VOL. 405, NOS. 1–2   31 AUGUST 2011   ISSN 0022-0981

ELSEVIER

Journal of
EXPERIMENTAL
MARINE BIOLOGY
AND ECOLOGY

Effects of nutrient enrichment and crab herbivory on a SW Atlantic salt marsh productivity

Juan Alberti [a,b,*], Just Cebrian [c,d], Agustina Méndez Casariego [a,b], Alejandro Canepuccia [a,b], Mauricio Escapa [a,b], Oscar Iribarne [a,b]

[a] Laboratorio de Ecología, Departamento de Biología (FCEyN), Universidad Nacional de Mar del Plata, CC 573 Correo Central, B7600WAG, Mar del Plata, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rivadavia 1917, CP C1033AAJ, Ciudad de Buenos Aires, Argentina
[c] Dauphin Island Sea Lab, 101 Bienville Blvd., Dauphin Island, AL 36528, United States
[d] Department of Marine Science, University of South Alabama, LSCB 25, 307 University Blvd., Mobile, AL 36688, United States

ARTICLE INFO

ABSTRACT

After intense debate it is now accepted that nutrients (a bottom-up process) and herbivores (a top-down process) are both important controls of plant productivity in many systems. Besides their direct effects, herbivores may also have profound positive or negative indirect effects that can be modulated by nutrients and time. The interactive relationships between time, nutrient availability and herbivore impacts (direct and indirect) on plant growth dynamics are an emerging research topic that merits further effort. Here we did several experiments in a SW Atlantic marsh to contribute towards that gap by focusing on the dominant plant, *Spartina densiflora*, and one of the dominant herbivores, the crab *Neohelice* (*Chasmagnathus*) *granulata*, in the marsh. Herbivory by the crab was highly seasonal, with most of the consumption occurring in fall. Even though crabs preferred nutrient enriched leaves, nitrogen content was not the driver of these seasonal variations. Crab herbivory had markedly indirect negative impacts on *S. densiflora* leaves, reducing their growth rates and increasing their senescence. These deleterious impacts may partially explain the seasonal decline in leaf growth and a net loss in leaf biomass observed in the fall. Fertilization did not seem to alter these processes. Adding nutrients increased leaf growth in the spring, where ambient herbivory was low, but it also increased herbivory in the fall, resulting in similar patterns as the ones observed under non-fertilized conditions. Herbivory by the crab also greatly affected the dynamics of *S. densiflora* stems. Increases in stem density in relation to initial conditions were larger in non-grazed than in grazed plots regardless of whether nutrients were added or not. Together, these results indicate that, in Southwestern marshes populated by *S. densiflora* and *N. granulata*, herbivory by the crab represents an important direct and indirect control of plant growth. Our results also emphasize the importance of considering impacts on growth rates and not only on biomass because not considering reduced growth after herbivory may lead to improper calculations of nutrient cycling or detritus production.

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1. Introduction

After intense and long-lasting debate about whether top-down (i.e. herbivores and their predators) or bottom-up forces (i.e. nutrients and physical factors) regulate plant productivity (see Hunter and Price, 1992), there is increasing agreement that both forces commonly operate together and that their net balance often depends on local characteristics (i.e. species involved, abiotic conditions, and habitat productivity; Lotze et al., 2001; Moran and Scheidler, 2002; Russell and Connell, 2005; Borer et al., 2006; Burkepile and Hay, 2006; Gruner et al., 2008). Nutrients generally increase plant biomass while herbivores decrease it (e.g., Burkepile and Hay, 2006; Gruner et al., 2008). However, it is also

known that increased nitrogen availability may lead to more palatable plants and increased consumption rates on them (Cebrian et al., 2009; Mattson, 1980). A recent review, however, found that the interaction of nutrients and herbivory controlling the biomass of primary producers is uncommon (Gruner et al., 2008). Nevertheless, at least in salt marshes, nutrient-enhanced herbivory may become intense and override the positive impact of nutrients on plant biomass, leading to lower biomass in fertilized in comparison with unfertilized conditions (Bertness et al., 2008; Sala et al., 2008).

