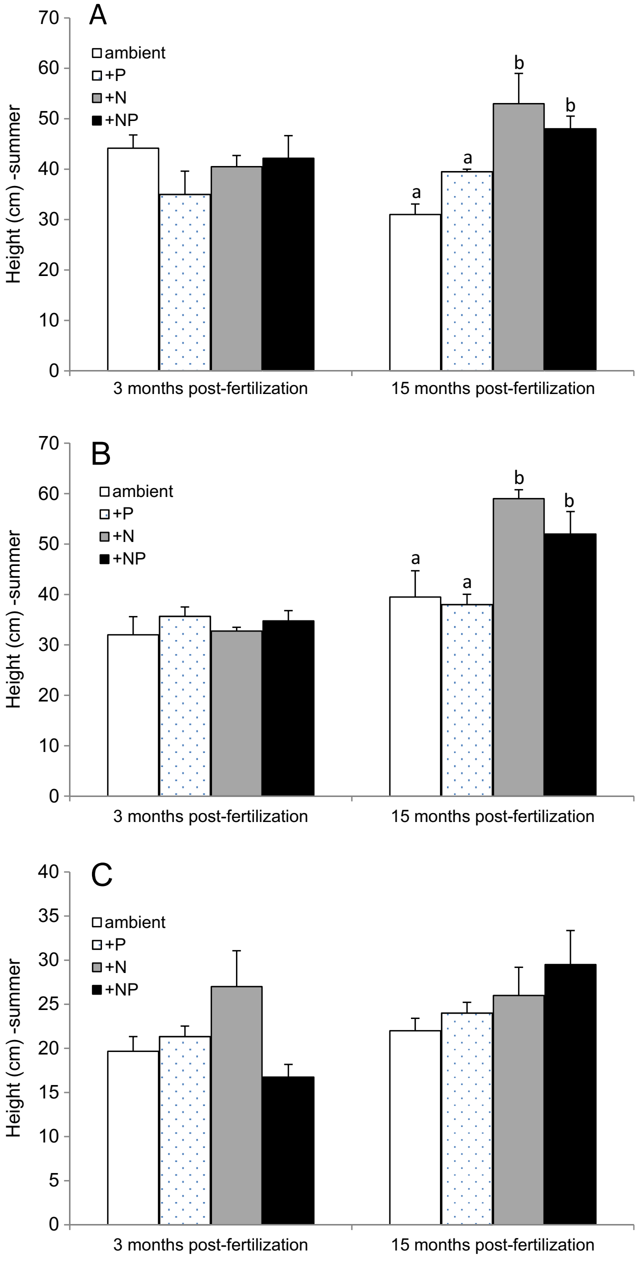

Fig. 1 Height (mean ± standard deviation) for (**a**) *Triglochin martima*, (**b**) *Distichlis spicata*, and (**c**) *Sarcocornia perennis* for each treatment after 3 and 15 months of fertilization. Letters indicate significant differences among fertilization treatments based on ANOVA and Tukey-HSD tests

Community level shifts were also observed in response to fertilization. Prior to fertilization, all plots had an average of 4.1 species and the relative cover of dominant species was variable but not statistically different among plots. *Distichlis spicata* had the highest cover (mean: 29 ± 21%), followed by *Triglochin maritima* (mean: 21 ± 20%), and *Sarcocornia virginica* (mean: 15 ± 13%). In addition to the three dominant species mentioned above, *Spergularia maritima* (All.) Chiov., *Jaumea carnosa* (Less.) A. Gray, and *Carex lyngbei* Hornem. occurred in several plots but at low coverages (never more than 15%). Most plots had some amount of bare area and wrack (dead green macroalgae) coverage. Relative cover did not differ among fertilization treatments after three months. After fifteen months of fertilization, *D. spicata* still had the highest coverage in the plots regardless of fertilization treatment (range 20–80%). However its relative cover decreased significantly in response to fertilization (+P, +N, +NP) as other less common species became more abundant (ANOVA $F_{3,12} = 4.1$, $p = 0.03$; Fig. 2). *S. perennis* and *T. maritima* had similar relative cover prior to fertilization. *T. maritima* cover responded positively to fertilization, with cover being significantly higher in the +NP treatment fifteen months post-fertilization (ANOVA $F_{3,12} = 3.9$, $p = 0.04$). No other species responded significantly to nutrient additions. However, three species, *Atriplex patula* L., *Hordeum spp.*, and *Grindelia stricta* DC., found at very low relative cover, appeared in only the fertilized plots by the end of the study. Fertilization influenced dominance by a single species but not measures of community diversity. Dominance by a single species ranged between 45 and 80% in all plots post-fertilization and was significantly lower in the +NP treatment (ANOVA $F_{3,12} = 3.7$, ANOVA $p = 0.04$). Species richness per plot ranged between 3 and 6 in 2015 and did not vary between fertilization treatments. Shannon Diversity index was low, ranging between 0.42–1.38 per plot, throughout the study.