The impacts of herbivores on plants are highly variable, as the amount they consume depends on many abiotic and biotic conditions (e.g., Goranson et al., 2004). Herbivores may also interact with pathogens or mutualists, which may modify their impacts on plants. For example, herbivores may induce plant susceptibility (decreased resistance or tolerance to other herbivores or pathogens), which may magnify the negative effects of herbivore consumption *per se* (Nykänen and Koricheva, 2004; Silliman and Newell, 2003). Herbivores may also

* Corresponding author at: Laboratorio de Ecología, Departamento de Biología (FCEyN), Universidad Nacional de Mar del Plata, CC 573 Correo Central, B7600WAG, Mar del Plata, Argentina. Tel.: +54 223 4753554; fax: +54 223 4753150.
E-mail address: jalberti@mdp.edu.ar (J. Alberti).

promote mycorrhizal colonization, increasing nutrient availability for plants and thus increasing their growth (Kula et al., 2005). Herbivores themselves may become mutualists by, for instance, triggering plant growth through moderate and partial consumption (i.e. overcompensation; see de Mazancourt et al., 1998; Agrawal, 2000; Hawkes and Sullivan, 2001). Overcompensation may occur through the promotion of nutrient cycling and enhanced photosynthetic activity due to removal of old, less productive tissues and increased light availability for basal parts of grasses (Tiffin, 2000; Yamauchi and Yamaura, 2004; Zieman et al., 1984). Plant nutritional quality and phenology influence herbivory and plant compensatory responses (Mattson, 1980; Yamauchi and Yamaura, 2004). Many studies have focused on nutrient–plant–herbivore interactions (Borer et al., 2006; Burkepile and Hay, 2006; Gruner et al., 2008; Mattson, 1980), but relatively fewer studies have explicitly addressed these issues through time (Lotze et al., 2001; Vinueza et al., 2006).

Salt marshes are vegetated intertidal areas where nitrogen availability has long been recognized as a major limiting factor for plant productivity (Dai and Wiegert, 1996; Pomeroy and Wiegert, 1981; Valiela et al., 1976). However, recent results show that herbivores can also limit marsh productivity by reducing plant density and/or height (Bortolus and Iribarne, 1999; Jefferies et al., 2006; Kuijper and Bakker, 2005; Silliman et al., 2005). Vast areas of Southwestern Atlantic salt marshes (located between southern Brazil and the northern Argentinean Patagonia) are vegetated by the cordgrass *Spartina densiflora* Brongn (Isacch et al., 2006). The productivity of this plant may be strongly limited by nutrients, as fertilizer supply may result in up to 6-fold increases in plant biomass (Alberti et al., 2010). These marshes are also characterized by the presence of the burrowing crab *Neohelice* (*Chasmagnathus*) *granulata* Dana (Alberti et al., 2007a; Iribarne et al., 2005). The crabs are mainly herbivorous in the marsh and herbivorous/detritivorous in the mudflat (Bortolus and Iribarne, 1999; Botto et al., 2005; Iribarne et al., 1997). Crab herbivory typically removes the top part of cordgrass leaves (J. Alberti unpubl. data), facilitating fungal infections (Daleo et al., 2009). However, evidence suggest that the relative importance of nutrients and herbivory might be seasonally variable given that growth as well as herbivory vary throughout the year (Alberti et al., 2008). The objective of the present study was to contribute to our understanding of the relationships between nutrient enrichment, herbivory by *N. granulata*, and the growth dynamics and mortality of *S. densiflora* through time. In particular, we evaluated (1) the effects of fertilization on herbivory and leaf growth rates, (2) the single and interactive short-term effects of fertilization and recent herbivory on length-specific leaf growth and senescence and, (3) the single and interactive effects of fertilization and herbivory on stem density dynamics.

2. Materials and methods

2.1. Study site

This study was carried out at the Mar Chiquita coastal lagoon (37° 45′ S, 57° 26′ W Argentina), an UNESCO Man and the Biosphere Reserve. This is a body of brackish water (salinity range between 0.5 and 34‰) with low-amplitude tides (≤1 m; Reta et al., 2001). The surrounding area is characterized by halophytic vegetation, dominated by *S. densiflora* and *Sarcocornia perennis* (Isacch et al., 2006). The burrowing crab *N. granulata* is one of the most important macro-invertebrates of SW Atlantic salt marshes and occurs in high abundance in the tidal flats and marshes of the Mar Chiquita coastal lagoon (Iribarne et al., 1997).

2.2. Effects of nutrient enrichment on leaf growth and herbivory

We conducted a 19-month experiment (from January 2006 through July 2007) to evaluate if fertilizer supply affected the growth rates of *S. densiflora* and herbivory by *N. granulata*. This experiment consisted of two treatments (with and without nutrient addition) with 8 replicates each (75×75 cm open plots), located in the low marsh where crab herbivory is frequent (Alberti et al., 2007a). Nutrient additions started in January 2006 and consisted of doses of 60 g of a slow-release pelletized fertilizer (29% N, 5% P, and 5% K) per plot per month. Similar dosing has been used in other nutrient addition studies in salt marshes (Daleo et al., 2008; Emery et al., 2001; Levine et al., 1998).

Seven months after the beginning of fertilization (August 2006), we measured the length and width at the base of all live leaf blades (thereafter called "leaves") in two stems chosen randomly in each plot. We considered live leaves those exhibiting some green length. The crab *N. granulata* consumes the leaves from the top, cutting their tips and rendering them trapezoidal in shape. Thus, if the leaf had been grazed by the crab, we also measured the width at the top of the leaf. In addition we made a small mark at the base of each leaf with a permanent fine point marker. Upon revisiting the plots the following month, in all marked leaves we measured the length from the base to the top of the leaf, the length from the base to the mark, and width at the base, mark, and top of the leaf if it had been grazed. We repeated this process monthly throughout the conclusion of the experiment (i.e. every month we marked and measured green leaves in two randomly-chosen new stems in each plot and measured them again 1 month later as previously explained).

From these measurements the area consumed by the crab on each leaf through the month (H, cm^2 per leaf per month) was quantified as:

$$H = \text{total leaf area in month t (A)} + \text{leaf area grown from t to t} + 1(B) \quad (1)$$
$$- \text{total leaf area in month t} + 1(C)$$

where leaf areas were calculated using equations for triangular or trapezoidal areas. For instance, the leaf area grown from t to t + 1 corresponds to the area of the trapezoid with height = length from base to mark of the leaf, length of bottom side = width at the leaf base, and length of top side = width at the mark (see Fig. 1). Grazing by other herbivores, such as snails and the wild guinea pig *Cavia aperea* did not occur at our experimental units or were minimal and the marks left by those herbivores were different from the marks left by the crab and did not hinder calculation of the leaf area consumed by the crab.
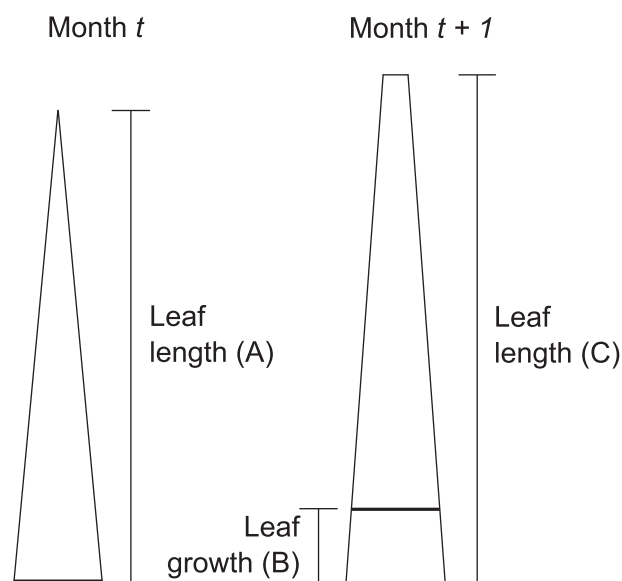

Fig. 1. Schematic representation of the measurements taken to estimate the leaf area consumed by the crab *N. granulata*. See text and Eq. (1) for details.

Leaf biomass was estimated from leaf area based on regressions between leaf biomass and area built with additional leaves collected throughout the duration of the experiment. Separate regressions were built for intact and grazed leaves ($r^2 > 0.94$ for both regressions). The biomass grown or consumed per leaf was summed for all leaves in the stem to derive leaf growth, consumption and the net change in biomass (i.e. difference between leaf growth and consumption) per stem, and the two stems per plot were averaged to avoid pseudoreplication (Hurlbert, 1984).