In addition to responses of individual species, the overall macrophyte community shifted with fertilization (Fig. 3).

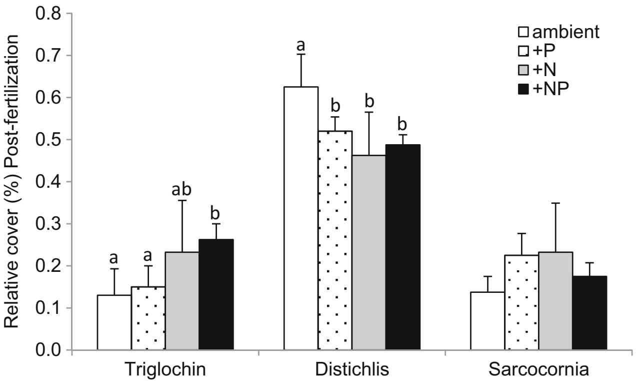

Fig. 2 Relative cover (mean ± standard deviation) for *Triglochin martima, Distichlis spicata*, and *Sarcocornia perennis* after 15 months of fertilization. Letters indicate significant differences among fertilization treatments based on ANOVA and Tukey-HSD tests

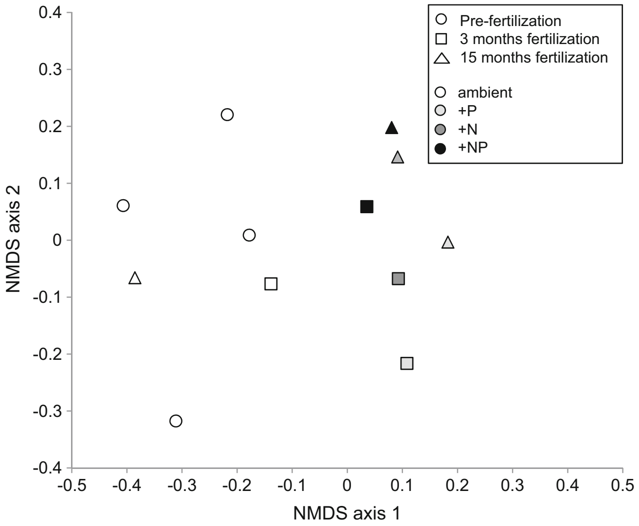

Fig. 3 Non-metric multidimensional scaling ordination plot based on relative cover of emergent macrophyte species pre-fertilization (May 2014), after three months of fertilization (August 2014), and after 15 months of fertilization (August 2015). Points represent the average of the four fertilization treatment plots for each sampling data

MDS analysis produced a two-dimensional solution with a stress of 0.06. The first ordination axis represents a gradient of nutrients, with all plots prior to fertilization (May 2014) and ambient plots three months post-fertilization (August 2014) and fifteen months post-fertilization (August 2015) clustering at the negative end of this axis. All fertilized plots clustered at the positive end of axis 1.

The increased height of dominant species and taller overall vegetation canopy in response to nutrient additions resulted in a different physical environment in fertilized plots as compared to ambient plots. The dense vegetation canopy resulted in a light extinction ranging from 67 to 97% in all plots throughout the study. While light levels at the sediment surface were not significantly different among plots pre-fertilization or three months post-fertilization, light levels fifteen months post-fertilization were significantly lower in all fertilized plots (treatment averages 86–92%) compared with ambient plots (average 74%; ANOVA $F_{3,12} = 5.3$, $p = 0.01$). Other environmental variables, including salinity, soil conductivity, and soil temperature, were not significantly affected by fertilization.