We also measured leaf nitrogen content. We collected one leaf per plot and randomly pooled them into 2 replicates per treatment, with each replicate assembling 4 leaves. Only the second live leaf (intermediate age) on the stem was sampled to minimize impacts related to leaf age. Leaves were dried overnight at 80 °C and nitrogen content (% DW) measured using a CHN Carlo Erba autoanalyzer (Strickland and Parsons, 1972).

Differences in leaf growth per stem, leaf consumption per stem, the net change in leaf biomass per stem, and leaf nitrogen content between treatments (nutrient addition vs. control) and dates were evaluated using a mixed two-way ANOVA model (Zar, 1999) with fertilization as a fixed factor and time as random factor. Whenever the assumptions of normality and homoscedasticity could not be met, we considered the differences marginally significant if $0.05 > P \geq 0.005$ and significant if $P < 0.005$ to reduce the probability of committing type I error (Zar, 1999; see Antón et al., 2011 for a similar approach).

2.3. Short-term effects of nutrient enrichment and recent herbivory on length-specific leaf growth and senescence

To determine if nutrient enrichment and herbivory by *N. granulata* affected subsequent *S. densiflora* leaf growth, we compared growth between ungrazed (i.e. intact) and recently grazed leaves in non-fertilized and fertilized plots using the same experiment described above. In the late summer of 2007 (a period with high leaf expansion and intense herbivory; see Results), we randomly tagged 15 intact (i.e. ungrazed), young leaves (i.e. one of the two youngest leaves in the stem, with each tagged leaf belonging to a different stem) in each of the plots. Two weeks later we recorded which of the tagged leaves had been grazed. We then measured the growth of all tagged leaves over the following 4 weeks and measured the total length of all tagged leaves at the end of the four-week period as explained previously (Fig. 1). We discarded the leaves that were intact 2 weeks after tagging but that were grazed in the following 4 weeks. The minimum number of leaves per plot that were grazed within 2 weeks after tagging or that remained intact during the six-week interval was 2. Therefore, to obtain a balanced statistical design, in each plot we randomly chose the growth calculations for the two leaves that were grazed within 2 weeks after tagging and the growth calculations for the two leaves that remained intact throughout the 6 weeks.

We followed a similar approach to determine whether recent herbivory and nutrient enrichment affected senescence rates. For this we tagged old, ungrazed leaves and senescence was quantified as the length of senesced tissue 6 weeks after tagging the leaves. Both leaf growth and senescence were reported as proportion of total leaf length measured at the end of the four-week period. The two growth and senescence calculations done per plot were averaged to avoid pseudo-replication and the impact of nutrient addition and recent herbivory was analyzed with a fixed two-way ANOVA model (Zar, 1999) after confirming that the data complied with the assumptions of normality and homoscedasticity.

2.4. Effects of nutrient enrichment and herbivory on stem density dynamics

We also did an experiment to evaluate the effects of nutrient enrichment and herbivory on the dynamics of *S. densiflora* stems. The experiment consisted of a fully factorial design (2×2): with and without herbivory (controls and exclosures 75×75 cm), and with and without nutrient addition. We also set up controls for cage effects (exclosures with three sides instead of four). The experiment ran from January 2006 to January 2007 and each treatment had 8 replicates. The open plots were the same ones used for the experiment described above. Nutrient addition was also as described for the previous experiment. Crab exclusion plots were constructed with 1 cm-pore size plastic mesh fence and were 40 cm high. The mesh also excludes the herbivorous wild guinea pig *C. aperea*, and potential herbivorous fishes. Grazing by these other herbivores, however, is not important in our experimental area (Cousseau et al., 2001; A. Canepuccia, J. Alberti, J. Pascual, G. Álvarez, J. Cebrian, O. Iribarne, unpubl. data).

Seasonally throughout the experiment we counted the number of *S. densiflora* live stems in a fixed 100 cm^2 area within each plot. The seasonal dynamics of stems with and without fertilization and with and without herbivory by *N. granulata* was analyzed with a three way repeated measures ANOVA model, with fertilization and herbivory as the among-subjects factors and season as the within-subjects factor, since we repeatedly counted the same plots throughout the experiment. Data were transformed to comply with the assumptions of normality and homoscedasticity. We used the Greenhouse–Geiser correction because the assumption of sphericity was not met (Crowder and Hand, 1990). Finally, open, non-fertilized plots and cage controls were compared separately on each season with the Welch t_c-tests due to the unequal variances of the groups compared (Zar, 1999).

3. Results

3.1. Effects of nutrient enrichment on leaf growth and herbivory

Fertilization increased the nitrogen content of *S. densiflora* leaves throughout the year (Fig. 2A). The increase was largest in winter (fertilization × time interaction: $P < 0.05$; see post-hoc Tukey tests depicted on the figure), a season where ambient (i.e. non-fertilized) leaf nitrogen content was high in comparison with the rest of the year (Tukey tests for non-fertilized plots between sampling dates in the winter and sampling dates in other seasons: $P < 0.05$). Fertilization increased the growth rate of *S. densiflora* leaves in spring, summer and early fall (Fig. 2B; fertilization × time interaction: $P < 0.005$; see Tukey tests depicted on the figure). The increase was largest in spring, a season where ambient growth rates were high in comparison with the rest of the year (Tukey tests for non-fertilized plots between sampling dates in the spring and sampling dates in other seasons: $P < 0.005$). Fertilization also increased herbivory by *N. granulata* in summer and fall (Fig. 2C; fertilization × time interaction: $P < 0.005$; see Tukey tests depicted on the figure). The increase was largest in fall, a season where ambient herbivory tended to be high in comparison with the rest of the year (Tukey tests for non-fertilized plots between sampling dates in fall and sampling dates in other seasons: $0.05 > P \geq 0.005$).

It may have appeared that the seasonally-lagged impacts of fertilization on leaf growth and herbivory by *N. granulata* resulted in concomitant impacts on net leaf biomass change (Fig. 2D). In spring, a season of ambient high leaf growth and low herbivory where fertilization had the largest impact on growth but did not affect herbivory, fertilization would have led to larger increments in leaf biomass. In fall, a season of ambient low leaf growth and high herbivory where fertilization affected leaf growth little but had the largest impact on herbivory, fertilization would have lead to larger decrements in leaf biomass. We failed, however, at acquiring full statistical support for these seemingly possible contrasts; even though we obtained a significant fertilization × time interaction ($P < 0.005$), we found no significant differences when comparing non-fertilized vs. fertilized plots on the same given date using Tukey tests (Fig. 2D; all of those Tukey tests: $P \geq 0.05$).
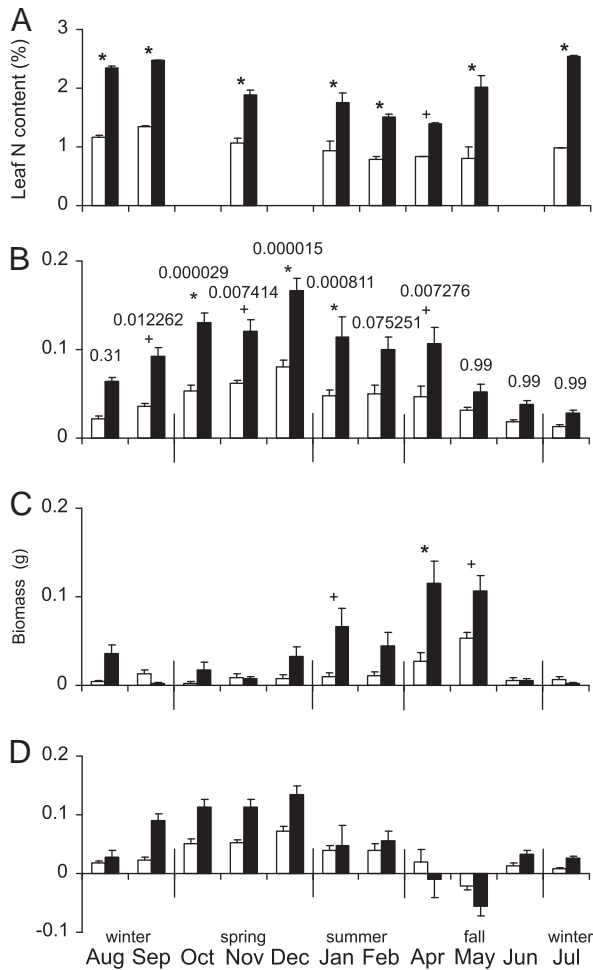
Fig. 2. The impacts of fertilization on (A) the nitrogen content, (B) growth, (C) consumption by *N. granulata*, and (D) the net change in biomass of *S. densiflora* leaves. Bars (open: non-fertilized; solid: fertilized) represent mean values ± SE. Asterisks on top of the bars denote significant differences between non-fertilized and fertilized plots on the given date (for nitrogen content: P<0.05; for growth and herbivory: P<0.005 due to non-compliance with the assumptions of normality and homoscedasticity, see "Materials and methods") obtained with post-hoc Tukey tests. Crosses on top of the bars denote marginally-significant differences between non-fertilized and fertilized plots on the given date (for nitrogen content: 0.1>P≥0.05; for growth and herbivory: 0.05>P≥0.005 due to non-compliance with the assumptions of normality and homoscedasticity, see "Materials and methods") obtained with post-hoc Tukey tests.