Fertilization also had significant impacts on benthic microalgal biomass and community structure. Benthic algal biomass fifteen months post-fertilization (mean: 31.0 mg chl *a* m^{-2}) was lower in all plots compared to three months post fertilization (mean: 73.9 mg chl *a* m^{-2}). There was no significant difference in biomass among treatments after three months of fertilization (Fig. 4). After 15 months of fertilization, biomass was significantly lower in all fertilized plots compared with ambient plots (ANOVA $F_{3,12} = 3.9$, $p = 0.04$). Microalgal biomass averaged 49.7 mg chl *a* m^{-2} in ambient plots and between 17.2–30.6 mg chl *a* m^{-2} in all fertilized plots after fifteen months of fertilization.

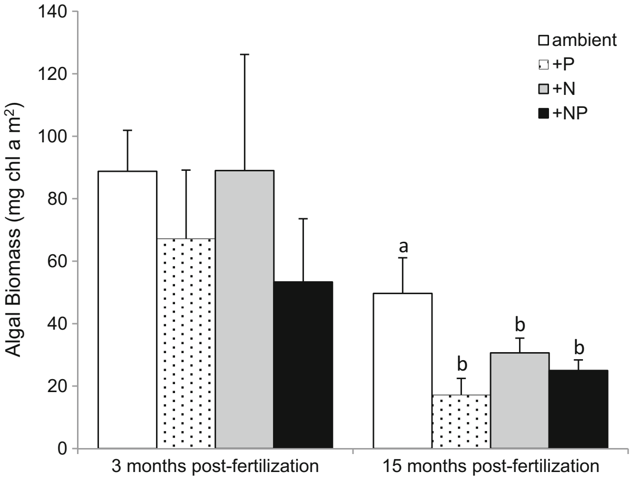

Fig. 4 Benthic microalgal biomass (mean ± standard deviation) for each treatment after 3 and 15 months of fertilization. Letters indicate significant differences among fertilization treatments based on ANOVA and Tukey-HSD tests

Reductions in benthic algal biomass in fertilized plots were coupled with shifts in the benthic microalgal community. The benthic microalgae community was diverse, with 89 distinct taxa being identified throughout the study. Communities within the plots were diverse, with 37.6 taxa, on average occurring in each plot (range: 27–44; Table 1). Frequently occurring taxa included *Achnanthidium delicatulum* Kützing, *Navicula gregaria* Donkin, and *Nitzschia frustulum* Kützing. Fifteen months of fertilization had a significant effect on the relative abundance of only two taxa. The relative abundance of *A. delicatulum* was significantly lower in the +NP plots compared with all other treatments (ANOVA $F_{3,12} = 5.6, p = 0.01$). *Nitzschia frustulum* was also significantly less abundant, in fact rarely present, in plots fertilized with +NP (ANOVA $F_{3,12} = 3.8$, $p = 0.04$). While the abundance of only these two dominant species changed significantly with nutrient additions, there were shifts in overall community structure in response to fertilization. Measures of community diversity were significantly impacted by nutrient treatment, with fertilization promoting species diversity. Species richness and H′ were significantly higher in all fertilized plots compared with ambient plots after fifteen months of fertilization (ANOVA $F_{3,12} = 8.0$, $p = 0.003$ and ANOVA $F_{3,12} = 6.7$, $p = 0.007$, respectively; Table 1). Dominance by a single species was significantly lower in all fertilized plots compared with ambient plots (ANOVA $F_{3,12} = 7.7$, $p = 0.004$). Non-metric multidimensional scaling showed shifts in the overall benthic diatom community structure with fertilization (Fig. 5). MDS analysis produced a two-dimensional solution with a stress of 0.13. The benthic algal community in the +NP plots was distinct from all other treatments (ANOSIM $R = 0.72$, $p = 0.001$), clustering at the negative end of axis 1. The community in the ambient plots was also distinct, clustering at the negative end of axis 2. The benthic algal community in the +N and +P plots were not distinct from one another.