3.2. Short-term effects of nutrient enrichment and recent herbivory on length-specific leaf growth and senescence

Recent herbivory by *N. granulata* decreased the length-specific growth of *S. densiflora* leaves in the experiment done in late summer 2007 (Fig. 3A), regardless of whether the leaves had been fertilized or not (main herbivory effect: $F_{1, 28} = 46.8$, P<0.001; fertilization × herbivory interaction: $F_{1, 28} = 1.2$, P=0.3). Fertilization increased length-specific leaf growth (main nutrient effect: $F_{1, 28} = 5.0$, P<0.05), but the positive effect of fertilization was small in comparison with the negative effect of herbivory as demonstrated by the magnitude of the respective F ratios.

Recent herbivory increased length-specific senescence of *S. densiflora* leaves (Fig. 3B), regardless of whether the leaves had been fertilized or not (main herbivory effect: $F_{1, 28} = 46.4$, P<0.001; fertilization × herbivory interaction: $F_{1, 28} = 0.1$, P=0.7). Fertilization had no impact on length-specific senescence of *S. densiflora* leaves in this experiment (main nutrient effect: $F_{1, 28} = 1.6$, P=0.2).
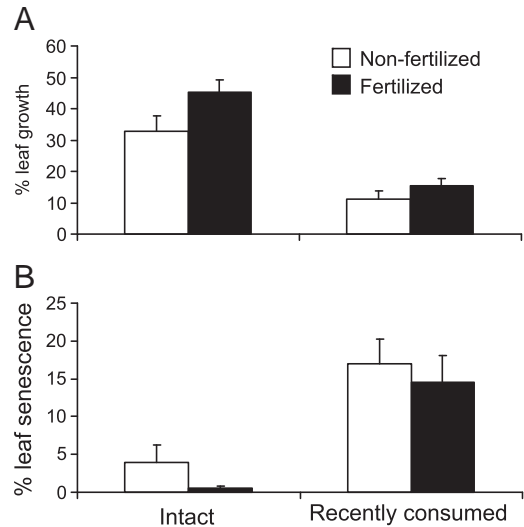
Fig. 3. (A) Percent leaf growth and (B) percent leaf senescence in intact and recently grazed leaves from non-fertilized (open bars) and fertilized (solid bars) plots. Bars represent mean values ± SE. See text for results from post-hoc Tukey tests.