Discussion

This study is the first of its kind to look at the concurrent response of emergent macrophytes and benthic microalgae to nutrient additions in Pacific Northwest tidal wetlands. Our findings indicate an interactive effect between these two communities in response to nutrients. Height of dominant macrophyte species increased significantly in plots amended with nitrogen. While we had hypothesized potential phosphorus limitation due to naturally high levels of nitrogen in this system, our data does not support this, with heights of all species being greatest and relative cover shifts occurring in plots fertilized with nitrogen. The benthic algal community also responded to nutrients, although it is unclear if this response was directly to the nutrients themselves or light limitation in fertilized plots in response to the taller plant canopy. Algal biomass was significantly lower in all fertilized plots, supporting our hypothesis that increased macrophyte growth would suppress the response of benthic microalgae to nutrient enrichment. Shifts in overall community structure and relative

Table 1 Relative abundance of common diatom taxa and diatom community metrics after 15 months of fertilization (August 2015)

	Overall	Ambient	+P	+N	+NP	ANOVA p
Achnanthidium delicatulum Kützing	24 ± 8	33 ± 8^{a}	20 ± 5^{a}	25 ± 2^{a}	18 ± 2^{b}	0.012
Navicula gregaria Donkin	8 ± 3	6 ± 1	9 ± 3	7 ± 4	10 ± 2	
Nitzschia frustulum Kützing	4 ± 4	5 ± 3^{a}	7 ± 3^{a}	6 ± 5^{a}	0 ± 1^{b}	0.04
Achnanthes nodosa Cleve	5 ± 2	4 ± 1	5 ± 3	4 ± 2	6 ± 4	
Staurosirella pinnata (Ehrenberg) Williams and Round	5 ± 2	3 ± 2	6 ± 3	5 ± 2	5 ± 2	
Richness	37.6 ± 5.1	30.5 ± 4.1^{a}	40.8 ± 2.1^{b}	39.5 ± 3.0^{b}	39.3 ± 3.4^{b}	0.003
H′	3.0 ± 0.2	2.7 ± 0.2^{a}	3.1 ± 0.0^{b}	3.0 ± 0.2^{b}	3.1 ± 0.1^{b}	0.007
Dominance (%)	24 ± 8	37 ± 1^{a}	22 ± 3^{b}	26 ± 5^{b}	17 ± 3^{b}	0.004

Superscripts indicate fertilization treatments that are significantly different based on ANOVA and Tukey-HSD tests

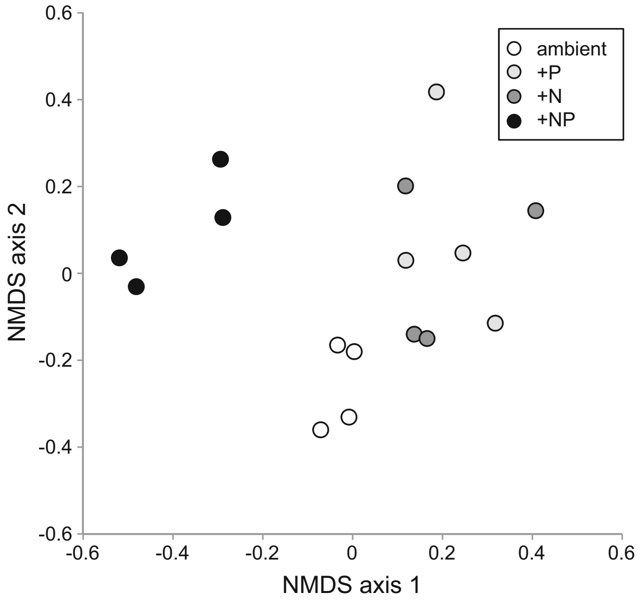

Fig. 5 Non-metric multidimensional scaling ordination plot based on relative abundance of diatom species after 15 months of fertilization

abundance of dominant diatom species were only observed in plots fertilized with nitrogen and phosphorus, suggesting that along with light limitation, the microalgae community is secondarily limited by nitrogen and phosphorus, partially supporting our hypothesis of phosphorus limitation.