3.3. Effects of nutrient enrichment and herbivory on stem density dynamics

The dynamics of stem density reflected seasonal patterns of ambient herbivory and plant growth, as well as impacts of fertilization on these patterns, that were consistent overall with the other year-long experiment run from August 2006 to July 2007 (Fig. 2). Indeed seasonal changes in stem density varied largely depending on the treatment considered (Fig. 4; season × fertilization × herbivory interaction, P<0.05). In relation to initial conditions, stem density in open, non-fertilized plots did not change significantly during summer and fall (all post-hoc Tukey tests P>0.9), seasons where ambient growth rates drop and ambient herbivory raises. Stem density in caged, non-fertilized plots was not higher in the summer than at the beginning of the experiment (Tukey test, P=0.8), but it was marginally higher in the fall, the season with the highest ambient herbivory, than at the beginning of the experiment (Tukey test, P=0.08). Stem density in open, fertilized plots was higher in summer, a season where fertilization had a positive effect on leaf growth, than at the beginning of the experiment (Tukey test, P<0.05). However, stem density in open, fertilized plots remained unchanged through the fall (Tukey test, P=0.9), a season of intense herbivory on fertilized plants. Stem density in caged, fertilized plots increased from the beginning of the experiment to summer to fall (all Tukey tests, P<0.05), reflecting the compounded impacts of fertilization and herbivory prevention during those seasons.
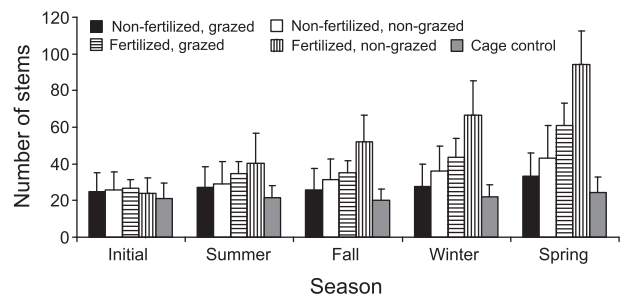
Fig. 4. Seasonal changes in stem density in open, non-fertilized plots (solid bars); caged, non-fertilized plots (open bars); open, fertilized plots (cross bars); and caged, fertilized plots (hatch bars). Bars represent mean values ± SE. See text for results from post-hoc Tukey tests.

All treatments increased their stem density in winter and spring in relation to initial conditions (Fig. 4). The magnitude of those increases augmented from open, non-fertilized to caged, non-fertilized to open, fertilized to caged, fertilized plots as revealed by the value of the corresponding Tukey tests. All comparisons between open, non-fertilized plots and cage controls were non-significant (t_c-test, P>0.1 for all comparisons).

4. Discussion

Our results exemplify a number of interactions between *S. densiflora*, the herbivorous crab *N. granulata* and nutrient availability. Fertilization did clearly increase the consumption of *S. densiflora* leaves by the crab in summer and, even to a greater extent, in fall. The higher levels of leaf consumption found in fertilized plots were associated with higher nitrogen contents in the leaves, suggesting that enhanced herbivory was a response to the higher nutritional quality of fertilized leaves. Indeed many others have also found higher herbivory rates on plants with higher nitrogen contents (e.g., Griffin et al., 1998; Sterner and Elser, 2002; Goecker et al., 2005; Cebrian et al., 2009). However, the seasonal herbivory by *N. granulata* on *S. densiflora* leaves (most annual consumption occurring in fall) did not exhibit the same pattern that the seasonal variation in N-content in leaves (highest %N in winter). These results suggest that even though crabs prefer N-rich leaves, other factors are driving the seasonal variation in herbivory. This seasonal pattern could result from higher plant exposure to the herbivore in fall due to longer tidal submersion intervals (Alberti et al., 2007b), ontogeny of the crab and associated nutritional requirements, which may vary among seasons (see Westoby, 1998 for large herbivores), and availability of more palatable food sources in other seasons (Persson and Brönmark 2002).

Whatever the causes that determine herbivory rates, partial consumption of leaves can have positive and negative effects on subsequent plant growth. Some herbivores can promote leaf growth through moderate consumption (i.e. compensation; Zieman et al., 1984; de Mazancourt et al., 1998; Agrawal, 2000; Tiffin, 2000; Hawkes and Sullivan, 2001; Yamauchi and Yamaura, 2004), while others magnify the direct negative effects of consumption by increasing the loss of live tissues through promoted leaf abscission (Moore et al., 2003; Risley, 1993) and/or increased infestation by pathogens (Daleo et al., 2009). The experiment done in late summer 2007 showed that herbivory by *N. granulata* reduces length-specific growth rates of *S. densiflora* leaves to a similar extent in non-fertilized and fertilized leaves. Namely, length-specific growth rates in non-fertilized leaves decreased from ca. 35% to 10% when the leaves were grazed upon by the crab, and from ca. 45% to 15% when fertilized leaves were grazed upon. Herbivory by the crab also had a negative impact on leaf length-specific senescence, increasing it from ca. 5% to 15% in non-fertilized leaves and from 1% to 15% in fertilized leaves. Reduced growth rates and increased senescence in grazed leaves in comparison with non-grazed leaves may have partially resulted from enhanced fungal infectiveness following the wounds caused by the crab, as other authors have found in other salt marshes (Daleo et al., 2009; Silliman and Newell, 2003). Indeed, fungal infections were found in senescent tissue around wounds produced by crabs on *S. densiflora* and not in ungrazed leaves (P. Daleo, N. Peña, J. Alberti and O. Iribarne unpubl. data). Regardless of the cause of reduced growth and increased senescence after grazing, our results suggest that the profound impacts of invertebrate herbivores throughout the western Atlantic salt marshes (Alberti et al., 2007a; Bertness et al., 2008; Holdredge et al., 2009; Rand, 2002; Silliman and Bortolus, 2003) might involve these overlooked mechanisms.

The negative impact of herbivory by *N. granulata* on the growth of *S. densiflora* leaves may partially explain the seasonal patterns of leaf growth observed here. In spring, when ambient herbivory is small and fertilization does not have a noticeable impact on herbivory, ambient leaf growth rates are high and fertilization enhances them largely. In summer, ambient herbivory continues to be small but fertilization increases herbivory noticeably. This could have a negative impact on fertilized leaves, which in summer do not show such higher growth rates in comparison with non-fertilized leaves as they do in spring. In fall, ambient herbivory increases and the stimulatory impact of fertilization on herbivory is largest, which coincides with a reduction of the growth rates of both non-fertilized and fertilized leaves and the eventual disappearance of statistically-significant differences in growth rates between the two types of leaves. Thus, along with unfavorable environmental conditions such as reduced photoperiod and lower temperature, our results suggest that elevated herbivory by *N. granulata* in fall, through direct (e.g. removal of photosynthetic tissue) and indirect (e.g. more fungal infections) deleterious impacts, may be responsible for concomitant seasonal drop in the growth rates of *S. densiflora* leaves regardless of whether the leaves are fertilized or not. Our results also suggest that intense herbivory in the fall may lead to a net decrease in stem leaf biomass, even more pronounced under fertilized conditions, as opposed to a net increase that could be found in spring when herbivory is small. The seasonal strong impact of *N. granulata* on *S. densiflora* leaves translates to the dynamics of *S. densiflora* stems (comparing with and without herbivory and/or fertilization).

There is increasing consensus that both top-down and bottom-up forces are prevalent in many systems (see review by Gruner et al., 2008). An important goal of contemporary ecology is to determine how these two forces interact and when and how one of them is relatively more important than the other (e.g., Borer et al., 2006; Burkepile and Hay, 2006). Even though both forces usually control the diversity of primary producers interactively (Hillebrand et al., 2007), the interaction between those two forces controlling the biomass of primary producers is uncommon (Gruner et al., 2008). Here, we show that grazing by the crab *N. granulata* on *S. densiflora* not only interacts with nutrients but also with time. Herbivory is highly seasonal, leading to shifts in the relative importance of herbivory (most important in fall) and nutrients (most important in spring) throughout the year. Herbivory by the crab reduces leaf growth rates and enhances senescence, which may be partially responsible for the seasonal decline in leaf growth and for the net loss of stem leaf biomass observed in the fall. These effects also appear to occur under fertilized conditions because, despite the fact that leaf growth rates increase with fertilization, herbivory by the crab increases too. These results might also have important consequences for the estimation of nutrient cycling by marsh herbivores, as many marshes around the world are inhabited by herbivores that consume just parts of the leaves of closely related plant species (Alberti et al., 2007a; Bertness et al., 2008; Holdredge et al., 2009; Silliman and Bortolus, 2003; Silliman and Zieman, 2001). Given that nutrient cycling calculations sometimes involve biomass comparisons between controls and exclosures (Belovsky and Slade, 2000; Ritchie et al., 1998), ignoring the potential effects of herbivores on leaf growth and senescence rates might lead to improper estimations of the amount of biomass consumed and thus cycled by herbivores.

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