Our research examined the effects of both nitrogen and phosphorus enrichment in a mixed macrophyte community. Despite naturally high levels of nitrogen loading to these wetlands (Brown and Ozretich 2009) and only weak relationships between vegetation communities and nitrogen being observed in previous studies in the Pacific Northwest (Weilhoefer et al. 2013), dominant plant species were more limited by nitrogen than phosphorus, with greatest increases in growth occurring in the +N and +NP plots. This finding of nitrogen limitation agrees with previous work along the Atlantic coast (Valiela and Teal 1974; Wigand et al. 2003; Crain 2007) and the Gulf of Mexico (e.g., Pennings et al. 2002). Nutrient limitation in southern California appears to depend both on the species in question and the ambient nutrient levels, with strong evidence of nutrient limitation for *S. virginica* and mixed results for *S. foliosa* (Covin and Zedler 1988; Page 1995; Boyer et al. 2001). Our findings suggest that nitrogen limitation may be a near universal feature in tidal wetlands despite differing physical factors, macrophyte assemblages, and ambient nutrient levels.

Nutrient enrichment also resulted in shifts in the overall macrophyte community, with the relative cover *Distichlis spicata*, the dominant species under ambient conditions, decreasing in the +NP treatment, while relative cover of *Triglochin maritima* significantly increased. It is well established that vegetation zonation in estuarine wetlands is governed by the interplay of stress tolerance and competitive ability (Bertness and Ellison 1987; Pennings et al. 2002; Pennings et al. 2005). The zone of highest stress depends on climate, with the low marsh being the high stress zone in Atlantic coast wetlands of temperate climate and both the low and high marsh being stressful zones in southern California marshes with Mediterranean climate (Zedler 1982; Callaway et al. 1990; Pennings and Callaway 1992). Tidal wetlands of the Pacific Northwest are more similar to the Atlantic coast in that they exhibit a monotonic gradient of decreasing stress from low to high marsh. While studies in Atlantic coast (Bertness and Ellison 1987; Levine et al. 1998; Emery et al. 2001) and Gulf coast (Pennings et al. 2002) salt marshes have demonstrated nutrient induced reversals of competitive hierarchies and studies in southern California have demonstrated species shifts in response to nutrients (Covin and Zedler 1988; Boyer and Zedler 1999), we had not expected to observe community shifts within the short time frame of this study because the low marsh vegetation in Pacific Northwest wetlands does not exhibit clear zonation of macrophytes based on environmental tolerances (Weilhoefer et al. 2013; Janousek and Folger 2014); many species of similar salt tolerances grow intermixed. Both *D. spicata* and *T. martima* are considered salt tolerant but competitively inferior species in Atlantic coast marshes (Bertness and Ellison 1987; Fogel et al. 2004), with *T. martima* often being restricted to the marsh panne habitat (Ewanchuk and Bertness 2004; Fogel et al. 2004). However, in Pacific Northwest wetlands *D. spicata* is often an abundant plant in the low marsh, growing intermixed with *Sarcocornia perennis*, while *T. maritima*, although not restricted to the edge of pannes, is usually much less common in the low marsh (Eilers 1974; Jefferson 1975; Weilhoefer et al. 2013). Our results suggest that in Pacific Northwest wetlands, *D. spicata* maybe the competitively dominant species under ambient conditions and nutrient enrichment might result in a reversal of the competitive hierarchy, allowing *T. maritima* to increase in height and cover. Further support for a nutrient induced reversal of competitive hierarchy includes the observation of several species normally restricted to the more benign high marsh (*Atriplex patula*, *Grindelia stricta, Hordeum* spp.) in the +NP plots by the end of the study.

Despite community shifts and recruitment of several high marsh species into +NP plots, we did not observe significant changes in richness or diversity in fertilized plots. Several studies have documented decreased species richness with increased nitrogen loading from the surrounding landscape (e.g., Bertness et al. 2002; Wigand et al. 2003; Silliman and Bertness 2004). There are several possible explanations for the different response we observed in Pacific Northwest wetlands. First, the macrophyte community within the low marsh zone in this study and in tidal wetlands of the Pacific Northwest is diverse and doesn't exhibit striking zonation dominated by a single species (Weilhoefer et al. 2013; Janousek and Folger 2014); in this study an average of 4.1

species grew intermixed in a 1-m^2 plot. In addition, the Pacific Northwest assemblage characteristically lacks *Spartina alterniflora*, the species that often exhibits the strongest response to nutrients in Atlantic coast marshes (Pennings et al. 2002). Finally, in studies along the Atlantic coast of the U.S., long term nutrient loading often results in dominance by the invasive species *Phragmites australis* (Cav.) Trin. ex Steud. as nutrients ameliorate the harsh physical conditions of the salt marsh (Bertness et al. 2002; Silliman and Bertness 2004; King et al. 2007; Wigand et al. 2007). It is possible that the vegetation in Pacific Northwest tidal wetlands is less susceptible to dominance by a single species with nutrient additions because it currently lacks invasive species (Weilhoefer et al. 2013).

While the benthic microalgal community also responded to nutrients, this response appeared to be mediated by the increased growth of macrophytes reducing light at the sediment surface, supporting our second hypothesis. While previous studies have documented stimulation of benthic algal production in response to fertilization (Sullivan and Daiber 1975; Van Raalte et al. 1976b) we observed significantly lower biomass in all fertilized plots which we attribute to lower light levels in these plots. The benthic microalgal community has been shown to be sensitive to light levels (Whitney and Darley 1983; Whitcraft and Levin 2007; Baas et al. 2014) and some work has demonstrated an interaction of light and nutrients on algal production (Van Raalte et al. 1976a, b; Sullivan and Daiber 1975; Sullivan 1981). In addition to light limitation in fertilized plots, the benthic microalgal community appeared to be secondarily limited by nutrients, evidenced by significant shifts in relative abundance of dominant taxa and shifts in overall diatom community structure in the +NP plots. Sullivan (1976) also demonstrated an interaction of light and nutrients on the structure of the salt marsh diatom community, with the importance of each variable depending on the individual taxa.

We found significantly higher diversity and species richness in plots amended with +NP, contrasting other studies that have demonstrated a negative impact of nitrogen on species richness (Sullivan 1976; Van Raalte et al. 1976a; Hankin et al. 2012), suggesting that elevated nutrients might ameliorate the effects of low light and allow less competitively dominant species to thrive. Or it is possible that observed shifts in the benthic algal community in +NP plots are in response to changes in macrophyte community composition in these plots, as cover of *Trichlogin martima* and rare species increased and *Distichlis spicata* cover decreased in +NP plots. While this study lends support to both light and nutrient limitation of tidal wetland microalgae, further studies are necessary to parse the direct effects of nutrients on microalgae versus the indirect effects mediated by the macrophyte response.

Our results clearly demonstrate nutrient limitation of primary producer communities in Pacific Northwest estuarine tidal wetlands, which may have cascading impacts on the wetland foodweb. Benthic microalgae, particularly diatoms, are the preferred food source of invertebrates and fish in these ecosystems (Kwak and Zedler 1997; Sullivan and Currin 2000). As human populations grow and nutrient loading to these systems increases, resulting increased plant growth and subsequent sediment shading may cause a decrease in algal biomass, decreasing available food. In addition, shifts in the benthic microalgae community in response to nutrients, whether a direct response or an indirect response, may alter the quality of food available to higher trophic levels as algal species vary in their nutritional content. Shifts in primary producer communities may also alter the salt marsh foodweb through changes in the wetland physical environment. For example, *Triglochin martima* is considered an ecological engineer, with its rhizomatous growth raising the sediment surface, lowering soil salinity, and increasing oxygen levels (Fogel et al. 2004). Increases in the growth of this species in response to nutrient additions may allow less stress tolerant plants to colonize the low marsh. Finally, while long term fertilization studies have demonstrated salt marsh loss as a result of eutrophication on the Atlantic coast of the U.S. (Deegan et al. 2012), it is unclear if this paradigm will hold for Pacific Northwest tidal wetlands with their different species assemblages and nutrient regimes. In this study, short term fertilization produced significant changes in the primary producer communities, however further study over longer time scales is warranted so that we can accurately predict how these systems will respond to increases in nutrients brought on by cultural eutrophication.

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